J. Microbiol. Biotechnol. (2012), **22**(1), 25–33
http://dx.doi.org/10.4014/jmb.1107.07029
First published online November 11, 2011

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Virulence, Resistance Genes, and Transformation Amongst Environmental Isolates of *Escherichia coli* and *Acinetobacter* spp.

Doughari, Hamuel James*, Patrick Alois Ndakidemi, Izanne Susan Human, and Spinney Benade

Applied Sciences Faculty, Cape Peninsula University of Technology, PO Box 652, Cape Town 8000, South Africa

Received: July 13, 2011 / Revised: September 13, 2011 / Accepted: September 26, 2011

The association of verotoxic *E. coli* and *Acinetobacter* spp. with various antibiotic-resistant, diarrhogenic, and nosocomial infections has been a cause for concern worldwide. *E. coli* and *A. haemolyticus* isolated on a number of selective media were screened for virulence factors, antibiotic resistance, and transformation of resistance genes. Out of 69 *E. coli* isolates obtained, 25 (35.23%), 14 (20.30%), and 28 (40.58%) were positive for Vtx1&2, Vtx1, and Vtx2, respectively, 49 (71.015%) for extended-spectrum beta-lactamases (ESBLs), 34 (49.28%) for serum resistance, 57 (82.61%) for cell surface hydrophobicity, 48 (69.57%) for gelatinase production, and 37 (53.62%) for hemolysin production. For the 14 *A. haemolyticus* isolates, only 2 (14.29%) in each case from all the samples investigated were positive for Vtx1, Vtx2 and Vtx1&2 respectively, 8 (57.14%) for ESBLs, 7 (50.00%) for serum resistance, 11 (78.57%) for cell surface hydrophobicity, 4 (28.57%) for gelatinase production, and 8 (57.14%) for hemolysin production. Although transformation occurred among the *E. coli* and *Acinetobacter* isolates (transformation frequency: 13.3×10^{-7}–53.4^{-7}), there was poor curing of the plasmid genes, a confirmation of the presence of stable antibiotic-resistant genes (DNA concentration between 42.7 and 123.8 µg) and intragenetic transfer of multidrug-resistant genes among the isolates. The isolates were potentially virulent and contained potentially transferable antibiotic resistance genes. Detection of virulence factors, antibiotic resistance genes, and transformation among these isolates is a very significant outcome that will influence approaches to proactive preventive and control measures and future investigations. However, continued surveillance for drug resistance among these bacteria and further investigation of the mechanism of action of their virulence factors are a necessity.

*Corresponding author
Phone: +27-(0)-73355-0274, 234-(0)-703-559-9712;
E-mail: jameshamuel@yahoo.com, dougharijames@gmail.com

Keywords: Cell surface hydrophobicity, extended-spectrum beta-lactamases, gelatinases, hemolysins, resistance genes, transformation, virulence factors

Pathogenic bacteria utilize a number of mechanisms to cause disease in human hosts [31]. These mechanisms are often expressed in a wide range of molecules that enable adhesion of bacteria to host cell targets in order to initiate the infection process, and as a result trigger a variety of different host responses [26]. The virulence factors are of two main types; those produced on the surface of the cell, and those produced within the cell and then exported to the site of action. Those on the surface include different sorts of fimbriae (s, p, or type F 1 or curli fimbriae), certain other mannose-resistant adhesins, and K capsules or cell surface lipopolysaccharides (LPS) that have a role in adhesion to the surface of host cells, but may also have additional roles such as tissue invasion, biofilm formation, or cytokine induction [6]. Virulence factors secreted within the cell and exported into the medium or host cell protoplast include enzymes such as hemolysins, gelatinases, and beta-lactamases. Just as in other bacteria, *Escherichia coli* and *Acinetobacter haemolyticus* depend largely on several virulence factors for survival in host tissue and for pathogenicity. Verotoxic *E. coli* (VTEC) has been a source of public concern, as it is responsible for outbreaks of bloody and traveler's diarrhea [30], whereas *Acinetobacter* spp. that were considered nonpathogenic are now associated with notorious multidrug-resistant nosocomial infections as well as bloody diarrhea [7]. Although *E. coli* O157:H7 is responsible for approximately half of all confirmed VTEC infections in Europe, there is growing concern about the risk of non-O157 VTEC serotypes for humans [3]. The mechanisms involved in VTEC adherence to epithelial cells and colonization are yet to be understood [2]. A key to fighting these bacterial pathogens is the identification and characterization of all the virulent factors

that make them so potent. This is crucial for the effective diagnosis of the infection, the surveillance of animal reservoirs, the assessment of public health risks, and the development of controlled interventions. There are growing concerns regarding the increasing significance of both *Acinetobacter* spp. and *E. coli* as causative agents of notorious antibiotic-resistant infections in both hospital and community set-ups. Recently there was a diarrheal outbreak associated with an *E. coli* infection in Germany and other parts of the world [5]. There is therefore a need for surveillance for the presence of these organisms, the characterization of their virulent potentials, and the determination of their potential to transfer resistant genes to other bacteria, especially in the developing countries. Thus, this novel work reports on virulence factors, antibiotic resistance genes, and the potential for the transfer of resistance amongst environmental isolates of verotoxic strains of *E. coli* and *Acinetobacter* spp. isolated from a number of waste and river water samples in South Africa.

Materials and Methods

Sources of Media, Antibiotics, Chemicals, and Plant Materials

Eosin Methylene Blue (EMB), Nutrient Broth (NB), modified Trypton Broth (mTSB), Mueller–Hinton Agar (MHA), and antibiotic discs were all Oxoid grade, and were purchased from Quantum Biotechnologies, Cape Town, South Africa (SA). Glisa Duopath Verotoxins test kits were sourced from Merk (Darmstadt, Germany), and oxidase test strips and all the laboratory-grade chemicals used in this study, unless otherwise indicated, were procured from Merck (Johannesburg, SA). *E. coli* polyvalent antisera 2, 3, and 4, and REMEL RapID NF plus test kits were obtained from Bioweb (Randburg, SA).

Sample Collection

Sixty-two water samples (18 wastewater samples each from a wastewater treatment plant and an abattoir, and 14 river water samples each from the River Berg and the River Plankenburg), all in Cape Town, South Africa, were collected using the shoreline sampling method [16]. For microbiological analysis, 1 l volume sized sterilized sample bottles were held at the base and dipped downwards below the water's surface (20–30 cm deep), opened, and allowed to fill up and then corked while still under water [9]. The collected water samples were placed in a cooler box with the temperature maintained between 4 and 10°C using ice packs. The samples were then immediately transported to the Microbiology Laboratory of the Biotechnology Department of Cape Peninsula University of Technology, Cape Town, South Africa, where they were analyzed within 3–6 h.

