Low prevalence of antibiotic-resistant gram-negative bacteria isolated from rural south-western Ugandan groundwater

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Abstract

The objective of this study was to determine antibiotic resistance patterns and specific resistance genes in Gram-negative enteric bacteria recovered from 42 different drinking water sources servicing 2 rural villages in south-western Uganda. These water sites were prone to contamination by both human and cattle activity. Of the 52 isolates examined, 26 carried antibiotic resistance genes with 25 being ampicillin resistant, 21 carrying the bla_{TEM} gene, and no isolate carrying genes coding for extended-spectrum β−lactamases. Twelve isolates were tetracycline resistant and these bacteria carried between 1 and 3 different *tet* genes, with the *tet*(A) gene the most common. Six isolates carried the macrolide resistance *mef*(A) and/or the macrolide-lincosamide-streptogramin B resistance *erm*(B) genes. Four isolates carried the *sul1* gene, and 4 isolates carried the *sul1* and *int1* genes indicating the presence of Class 1 integrons. The Ugandan isolates in this study had lower than expected carriage rates of antibiotic and multi-drug resistance genes, carriage of Class 1 integrons and lacked genes coding for extended-spectrum β−lactamases as compared to antibiotic resistance carriage in clinical African isolates.

Keywords: drinking water, antibiotic resistance, resistance genes, Gram-negative, conjugal transfer

Introduction

Pearson et al. (2008) reported on the isolation and biochemical characterisation of water-borne Gram-negative bacteria isolated from boreholes, ponds and valley water tanks used by 2 rural villages, from Nyabushozi County in the Mbarara District of south-western Uganda. Both humans and animals used the water sources and the *E. coli* counts confirmed that 38 of the 42 water sites did not meet international drinking water standards. The inhabitants of these villages were settled and semi-nomadic Bahima pastoralists, Bairu agriculturalists and a few internal migrants. The villages had undergone dramatic water resource changes as a result of land privatisation and the creation of a national park in 1989. The villagers were 10 to 15 km from the nearest local private medical clinic, which charged money for its services and 40 to 50 km from the nearest hospital. These communities lacked the infrastructure required for basic services, such as health care and a municipal drinking water supply. As a result, these people had little interaction with western medicine and relied on traditional herbal treatments for human and livestock diseases (authors' unpublished observations).

The currently published literature suggests that levels of antibiotic-resistant bacteria are high and continue to rise in Africa (Okeke et al., 2007). There are no data on antimicrobial susceptibilities of water bacteria from Uganda. Therefore it was of interest to characterise the level of antibiotic resistance and corresponding resistance genes in the Ugandan bacteria previously isolated from 42 of the 47 Ugandan water sites tested which serviced the 2 villages. In this study, the antibiotic resistance phenotypes and genotypes of 52 randomly selected Gram-negative enteric bacteria from Nyabushozi County in the Mbarara District of south-western Uganda were characterised.

Materials and methods

Bacterial isolates

Previously, water-borne Gram-negative bacteria were recovered from 1 mℓ water samples taken from 47 water sites within 2 villages in Nyabushozi County in the Mbarara District of south-western Uganda. The water samples were plated onto EC 3M™ Petrifilm™ according to the manufacturer's instructions and incubated at 37°C for 24 h. Forty-two of the 47 water sites were positive for enteric bacteria. Random isolates were taken from the Petrifilms and biochemically identified and then representative isolates were verified by sequencing the variable region of their 16S rRNA gene as previously reported (Pearson et al., 2008). Many of the 16S rRNA gene sequences obtained allowed identification to the genus but not species level. From the initial study, 52 genetically distinct archived bacteria were available for further study. These included: 2 *Citrobacter* spp., 20 *E. coli*, 7 *Enterobacter* spp., 5 *Klebsiella* spp., 3 *Morganella morganii*, 2 *Proteus* spp., 3 *Providencia rettgeri*, 4 *Pseudomonas* spp., 4 *Salmonella* spp., and 2 *Serratia odorifera*. Because *E. coli* 0157:H7 had previously been reported in the area (Majalija et al., 2008), we cultured the 20 *E. coli* isolates on Sorbitol-MacConkey medium-SMAC (Remel, Inc., Lenexa, KS, USA; March and Ratnam, 1986). No growth was observed indicating that they were not *E. coli* 0157:H7. Two laboratory strains, *E. coli* HB101 and *E. coli* DH5α were used as recipients in conjugation experiments.