Cultivation, Isolation, and Identification of Bacteria

For the isolation of *E. coli*, water samples were serially diluted up to 10^5, and 1 ml was then inoculated into a Brilliance *E. coli*/coliform selective medium (BECSM) (Oxoid, Basingstoke, UK) by the agar dilution method, and the plates were incubated at 37°C for 24 h. After incubation, discrete colonies were separated and inoculated onto plates of EMB and incubated at 37°C for 24 h. Isolates were further purified by picking discrete colonies (green metallic sheen) and subculturing onto fresh plates of EMB, and once again incubating for 18–24 h at 37°C. After incubation, 5–10 discrete colonies were characterized using an IMViC (DIFCO, Sparks, USA) test kit, oxidase test strips, and Ehrlich's reagent. Isolates that were indole positive, nonmotile, as well as being negative for methyl red, through Voges–Proskauer and citrate utilization tests, were identified as *E. coli.* Slide agglutination tests were performed on selected 5–10 presumptive single colonies using polyvalent *E. coli* antisera 2, 3, and 4 (Bioweb, Randburg, SA). Differences between colonial isolates were determined by the characterization of their antibiotic susceptibility profiles. *E. coli* ATCC 25922 was used as the control. Serotyped (confirmed) *E. coli* isolates were inoculated onto tryptic soy (TS) slants and incubated for 24 h at 37°C, and then stored at 4°C until use [23, 28].

To isolate and identify *Acinetobacter* spp., 1 ml of 10^5 dilutions of the water samples, as with *E. coli,* was inoculated into 9 ml tubes containing Baumann's enrichment medium (BEM) with a composition of 2 g sodium acetate (trihydrate), 2 g KNO_3, and 0.2 g $MgSO_4 \cdot 7H_2O$, dissolved in a 0.04 M KH_2PO_4–Na_2HPO_4 buffer (pH 6.0), and made up to a total volume of 1 l, instead of EMB. The inoculated BEM was shaken vigorously by vortexing and then incubated at 37°C for 24 h in a shaker incubator with vigorous agitation. After incubation, 2 drops of the BEM culture were further inoculated into mTSB in a test tube and incubated at 37°C for 24–48 h. After this, 1–2 loopfuls of BEM or mTSB cultures was inoculated onto EMB (Oxoid, Basingstoke, UK) or Leeds *Acinetobacter* medium (LAM) (Hardy Diagnostics, Santa Maria, USA), with a composition of 10 g agar, 15 g acid hydrolyzate of casein, 5 g soy peptone, 5 g NaCl, 5 g D-fructose, 5 g sucrose, 5 g D-mannitol, 1 g L-phenylalanine, 0.4 g iron ammonium citrate, and 0.02 g phenol red. This was then made up to 1 l with distilled water, autoclaved, and cooled to 50°C. To this were added antibiotic solutions of 10 g/l vancomycin, 15 g/l cefsulodin, and 50 mg/l cefradine to give the final concentration. This was then further incubated at 37°C for 18–72 h. After incubation, pink colonies on EMB or pink/purple colonies on LAM were Gram stained to observe for large Gram-negative coccobacilli cells, while 5–10 discrete colonies were inoculated into SIM for a motility testing medium, and also subjected to oxidase tests using oxidase test strips (Oxoid, Basingstoke, UK). The isolates were also subjected to biochemical biotyping using a REMEL RapID NF plus (Bioweb, Randburg, SA). The strains were further purified by inoculation onto TS slants, incubated for 24 h at 37°C, and then stored at 4°C until use [20, 24]. *A. haemolyticus* ATCC 19002 was used as the control.

Detection of Virulence Factors on the Bacterial Isolates

Screening of isolates for verotoxin production. All the bacterial isolates were screened for verotoxin production using antibody-based rapid slide agglutination assays with a Duoperth kit (Merck, Johannesburg, SA) according to the manufacturer's instructions. The bacterial isolates were first precultured in 1 ml of casaminacid yeast extract (CAYE) broth consisting of 20 g of casamino acid, 6 g of yeast extract, 2.5 g of NaCl, 8.71 g of KH_2PO_4, and 1 ml of trace salt solution (0.5% $MgSO_4$, 0.5% $MnCl_2$, and 0.5% $FeCl_3$, dissolved in 0.0005 M H_2SO_4). They were then incubated at 37°C with rotation at 100 rpm for 24 h. After incubation, 10 µl of the precultured broth (approximately 1×10^7 cells/ml) was inoculated into fresh CAYE broth, and further incubated for 16 h with rotation at 100 rpm at 37°C. The culture was then centrifuged at 5,000 rpm for 5 min to

separate the supernatant and cell pellets. The cell pellets were then washed three times with phosphate-buffered saline (PBS, 5 ml), and suspended in 0.25 ml of 0.01 M Tris-HCl (pH 7.5). To the pellets, 0.5 ml of distilled water containing 50 µg/ml polymyxin B was added, and the suspension incubated at 37°C for 30 min. Two hundred µl of the culture suspension was then transferred onto the test device using a sterile Pasteur pipette, and the result read after 10 min. The appearance of red bands, on the Vtx1 or Vtx2 bands, denoted the presence of either one or both verotoxins.

Screening of isolates for hemolysin production. The plate hemolysis technique was used to screen for the presence of cytolytic protein toxins, known as alpha-hemolysin, secreted by most hemolytic bacteria [26]. In this procedure, discrete bacteria colonies (2–3) from nutrient agar (NA) plates were subcultured onto 5% sheep blood agar plates (supplemented with 10 mM $CaCl_2$ for *A. haemolyticus*), and incubated at 37°C for 24 h. After incubation, enterohemolysin production was detected by the appearance of a zone of complete lysis of the erythrocytes around each bacterial colony on the plates, and clearing of the medium.

Cell surface hydrophobicity test. The cell surface hydrophobicity of the bacterial isolates was determined using the salt aggregation test (SAT) [20, 26]. Briefly, a loopful (10 µl) of bacterial suspension in 1 ml of phosphate buffer (pH 6.8) (equivalent to 5×10^9 colonies/ml) was mixed with equal volumes of ammonium sulfate solution of different molarities (1.4, 2.0, and 4.0 M) on a glass slide. The suspensions were rotated carefully for 1 min, and then microscopically observed for agglutination. The highest dilution of ammonium sulfate solution giving a visible agglutination of bacteria was scored as the SAT value. Bacterial suspension clumping at the lowest dilution (1.4 M) was considered auto-aggregative, whereas those with SAT values of ≤2 M were considered hydrophobic.

Gelatinase test. This was carried out in order to screen the bacterial isolates for gelatinase production [23]. Gelatin agar was inoculated with the test bacteria, and then incubated at 37°C for 24 h, after which the plate was then flooded with mercuric chloride ($HgCl_2$) solution. The development of opacity in the medium, and a zone of clearing around the bacterial colonies, was considered positive evidence of the presence of gelatinase.

Bactericidal serum resistance assay. In this method, bacteria were first grown on blood agar for 18–24 h at 37°C. The cells were then harvested and suspended in Hank's balanced salt solution (HBSS). Equal amounts (0.05 ml) of the bacterial suspension and serum were mixed in a test tube and then incubated at 37°C for 180 min. After incubation, 10 µl of the mixture was withdrawn and spread-inoculated onto blood agar plates, and once again incubated at 37°C for 18 h, and the viable count was determined. Resistance of the bacteria to serum bactericidal activity was expressed as the percentage of bacteria surviving after 180 min of incubation with serum, in relation to the original count. Bacteria were termed serum sensitive if the viable count dropped to 1% of the initial value, and resistant if >90% of the organisms survived after 180 min of incubation [22].

Screening of isolates for extended spectrum beta-lactamase (ESBL) production. The screening of isolates for ESBL was carried out using the disc diffusion method according to recommended criteria [14]. Briefly, two discs, 30 µg in each case, of ceftazidime and cefotaxime were placed on Mueller–Hinton agar plates previously seeded with test bacteria, and the plates incubated at 37°C for 18 h. After incubation, ESBL production was determined by the appearance of zone diameters of inhibition (≤ 22 mm for ceftazidime and ≤ 27 mm for cefotaxime) against the test bacteria.

Phenotypic confirmation of ESBL production. The Double Disc Synergy Test (DDST) for confirming ESBL production was used [10, 26]. Zero point one milliliter (0.1 ml) of each bacterial isolate suspension, equivalent to 0.5 MacFarland turbidity standard, was inoculated on the surface of Mueller–Hinton agar plates using a sterile swab stick. A combination disc, containing 20 µg of amoxicillin and and 10 µg of clavulanic acid, was placed at the center of the Petri-dish, and 30 µg of ceftaxidime and 30 µg of cefotaxime were placed 15 mm apart, center to center, on the plates. The culture plates were then incubated at 37°C for 18–24 h. An enhanced zone of inhibition (synergy, regardless of size) between any one of the beta-lactam discs, compared with the combined amoxicillin–clavulanic acid disc, was considered to be positive for ESBL enzyme production [10].

Determination of Antimicrobial Susceptibility Pattern of Isolates

This was carried out using the disc diffusion method as described elsewhere [18]. Molten Mueller–Hinton agar (MHA) plates were inoculated with the test organisms (0.5 McFarland turbidity standard) using a sterile swab stick and the plates were incubated at 37°C for 18 h. After incubation, susceptibility to antibiotics was determined by measurement of zone diameters of inhibition (mm) against test bacteria. Antibiotics tested included ampicillin (10 µg), cefuroxime (30 µg), cephalexin (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), aztreonam (30 µg), nalidixic acid (30 µg), amikacin (30 µg), tetracycline (30 µg), gentamicin (10 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), and impenim (5 µg) (Oxoid, Basingstoke, UK). Antibiotics were selected based on the recommended criteria for the surveillance of antibiotic resistance in Enterobacteriaceae and *Acinetobacter* spp. [11, 13, 15, 25, 31]. Isolates that were resistant to 3–7 antibiotics were selected and used for further studies.

Extraction and Quantification of Bacterial DNA

Extraction of plasmid DNA was carried out as earlier described [19]. Briefly, bacterial cultures (2 ml) grown at 37°C for 24 h in MHB was transferred into a microcentrifuge and centrifuged at 5,000 rpm for 10 min, and the supernatant decanted and discarded. The cell pellets were mixed with universal DNA extraction buffer (Promega, Fitchburg, USA) and 2 µl of proteinase K (20 mg/ml) before shake-incubating for 30 min at 37°C. To this, 300 µl of 20% SDS (Beckton-Dickson & Company, Oxford, UK) was added and the mixture was incubated for 2 h at 65°C before being centrifuged at 13,000 rpm for 3 min. This was followed by the addition of an equal amount of chloroformamyl alcohol (24:1) (Merck, Darmstadt, Germany), and the suspension properly shaken to mix. The aqueous part was transferred to a new tube, and isopropanol and 70% ethanol (Merck, Darmstadt, Germany) were added to wash the pellet obtained. Ten µl of TE buffer (Sigma-Aldrich, St. Louis, USA) was then added to dissolve the DNA. The concentration of the extracted DNA was measured using a Spekol 1300 analytik jena UV–visible spectrophotometer (United Scientific, Johannesburg, SA) at 260 nm.

Transformation Test

Equal volumes (50 ml) of bacterial broth culture and lysed cells, or DNA isolated from either *Acinetobacter* sp. or *E. coli,* were mixed in an Eppendorf tube and then spread onto a nitrocellulose filter

(Millipore, Billerica, USA). The nitrocellulose filter was then placed on a Luria–Bertani agar (LBA) plate supplemented with ampicillin and rifampin, and incubated at 30°C for 18 h. The DNA used was either purified bacterial DNA at concentrations of 0.1, 1, 10, and 50 mg per 50 ml of broth, or cell lysates at concentrations of 1, 10, and 100 ml per 50 ml of broth. After incubation, the overgrown filter was transferred to a 50 ml Falcon tube and vortexed with 2 ml of a solution containing 0.85% NaCl and 50 ml of DNase I (5 mg/ml). Tenfold dilutions were plated onto LBA plates supplemented with ampicillin and impenim (recipient counts), and ampicillin, impenim, amikacin, and ceftriaxone (transformant counts), and colony forming unit (CFU) counts were determined after incubation of the plates at 30°C for 72 h. Plates obtained from filters containing either a culture suspension in 50 ml of water (for occurrence of spontaneous impenim mutants and bacterial contamination), only DNA (10 ml), or 100 ml of lysate (to check for sterility), were used as controls. Transformation frequencies (presented as mean triplicate values) are given as the number of *Acinetobacter* spp. or *E. coli* colonies growing on transformant-selective LBA plates divided by the number of colonies on recipient-selective plates after the filter transformations [19].

Transfer of Resistance Genes Between *E. coli* and *Acinetobacter* spp.