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TABLE 1
List of oligonucleotide primers used in this study

Resistance gene	Primer name	Sequence (5′ → 3′)	Amplicon size (bp)	Reference
ere(A)	*ere*A-fw	TCA CTG GCT AGA GCT AGT CTT	993	Soge et al., 2006a
	*ere*A-rv	CAT TCG CTT TGC TTC CAT GGG		
	*ere*A-int	GAG TTG GAA ACG GCT CAG CAG GAG		
ere(B)	*ere*B-fw	TTG GAG ATA CCC GAG TTG TAG	982	Soge et al., 2006a
	*ere*B-rv	GCT TTC TCG ACA GAA CCT TCA		
	*ere*B-int	CAG AAA TGG AGG TTC ATA CTT ACC		
erm(B)	*erm*B-fw	GAA AAG GTA CTC AAC CAA ATA	639	Ojo et al., 2004
	*erm*B-rv	AGT AAC GGT ACT TAA ATT GTT TAC		
	*erm*B-int	AGC CAT GCG TCT GAC ATC TAT		
mph(A)	*mph*A-fw	GAT ACC TCC CAA CTG TAC GCA	850	Soge et al., 2006a
	*mph*A-rv	CGA GGT ACT CTT CGT TAC CC		
	*mph*A-int	GCT GGC AAT GCT CAA GAA TCG		
mph(B)	*mph*B-fw	TTA AAC AAG TAA TCG AGA TAG C	889	Ojo et al., 2004
	*mph*B-rv	CCT TGT ACT TCC AAT GCT T C		
	*mph*B-int	GCG TAT GGA TGC AGT AAG AGC		
mph(C)	MPHC1	ATG ACT CGA CAT AAT GAA ATT ATT	900	Ojo et al., 2004
	MPHC2	CTA CTC TTT CAT ACC TAA CTC		
	MPHC int	GCT GAA ACA CTC GTA GAT TTA CAC		
mph(D)	*mph*D-fw	AGC CAA TTG CTA CAT GCG CTC T	756	Soge et al., 2006a
	*mph*D-rv	GGG TTT ACG AGC CAA GCA AGA A		
	*mph*D-int	TCA TTC GGC AAC AGC CTG TGC A		
mef(A)	MefF	TGT GCA TAT TTC TAT TAC G	324	Ojo et al., 2004
	MefR	CCA ATT GGC ATA GCA AG		
	MefInt	GCT GTG CAA TAA TGG GGC		
int1	IntI-fw	CAA GGT TCT GGA CCA GTT GC	907	Soge et al., 2006a
	IntI-rv	CAG CAC ATG CGT GTA AAT CA		
	IntI1-int	CAG GCT TAT GTC CAC TGG GT		
sulI	*sul1*-fw	TCA CCG AGG ACT CCT TCT TC	804	Soge et al., 2006a
	sul1-rv	GAT CTA ACC CTC GGT CTC TGG		
	Sul1-int	GCT CTT AGA CGC CCT GTC CG		
tet(A)	A1	CGA GCC ATT CGC GAG AGC	2027	Miranda et al., 2003
	A2	CGA ABC AAG CAG GAC CAT G		
	A3	GCC TCC TGC GCG ATC TGG		
tet(B)	BF	CAG TGC TGT TGT TGT CAT TAA	571	Miranda et al., 2003
	BR	GCT TGG AAT ACT GAG TGT AA		
tet(C)	CF	TTG CAT GCA CCA TTC CTT GCG	522	Miranda et al., 2003
	CR	ATG GTC GTC ATC TAC CTG CC		
tet(D)	DF	GGA TAT CTC ACC GCA TCT GC	436	Miranda et al., 2003
	DR	CAT CCA TCC GGA AGT GAT AGC		
tet(E)	EF	TCC ATA CGC GAG ATG ATC TCC	442	Miranda et al., 2003
	ER	CGA TTA CAG CTG TCA GGT GGG		
tet(G)	GF	GCT GGA TGA TGC ATT GCG CG	554	This study
	GR	ATG GTC TGC GTA GTA TTG GC		
tet(M/O/S)	M4	GAA GCC CAG AAA GGA TTY GGT	686	Miranda et al., 2003
	M6	GTT TAT CAC GGA AGY GCW A		
bla_{TEM-1}*	TEM INT	AGC CCT CCC GTA TCG TAG TT		Soge et al., 2006b
bla_{SHV-1}*	SHV INT	ATT TAT CTG CGG GAT ACC CC		Soge et al., 2006b

** Primers used as internal probes*

Antibiotic susceptibility

Antimicrobial susceptibilities were performed using disc diffusion on Mueller–Hinton agar (Remel, Inc., Lenexa, KS, USA) according to the CLSI guidelines (CLSI, 2003a). *E. coli* ATCC 25922 was used as a control. The antibiotic disks included ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), chloramphenicol (30 µg), kanamycin (30 µg), tetracycline (30 µg), trimethoprim/sulfamethoxazole (25 µg) (Remel, Inc., Lenexa, KS, USA). In addition, minimum inhibitory concentrations (MICs) were performed using an agar dilution method according to the CLSI guidelines (CLSI, 2003b) for ceftazidime, cefotaxime, aztreonam, piperacillin, cefepime, erythromycin, and imipenem for 26 ampicillin resistant (Ap^r) isolates.