Transfer of antibiotic-resistant genes between *E. coli* strains susceptible to 3–7 antibiotics and strains resistant to up to 7 antibiotics, as well as between resistant *E. coli* strains and susceptible *A. lwoffii* and *A. haemolyticus*, were tested. Mueller-Hinton broth (10 ml) was dispensed into four different sets of flasks and used for each of the bacterial isolates. The first flask was inoculated with 0.5 McFarland standard antibiotic susceptible *E. coli* strains (*E. coli* S), the second flask was inoculated with antibiotic resistant *E. coli* strains (*E. coli* R), resistant to the seven antibiotics (AMP-AMK-GEN-CEP-CRO-IMP-CXM), and the third flask was inoculated with both *E. coli* S and *E. coli* R strains to determine the transfer of resistance between *E. coli* strains. To the fourth flask, however, either resistant *A. lwoffii* or *A. haemolyticus* was inoculated instead of *E. coli* R in order to determine transfer of resistance between *E. coli* and *Acinetobacter* spp. All the flasks were then incubated at ambient temperature for 48 h. After incubation, a loopful of each broth culture was spread-inoculated onto plates of MHA and the susceptibility pattern assessed as described earlier [1] in order to determine recombinant isolates that have acquired resistance genes.

Plasmid Curing Test

Briefly, tubes containing 10 ml of peptone water, supplemented with 20 mg/ml acridine orange, were inoculated with 0.1 ml of overnight culture broth and incubated at 37°C for 24 h. Appropriate dilutions of the culture were inoculated on MHA to obtain single colony isolates. After overnight incubation at 37°C, the resulting colonies were tested for loss of antibiotic resistance on MHA plates containing appropriate concentrations of antibiotics [21]. Transformation of cured isolates was carried out using the $CaCl_2$ protocol described elsewhere, with cured cultures serving as competent recipients. To make the cured cultures competent, 0.1 ml of 2–4 h LB broth (grown at 37°C) was chilled in an ice bath, centrifuged for 5 min at 3,000 rpm, and the pellet suspended in 4 ml of ice-cold 50 mM $CaCl_2$ and incubated for 10 min on ice. The cells were then centrifuged at 3,000 rpm for 5 min, resuspended in 2 ml of ice-cold 50 mM $CaCl_2$, and then incubated for 5 min on ice. The cells were again centrifuged at 3,000 rpm for 5 min, and 125 µl of fresh LB broth was added to the pellets. To 50 µl of these competent cells, 5 µl of plasmid DNA was added in a vial and properly shaken to mix, and the vial was placed on ice for 3 min. The ice-cold vial was then transferred to a water bath containing water heated to 42°C for 3 min. To this mixture, 500 µl of fresh LB medium was added and the broth culture then incubated at 37°C for 2 h. After incubation, 100 µl of competent-plasmid DNA treated culture, or competent cells with no plasmid DNA (negative control), was spread-inoculated onto antibiotic-containing MHA plates and incubated for 18 h at 37°C.

Statistics

The significance in transformation and difference between the percentage values of antibiotic resistance among bacterial strains were evaluated using the Student *t* test ($p<0.05$) of the SIGMAPLOT statistical package (Systat Software, Chicago, USA).

RESULTS

Identification of *E. coli* Isolates and Detection of Virulence Factors

E. coli colonies on eosin methylene blue (EMB) were seen to be tiny with a green metallic sheen, and tested negative for the motility test, sulfide and oxidase production, and for Ehrlich's reagent, but positive for indole production. The various serotypes identified are shown in Table 3. For verotoxin production genes, results showed that out of the 69 *E. coli* isolates obtained, 25 (35.23%) produced Vtx1&2, 28 (40.58%) produced Vtx1, and 14 (20.30%) produced Vtx2 (Table 1). The highest rate (40%) of production of Vtx1&2 was obtained from the river water isolates, followed by isolates from the treatment plant wastewater (36%) and abattoir wastewater (33%). Results also showed that 49 (71.015%) were positive for extended-spectrum beta-lactamases (ESBLs), 34 (49.28%) for serum resistance, 57 (82.61%) for cell surface hydrophobicity, 48 (69.57%) for gelatinase production, and 37 (53.62%) for hemolysin production. Results also indicated that 19 of the 25 (76.00%) isolates from the treatment plant wastewater, 13 (54.17%) of the 24 from the abattoir wastewater, and 17 (80%) of the 20 isolates from the river waters, produced the ESBL enzymes. This shows that the highest percentage of isolates that produced the enzymes were from the river waters, followed by isolates from the treatment plant wastewater and the abattoir wastewater. The highest rate of hemolysin production was obtained in isolates from the treatment plant wastewater samples with 19 (76%) out of the 24 isolates producing hemolysins, followed by isolates from the river waters with 10 (50.00%), and the abattoir wastewater with 8 (33.33%). Results also indicated that all the isolates from all the water sources tested were positive for cell surface hydrophobicity, with the percentage occurrence of 19 (76.00%) in the treatment plant wastewater, 10

Table 1. Virulence characteristics of [a]*Escherichia coli* isolates obtained from water samples.

Sample source	No. of isolates	Number (%) positive for virulence factor					
		Verotoxin	ESBLs	Serum resistance	Cell surface hydrophobicity	Gelatinase	Hemolysin
Wastewater	25	Vtx1: 10(40.00) Vtx2: 6(24.00) Vtx1&2: 9(36.00)	19(76.00)	16(64.00)	22(88.00)	12(48.00)	19(76.00)
Abattoir waste	24	Vtx1: 2(8.33) Vtx2: 12(50) Vtx1&2: 8(33.33)	13 (54.17)	9(37.50)	21(87.5)	18(75.00)	8(33.33)
River water	20	Vtx1: 2(10.00) Vtx2: 10(50.00) Vtx1&2: 8(40.00)	17 (85.00)	6(30.00)	14(70.00)	18(90.00)	10(50.00)

ESBLs = extended spectrum beta-lactamases.
[a]Green metallic sheen on eosin methylene blue (EMB), negative for motility test, sulfide and oxidase production, and for Ehrlich's reagent, but positive for indole production.

(50.00%) in the river waters, and 8 (33.33%) in the abattoir wastewater sample. For serum resistance, the highest percentage, at 16 (64%) of 25, of positive isolates were from the treatment plant wastewater, followed by 9 (37.50%) of the 24 isolates from the abattoir wastewater, and 6 (30%) of the 20 isolates from the river water samples. Results for the gelatinase test showed that 12 (48.00%) out of the 25 isolates from the treatment plant wastewater produced gelatinase, followed by 18 (75.00%) out of 24 and 18 (90.00%) out of 20 isolates from the abattoir wastewater and river water samples, respectively. For the bactericidal serum resistance assay, the highest percentage resistance of 16 (64.00%) out of 25 isolates was recorded from the treatment plant wastewater, followed by 9 (37.50%) of the 24 isolates from the abattoir wastewater, and 6 (30.00%) of the 20 isolates from the river water samples examined.

Identification of *Acinetobacter* spp. and Detection of Virulence Factors

Colonies of *Acinetobacter* spp. were observed as a Gram-negative coccobacilli, appearing as tiny blue mucoid colonies on EMB and seen as pink defused colonies on Leeds *Acinetobacter* media (LAM). They also fermented glucose, urea, citrate, nitrite, and tryptophan (Table 2). Results for the detection of virulence factors in *A. haemolyticus* isolates obtained from the various water samples showed that out of the 14 *A. haemolyticus* isolates obtained, only 2 (14.29%) isolates for each of the samples were positive for Vtx1, Vtx2, and Vtx1&2 verotoxic genes,

Table 2. Virulence characteristics of [a]*Acinetobacter haemolyticus* isolates obtained from water samples.

Sample source	No. of isolates	Number (%) positive for virulence factor					
		Verotoxin	ESBLs	Serum resistance	Cell surface hydrophobicity	Gelatinase	Hemolysin
Wastewater	4	Vtx1: 1(25.00) Vtx2: 0(0.00) Vtx1&2: 0(0.00)	3(75.00)	3(75.00)	4(100.00)	0(0.00)	4(100.00)
Abattoir waste	5	Vtx1: 1(20.00) Vtx2: 1(20.00) Vtx1&2: 1(20.00)	2 (40.00)	3(60.00)	4(80.00)	3(60.00)	4(80.00)
River water	5	Vtx1: 0 (60.00) Vtx2: 1(20.00) Vtx1&2: 1(20.00)	3(75.00)	1(20.00)	3(75.00)	1(20.00)	0(0.00)

ESBLs = extended spectrum beta-lactamases.
[a]Tiny blue mucoid colonies on EMB, pink colonies diffused into the medium on LAM, Gram-negative coccobacilli, and fermented glucose, urea, citrate, nitrite, and tryptophane.

whereas 8 (57.14%) were positive for ESBLs, 7 (50.00%) for serum resistance, 11 (78.57%) for cell surface hydrophobicity, 4 (28.57%) for gelatinase production, and 8 (57.14%) for hemolysin production (Table 2). But for the absence of hemolysin in 5 of the river water isolates and gelatinase in 4 of the wastewater isolates, all other virulent factors were present on isolates from all the water samples investigated. Results also showed that the most prevalent virulent factor among the isolates was cell surface hydrophobicity, which was present in all the 4 (100%) isolates from the wastewater samples, 4 (80.00%) of the 5 isolates from the abattoir wastewater, and 3 (75.00%) of the 5 isolates from the river water samples. Furthermore, results revealed that 3 (75%) out of the 4 isolates from the wastewater samples were positive for both ESBLs and serum resistance factors.

Determination of Antimicrobial Susceptibility Patterns of Isolates

Results of antimicrobial susceptibility (Tables 3 and 4) showed that the majority of the isolates were resistant to between 4 and 10 antibiotics. Only 3 *E. coli* isolates (*E. coli* FEW O124, *E. coli* FEW103:H2, and *E. coli* FEW2iii O145:NM), and 3 *Acinetobacter* isolates (*A. lwoffii* RWW2i, *A. haemolyticus* PST2i, and *A. haemolyticus* PST2i) were susceptible to all the antibiotics tested.

Extraction and Quantification of Bacterial DNA, Resistance Genes, and Transformation Rates Amongst Bacterial Isolates

Quantification of extracted bacterial DNA showed that there was an increase in DNA concentrations in all the transformed bacterial samples compared with the untransformed cells with transformation frequencies ranging from 13.3 – 53.4×10^{-7} (Table 3). Results showed a significant transformation frequency ($p<0.05$) among all the isolates irrespective of the sample source. Result also indicated that resistance genes among *E. coli* and *Acinetobacter* spp. isolates included among others, resistant genes against ampicillin, gentimicin, nalidixic acid, ceftriaxone, tetracycline, amoxicillin, ofloxacin, and ciprofloxacin.

Plasmid Curing Amongst *E. coli* and *Acinetobacter* spp. Isolates

Results of plasmid curing for *E. coli* and *Acinetobacter* spp. revealed that out of 14 multidrug-resistant *E. coli* isolates, 7 were cured of resistance against ampicillin,

Table 3. Resistance patterns and DNA concentrations of normal and transformed *E. coli* and *Acinetobacter* spp. obtained from river water and wastewater samples.

Bacterial isolate	Resistance pattern of untransformed/ normal cells	DNA Quantity (µg/ml) of normal cells	Resistance pattern of transformed cells	DNA quantity (µg/ml) of transformed cells	Transformation frequency
E. coli FEW O124	-	98.4	SXT, OFX, AMP, CN, AK, NA, CXM	102.7	46.7×10^{-7}
E. coli FEW103:H2	-	108.3	ATM, AK, CL, IPM, TE	110.3	33.3×10^{-7}
E. coli FEW2iii O145:NM	-	86.0	TE, CN, CXM, CIP, AMP, NA, CRO	92.4	46.7×10^{-7}
E. coli RWW 1ivO96:H9	AML	112.0	AML, OFX, TE, CN, AMP	115.2	33.3×10^{-7}
E. coli RWW1v O126	TE, CL	78.8	TE, CL, AMP, CIP, CXM	81.4	33.3×10^{-7}
E. coli RWW1vi O4	CFM, CRO	52.6	CFM, CRO	56.0	13.3×10^{-7}
E. coli RWW1vii O55	CIP	90.4	CIP, OFX, NA, AMP, TE	91.4	33.3×10^{-7}
E. coli PSW2iiO96:H9	CN, TE	68.7	AMP, CL, CRO, IPM, TE, CFM, CIP, SXT	73.4	53.4×10^{-8}
E. coli PRE1iO4	CL	42.7	CL, AMP, CN, OFX, NA, TE, AML, AK	45.7	53.4×10^{-7}
E. coli FSE1iiO145:H2	AK	73.0	AK, CL, AMP, TE	83.1	26.7×10^{-7}
E. coli PST1vO96:H9	CN, AMP	68.2	CL, CFM, CRO, CXM, AMP, AK	74.0	40.0×10^{-7}
E. coli PST2iiO124	AK	93.2	AK, AMP, NA, OFX, TE, SXT, IMP, CN	95.6	53.3×10^{-7}
E. coli RBD1iiiO86	AML, CRO, AK	67.4	CFM, CL, AMP, NA, SXT, TE	77.2	40.0×10^{-6}
E. coli PRK2iiO86	AK, AMP, CN	86.3	AK, AMP, CN, AML, TE	88.1	40×10^{-7}
A. lwoffii RWW2i	-	67.5	ATM, AK, CL, SXT, OFX	67.8	33.3×10^{-7}
A. lwoffii PSW1ii	IMP	103.7	CL, AMP, CRO	111.6	33.3×10^{-7}
A. haemolyticus PST2i	-	87.0	OFX, CN, CXM	92.3	20.0×10^{-7}
A. lwoffii PSW1i	TE, CL, IPM	56.0	ATM, AK, CL, IPM, TE	62.4	33.3×10^{-7}
A. haemolyticus FEW2iv	AML	123.5	AML, TE	123.8	13.3×10^{-7}
A. lwoffii PRE2i	AMP, CL, CRO	87.4	AML, OFX, TE, CN, AMP	89.4	33.3×10^{-7}
A. haemolyticus PST2i	-	94.2	TE, CL, AMP, CIP, CXM, AML	98.4	33.3×10^{-7}
A. haemolyticus PST2i	CL	119.3	CFM, CRO, CIP, TE, NA	120.4	40.0×10^{-7}
A. lwoffii RBI2ii	TE, AK, NA	67.8	OFX, NA, AMP, TE	68.2	40.0×10^{-7}