Detection of antibiotic resistance genes

Polymerase chain reaction (PCR) assays were used for the detection of tetracycline resistance genes [*tet*(A), *tet*(B), *tet*(C), *tet*(D), *tet*(E), *tet*(G) and *tet*(M)]; macrolide or combinations of

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TABLE 2
Phenotypic and genotypic characteristics of bacterial isolates from rural Ugandan water sources

Strain	Resistance phenotype[a]	Resistance genotype		
		tet genes[b]	MLS genes[c]	Other resistance gene(s)
E. coli 301	Ap, Tc, SXT, Erm	*tet*(A)	*mef*(A)	bla_{TEM-1}
E. coli 302	Ap, Tc, SXT	*tet*(A), *tet*(C), *tet*(G)	-	*sul1*, $bla_{TEM-1,}$ bla_{SHV-1}
E. coli 304	Ap, Tc, SXT, Erm	*tet*(A), *tet*(C), *tet*(G)	*erm*(B)	*sul1*, *intI1*, bla_{TEM-1}
E. coli 387	Ap, Tc, Erm	*tet*(A), *tet*(C), *tet*(G)	*mef*(A)	*sul1*, bla_{TEM-1}
E. coli 270	Ap, Tc, Cm, Erm	*tet*(A)	*mef*(A)	*sul1*, *intI1*, bla_{TEM-1}
E. coli 317	Ap	-	-	bla_{TEM-1}
Citrobacter sp. 283	Ap, Tc, Erm	*tet*(D)	-	bla_{TEM-1}
Citrobacter sp. 104	Kn, Ap, Erm	-	-	bla_{TEM-1}
Enterobacter sp. 267	Ap	-	-	*sul1*, *intI1*, $bla_{TEM-1,}$
Enterobacter sp. 275	Ap	-	-	bla_{TEM-1}
Enterobacter sp. 307	Ap, Erm	-	*erm*(B)	-
E. cloacae 325	Ap, Erm	-	*erm*(B), mef(A)	$bla_{TEM-1,}$
E. endosymbiont 173	Ap, Erm	-	*erm*(B)	*sul1*, bla_{TEM-1}
Klebsiella sp. 328	Ap	-	-	bla_{SHV-1}
K. pneumoniae 426	Ap	-	-	*sul1*, $bla_{TEM-1,}$ bla_{SHV-1}
M. morganii 261	Ap, Tc	*tet*(M)	-	bla_{TEM-1}
M. morganii 390	Ap, Erm	-	*erm*(B)	$bla_{TEM-1,}$ bla_{SHV-1}
M. morganii 421	Ap	-	-	bla_{TEM-1}
Proteus sp. 421	Ap, Tc	*tet*(A), *tet*(M)	-	-
Proteus sp. 222	Ap, Tc	*tet*(G)	-	bla_{TEM-1}
Providencia rettgeri 3	Tc	*tet*(B)	-	-
P. rettgeri 318	Ap, Tc	*tet*(A)	-	bla_{TEM-1}
S. paratyphi 38	Ap	-	-	bla_{TEM-1}
Salmonella sp. 223	Ap, Tc, Erm	*tet*(C), *tet*(G)	*mef*(A)	bla_{TEM-1}
Salmonella sp. 276	Ap	-	-	bla_{TEM-1}
Serratia odorifera 394	Ap	-	-	*sul1*, *intI1*, bla_{TEM-1}

[a] *Ap, ampicillin; Tc, tetracycline; Erm, erythromycin; Kn, kanamycin; SXT, trimethoprim/sulfamethoxazole. All isolates were susceptible to CAZ, Ceftazidime (MIC <4 mg/ℓ), and CTX, cefotaxime (MIC <4 mg/ℓ), ATM, Aztreonam ((MIC <4 mg/ℓ), FEP, cefepime (MIC <4 mg/ℓ)*
[b] *None of the isolates carried the tet(E) gene*
[c] *All isolates were negative for mph(A), mph(B), mph(C), mph(D), ere(A) and ere(B)*

macrolide-lincosamide-streptogramin [MLS] genes [*erm*(B), *mef*(A), *ere*(A), *ere*(B), *mph*(A), *mph*(B), *mph*(C), and *mph*(D)]; the *intI1* integrase for Class 1 integron and *sul1* gene coding for sulphonamide resistance were performed as previously described (Miranda et al., 2003; Soge et al., 2006a). Positive and negative controls were used for each PCR assay. The PCR products were verified by DNA-DNA hybridisation with internal probes as previously described (Soge et al., 2006a). The ampicillin resistance genes bla_{TEM} and bla_{SHV} were identified as previously described (Soge et al., 2006b). The PCR primers and probes are listed in Table 1.