SXT (30 µg) - Trimethroprim-Sulfamethaxazole; OFX (5 µg) - Ofloxacin; ATM (30 µg) - Aztreonam; AMP (10 µg) - Ampicillin; TE (10 µg) - Tetracycline; AK (30 µg) - Amikacin; CAZ (30 µg) - Ceftazidime; CL (30 µg) - Cefalexin; CRO (30 µg) - Ceftriaxone; CXM (30 µg) - Cefuroxime; AML (10 µg) - Amoxicillin; CN (10 µg) - Gentamicin; CFM (5 µg) - Cefixime; CIP (5 µg) - Ciprofloxacin; IMP – Impenem (30 µg); NA (30 µg) -Nalidixic acid. - = susceptible.

Table 4. Resistance patterns of plasmid cured cells of *E. coli* and *Acinetobacter* spp. isolates obtained from river water and wastewater samples.

Bacterial isolate	Resistance pattern before plasmid curing	Resistance pattern after plasmid curing	Resistance markers cured
E. coli FEW O124	SXT, OFX, AMP, CN, AK, NA, CXM	OFX, CN, AK, NA, CXM	SXT, AMP
E. coli FEW103:H2	ATM, AK, CL, IPM, TE	AK, IPM, TE, ATM	CL
E. coli FEW2iii O145:NM	TE, CN, CXM, CIP, AMP, NA, CRO	TE, CN, CXM, CIP, AMP, NA, CRO	-
E. coli RWW 1ivO96:H9	AML, OFX, TE, CN, AMP	AML, OFX, TE, CN, AMP	-
E. coli RWW1v O126	TE, CL, AMP, CIP, CXM	TE, CXM	CL, AMP, CIP
E. coli RWW1vi O4	CFM, CRO	CFM, CRO, CFM	-
E. coli RWW1vii O55	CIP, OFX, NA, AMP, TE	CIP, OFX, NA, TE	AMP
E. coli PSW2iiO96:H9	AMP, CL, CRO, IPM, TE, CFM, CIP, SXT	AMP, CL, CRO, IPM, TE, CFM, CIP, SXT	-
E. coli PRE1iO4	CL, AMP, CN, OFX, NA, TE, AML, AK	CL, AMP, CN, OFX, NA, TE, AML, AK	-
E. coli FSE1iiO145:H2	AK, CL, AMP, TE	AK, CL, AMP, TE	CL, CFM, AMP
E. coli PST1vO96:H9	CL, CFM, CRO, CXM, AMP, AK	AK, CRO, CXM	SXT, AK
E. coli PST2iiO124	AK, AMP, NA, OFX, TE, SXT, IMP, CN	IMP, CN	NA
E. coli RBD1iiiO86	CFM, CL, AMP, NA, SXT, TE	AMP, OFX, TE	-
E. coli PRK2iiO86	AK, AMP, CN, AML, TE	AK, AMP, CN, AML, TE	-
A. lwoffii RWW2i	ATM, AK, CL, SXT, OFX	ATM, AK, AML, TE	AMP, CRO
A. lwoffii PSW1ii	CL, AMP, CRO	CL	CN, CXM
A. haemolyticus PST2i	OFX, CN, CXM	OFX	CN, CXM
A. lwoffii PSW1i	ATM, AK, CL, IPM, TE	ATM, TE, CL, IPM	AK
A. haemolyticus FEW2iv	AML, TE	-	AML, TE
A. lwoffii PRE2i	AML, OFX, TE, CN, AMP	AML, OFX, TE, CN	AMP
A. haemolyticus PST2i	TE, CL, AMP, CIP, CXM, AML	TE, CL, AML, CIP, CXMAML	-
A. haemolyticus PST2i	CFM, CRO, CIP, TE, NA	CFM, CIP, TE, NA	-
A. lwoffii RBI2ii	OFX, NA, AMP, TE	OFX, AMP, TE, NA	-

SXT (30 μg) - Trimethroprim–Sulfamethaxazole; OFX (5 μg) - Ofloxacin; ATM (30 μg) - Aztreonam; AMP (10 μg) - Ampicillin; TE (10 μg) - Tetracycline; AK (30 μg) - Amikacin; CAZ (30 μg) - Ceftazidime; CL (30 μg) - Cefalexin; CRO (30 μg) - Ceftriaxone; CXM (30 μg) - Cefuroxime; AML (10 μg) - Amoxicillin; CN (10 μg) - Gentamicin; CFM (5 μg) - Cefixime; CIP (5 μg) - Ciprofloxacin; IMP – Impenem (30 μg); NA (30 μg) -Nalidixic acid. - = no resistance marker.

cephalexin, trimethroprim–sulfamethaxazole, ciprofloxacin, and nalidixic acid only (Table 4). For the 9 *Acinetobacter* spp., 6 were cured of ampicillin, amoxicillin, amikacin, tertracycline, cefuroxime, and ceftazidime. However, the spectrum of antibiotics cured was narrow when compared with the total number of antibiotics tested.

Transfer of Resistance Genes Between *E. coli* and *Acinetobacter* spp.

Table 5 shows results for transformation of some *Acinetobacter* spp. using resistant *E. coli* [resistant against trimethroprim–sulfamethaxazole (SXT), ofloxacin (OFX), ampicillin (AMP), gentamicin (CN), amikacin (AK), nalidixic acid (NA), cefuroxime (CXM)] as the donor. Results showed that the *A. lwoffii* and all the *A. haemolyticus* isolates tested acquired resistance genes from the *E. coli.* Resistance genes acquired by *A. lwoffii* included amikacin and ampicillin, whereas those acquired by *A. haemolyticus* isolates included ampicillin, gentamicin, and ofloxacin.

DISCUSSION

The virulence of bacterial pathogens including *E. coli* and *A. haemolyticus* largely depends on the presence of virulent factors. These factors enable the bacteria to cause

Table 5. Transformation of *Acinetobacter* spp. by resistant *E. coli* (*E. coli* R resistant to SXT, OFX, AMP, CN, AK, NA, and CXM).

Bacterial isolate	Resistance pattern before transformation with *E. coli*	Resistance pattern after transformation with *E. coli*	Resistance genes acquired
E. coli R	SXT, OFX, AMP, CN, AK NA, CXM	N/T	N/T
A. lwoffii PSW1ii	CL, AMP, CRO	CL, AMP, CRO, AK, AMP	AK, AMP
A. haemolyticus PST2i	OFX, CN, CXM	OFX, CN, CXM, CN	CN
A. haemolyticus FEW2iv	AML, TE	AML, TE, AMP, CN, OFX	AMP, CN, OFX
A. haemolyticus PST2i	-	OFX, CN	OFX, CN

SXT (30 μg) - Trimethroprim–Sulfamethaxazole; OFX (5 μg) - Ofloxacin; ATM (30 μg) - Aztreonam; AMP (10 μg) - Ampicillin; TE (10 μg) - Tetracycline; AK (30 μg) - Amikacin; CAZ (30 μg) - Ceftazidime; CL (30 μg) - Cefalexin; CRO (30 μg) - Ceftriaxone; CXM (30 μg) - Cefuroxime; AML (10 μg) - Amoxicillin; CN (10 μg) - Gentamicin; CFM (5 μg) - Cefixime; CIP (5 μg) - Ciprofloxacin; IMP – Impenem (30 μg); NA (30 μg) -Nalidixic acid. N/T - not tested.

infection by overcoming the host's defence mechanisms, resulting in a variety of infections such as urinary tract infections, soft tissue infections, bacteremia, and neonatal meningitis. In this study, *E. coli* and a number of the *A. haemolyticus* isolates produced verotoxins. The verotoxins, also called Shiga toxins, act by the production of cytotoxins that inhibit protein synthesis [2]. The toxins cause hemorrhagic colitis and potentially fatal systemic sequelae in humans and are the leading cause of acute renal failure in children [2]. The presence of verotoxins in most of the isolates obtained from this study calls for more effective surveillance and control measures. ESBLs confer bacteria with resistance to beta-lactam antibiotics. Increase in the incidence of ESBL-producing strains of *E. coli* among clinical isolates has been steadily rising over the past few years, resulting in the limitation of therapeutic options [24]. The extensive use of antimicrobials, and the indiscriminate use of cephalosporins, is responsible for the high rate of selection of ESBL-producing microorganisms [20, 27]. Alpha-hemolysin is one of the very few proteins produced by members of the family Enterobacteriaceae that is released extracellularly. *E. coli* alpha-hemolysin is a protein that causes *in vitro* lysis of erythrocytes and the subsequent invasion of the cells in several species of animals [12]. Hemolysis, though not essential for the establishment of acute pyelonephritis, may contribute to tissue injury, survival in renal parenchyma, and entry into the blood stream. In this study, both *E. coli* and *A. haemolyticus* were noted as being associated with hemolysin production, an indication that they are potentially invasive if they colonize any cell. Gelatinase, a zinc-metallo-protease, is thought to contribute to virulence through the degradation of host proteins such collagen, fibrinogen, fibrin, and complementary components [29]. The enzyme is also known to contribute to biofilm formation [16]. Cell surface hydrophobicity protects the bacterium from phagocytosis by host cells and helps adherence to surfaces. Enzymes such as esterases, amino-peptidases, and acid phosphatases and lipopolysaccharide slimes on the bacterial cell surface are reported to confer the organisms with surface hydrophobicity [24]. Serum resistance is the ability of a bacterial cell to resist the lytic effects of serum *in vitro* and to invade and survive in the human blood stream [24]. This resistance to killing has been associated with the presence of capsular polysaccharides in *E. coli* [3].

The presence of various resistance genes among the bacterial isolates confirms that the antibiotic resistance genes are located on plasmids. The presence of resistant transformants also confirms that the resistance genes are carried on plasmids and is also an indication that the antibiotic resistance plasmid genes carry multidrug resistance markers. This can also mean that intrageneric resistance transfer is actively taking place among the *E. coli* isolates coexisting in the water samples. This was further confirmed by the exchange of antibiotic resistance between *E. coli* and *Acinetobacter* spp. The plasmid curing experiments showed a limited curing process among the *Acinetobacter* isolates, which is an indication of stability among the multidrug resistance genes. To the best of our knowledge, this is the first documented work on the exchange of resistance genes amongst *E. coli*, *A. lwoffii*, and *A. haemolyticus.*

Demonstration of virulence factors and multidrug resistance plasmid genes amongst the bacteria in this study leads us to conclude that *E. coli* and *Acinetobacter* spp. isolates from these water samples are developing high levels of resistance and virulence. Their spread into drinking water or food sources, if not controlled, could have disastrous consequences. The recent emergence of a very virulent strain of *E. coli*, in an outbreak in Europe [5], shows how rapidly these bacteria are transforming. This therefore calls for continued monitoring processes in both the developed and developing worlds in order to enable the research, design, and evolution of more proactive control and prevention measures.

Acknowledgment

The study was supported by the Cape Peninsula University of Technology, Cape Town, South Africa, through Research Fund RP03.

References

1. Bakar, A. F., A. S. Abdulamir, T. S. Yoke, and N. Nordin. 2010. Detection and quantification of probiotic bacteria using optimized DNA extraction, traditional and real-time PCR methods in complex microbial communities. *Afr. J. Biotechnol.* **9:** 1481–1492.
2. Bolton, D. J. 2011. Verocytotoxigenic (Shiga toxin-producing) *Escherichia coli*: Virulence factors and pathogenicity in the farm to fork paradigm. *Foodborne Pathog. Dis.* **8:** 357–365.
3. Cross, A. S., K. S. Kim, C. D. Wright, J. C. Sadoff, and P. Gemski. 1986. Role of lipopolysaccharide and capsule in the serum resistance of bacteremic strains of *Escherichia coli*. *J. Infect. Dis.* **154:** 497–503.
4. Davis, L. G., M. D. Dibner, and J. F. Battery. 1986. *In*: *Basic Methods in Molecular Biology*, pp. 135–150. Elsevier Science Publishing Co. Inc., New York.
5. CDC. 2011. Investigation update: Outbreak of Shiga toxin-producing *E. coli* O104 (STEC O104:H4) infections associated with travel to Germany. Available at http://www.cdc.gov/ecoli/2011/ecoliO104/index.html. Accessed on 2nd July, 2011.
6. Emödy, L., M. Kerényi, and G. Nagy. 2003. Virulence factors of uropathogenic *Escherichia coli*. Int. *J. Antimicrob. Agents* **22:** 29–33.
7. Grotiuz, G., A. Sirok, P. Gadea, G. Varela, and F. Schelotto. 2006. Shiga toxin 2-producing *Acinetobacter haemolyticus* associated with a case of bloody diarrhea. *J. Clin. Microbiol.* **44:** 3838–3841.

8. Guardabassi, L., A. Dalsgaard, and J. E. Olsen. 1999. Phenotypic characterization and antibiotic resistance of *Acinetobacter* spp. isolated from aquatic sources. *J. Appl. Microbiol.* **87:** 659–667.
9. Health Protection Agency. 2007. Identification of *Shigella* species. National Standard Method BSOP ID 20 Issue 2. Available at http://www.hpa-standardmethods.org.uk/pdf_sops.asp. Accessed 7th June 2011.
10. Iroha, I. R., A. E. O. Ji, and C. O. Esimone. 2008. Antimicrobial resistance pattern of plasmid-mediated extended-spectrum β-lactamase producing strains of *Escherichia coli. Sci. Res. Essay* **3:** 215–218.
11. Jones, R. N., W. A. Craigc, P. G. Ambrose, M. N. Dudley, and S. Pottumarthy. 2005. Reevaluation of Enterobacteriaceae MIC/ disk diffusion zone diameter regression scattergrams for 9 h-lactams: Adjustments of breakpoints for strains producing extended spectrum β-lactamases. *Diag. Microbiol. Infect. Dis.* **52:** 235–246.
12. Konig, B., W. Konig, J. Scheffer, J. Hacker, and W. Goebel. 1986. Role of *Escherichia coli* alpha-hemolysin and bacterial adherence in infection: Requirement for release of inflammatory mediators from granulocytes and mast cells. *Infect. Immun.* **54:** 886–892.
13. NARMS. 2004. The US National Antimicrobial Resistance Monitoring System Reports. http://www.ars.usda.gov/Main/ docs.htm?docid:6750. Accessed 17th November 2009.
14. National Committee for Clinical Laboratory Standards. 2005. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; 15th Ed. Approved standard (M100-S15). NCCLS, Wayne, PA.
15. National Nosocomial Infections Surveillance (NNIS) System Report. 2000. Data summary from January 1992–April 2000. *Am. J. Infect. Control* **28:** 429–448.
16. Obire, O., D. C. Tamuno, and S. A. Wemedo. 2005. Bacteriological water quality of Elechi Creek in Port Harcourt, Nigeria. *J. Appl. Sci. Environ. Mangt.* **9:** 79–84.
17. Park, S. Y., K. M. Kim, J. H. Lee, S. J. Seo, and I. H. Lee. 2007. Extracellular gelatinase of *Enterococcus faecalis* destroys a defense system in insect hemolymph and human serum. *Infect. Immun.* **75:** 1861–1869.
18. Perilla, G., G. M. S. Agello, C. M. S. Bopp, J. Elliot, R. Facklam, J. S. Knapp, *et al*. 2003. Manual for laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world, pp. 103–139. WHO.
19. Podar, M., C. B. Abulencia, M. Walcher, D. Hutchison, K. Zengler, J. A. Garcia, *et al*. 2007. Targeted access to the genomes of low-abundance organisms in complex microbial communities. *Appl. Environ. Microbiol.* **73:** 3205–3214.
20. Raksha, R., H. Srinivasa, and R. S. Macaden. 2003. Occurrence and characterization of *Escherichia coli* in urinary tract infections. *Indian J. Med. Microbiol.* **21:** 102–107.
21. Ramteke, P. W. and S. Tewari. 2007. Serogroups of *Escherichia coli* from drinking water. *Environ. Monit. Assesst* **130:** 215–220.
22. Ranjan, K. P., N. Ranjan, A. Chakraborty, and D. R. Arora. 2010. An approach to uropathogenic *Escherichia coli* in urinary tract infections. *J. Lab. Phys.* **2:** 70–73.
23. Roy, M. R., B. Anne-Marie, T. Marija, R. B. Vicki, R. Jacinta, O. Frances, *et al*. 2004. *Escherichia coli* and community-acquired gastroenteritis, Melbourne, Australia. *Emerg. Infect. Dis.* **10:** 1797–1805.
24. Russo, T. A., N. R. Luke, J. M. Beanan, R. Olson, S. L. Sauberan, U. C. MacDonald, *et al*. 2010. The K1 capsular polysaccharide of *Acinetobacter baumannii* strain 307-0294 is a major virulence factor. *Infect. Immun.* **78:** 3993–4000.
25. Seifert, H., R. Baginski, A. Schulze, and G. Pulverer. 1993. Antimicrobial susceptibility of *Acinetobacter* species. *Antimicrob. Agents Chemother.* **37:** 750–753.
26. Sharma, S., G. K. Bhat, and S. Shenoy. 2007. Virulence factors and drug resistance in *Escherichia coli* isolated from extraintestinal infections. *Indian J. Med. Microbiol.* **25:** 369–373.
27. Shukla, I., R. Tiwari, and M. Agarwal. 2004. Prevalence of extended spectrum β-lactamase in a tertiary care hospital. *Indian J. Med. Microbiol.* **22:** 87–91.
28. Tarawneh, K. A., N. M. Al-Tawarah, A. H. Abdel-Ghani, A. Al-Majali, M. Ahmed, and K. M. Khleifat. 2009. Characterization of verotoxigenic *Escherichia coli* (VTEC) isolates from faeces of small ruminants and environmental samples in Southern Jordan. *J. Basic Microbiol.* **49:** 310–317.
29. Thurlow, L. R., V. C. Thomas, S. Narayanan, S. Olson, S. D. Fleming, and L. E. Hancock. 2010. Gelatinase contributes to the pathogenesis of endocarditis caused by *Enterococcus faecalis. Infect. Immun*. **78:** 4936–4943.
30. WHO. 2002. Water for Health enshrined as a human right. Available at http://www.who.int/mediacentre/news/releases/prl/ en. Accessed 1st September 2010.
31. Wilson, J. W., M. J. Schurr, C. L. LeBlanc, R. Ramamurthy, K. L. Buchanan, and C. A. Nickerson. 2002. Mechanisms of bacterial pathogenicity. *Postgrad. Med. J.* **78:** 216–224.