Plasmids isolation

Plasmid DNA was extracted using a modified alkaline lysis procedure and electrophoresed through 0.7% agarose gel with the *E. coli* V517 (58 kb); R1 (100 kb) and R478 (274.5 kb) used as markers for plasmid size estimation as previously described (Soge et al., 2006a).

Conjugal gene transfer

Mating experiments were carried out using *E. coli* HB101 and/or *E. coli* DH5α as recipients. Both isolates have previously been selected for chromosomal resistance to streptomycin (500 mg/ℓ), fusidic acid (25 mg/ℓ), nalidixic acid (25 mg/ℓ), and rifampicin (25 mg/ℓ) [Strep^r, Fus^r, Rif^r, and Nal^r] as recipients (Soge et al., 2006a). *E. coli* 302, *E. coli* 304 (Ap^r, Tc^r, SXT^r), *E. coli* 387 (Ap^r, Tc^r, *Erm*^{r}), *Enterobacter cloacae* 325, *Morganella morganii* 390, *Serratia odorifera* 394, *Citrobacter* sp. 283 and *Proteus* sp. 222 were used as donors. Transconjugants were selected on one of the following; Luria-Bertani agar (Difco Laboratories, Kansas, MO, USA) supplemented with rifampicin (25 mg/ℓ) plus ampicillin (50 mg/ℓ), streptomycin (250 mg/ℓ) plus ampicillin (50 mg/ℓ), rifampicin (25 mg/ℓ) plus tetracycline (20 mg/ℓ), rifampicin (25 mg/ℓ) plus erythromycin (128 mg/ℓ)] as described previously (Soge et al., 2006a). Mating experiments were done with a ratio of 1:1 of the donor to recipient with *E. coli* as the parental strains and ratios of 1:10, 1:50, 1:100, and 1:200 donor to recipient with *Enterobacter cloacae, Morganella morganii, Serratia odorifera, Citrobacter* sp. and *Proteus* sp. parental strains. Transconjugants were confirmed biochemically and their antibiotic resistance genes verified by PCR assays followed by DNA-DNA hybridisation of the PCR products using radio-labelled internal probes as previously described (Soge et al., 2006a).

Results

Antibiotic susceptibility and antibiotic resistance genes

Of the 52 Gram-negative isolates examined, 26 were susceptible to all antibiotics and 26 isolates were resistant to ≥ 1 of the antibiotics examined, with 14 isolates exhibiting multidrug

TABLE 3
Conjugal transfer of resistance genes in Ugandan isolates

Donor	Number of plasmid	Recipient	Frequency[a]	Number of plasmid transferred	Genes transferred
E. coli 302	2 (65 kb, 125kb)	DH5α	8.38×10^{-5}	1 (65 kb)	*tet*(A), *tet*(C), *tet*(G), *sul1*, bla_{TEM-1}
E. coli 304	2 (58 kb, 95kb)	DH5α	1.37×10^{-6}	1 (58 kb)	*tet*(A), *tet*(C), *tet*(G), *sul1* bla_{TEM-1}
E. coli 387	2 (75 kb, 110 kb)	HB101	4.17×10^{-6}	1 (75 kb)	*tet*(A), *sul1*, bla_{TEM-1}

[a] *Transconjugants/number of recipient bacteria. All transconjugants were ampicillin and tetracycline resistant; 58 kb, 65 kb and 75 kb plasmids transferred in E. coli 304, E. coli 302, and E. coli 387 respectively*
No detectable transconjugants were obtained (transfer frequency $<10^{-9}$) for E. cloacae 325, M. morganii 390, S. odorifera 394, Citrobacter sp. 283 and Proteus sp. 222

resistance. Ap^r was found in 25 of the 26 antibiotic resistant isolates including 6 *E. coli*, 5 *Enterobacter* spp., 1 *Salmonella paratyphi*, 2 *Salmonella* spp., 2 *Klebsiella* spp., 2 *Citrobacter* spp., 2 *Proteus* spp., 3 *M. morganii*, one each of *P. rettgeri* and *S. odorifera*. All Ap^r isolates were susceptible to ceftazidime, cefotaxime, aztreonam, cefepime with MIC < 4 mg/ℓ for all 4 drugs tested suggesting that they did not carry genes coding for extended-β-lactamases. Nineteen of Ap^r isolates carried a bla_{TEM} gene, 1 *E. coli*, *Klebsiella* sp. and *M. morganii* carried both bla_{TEM} and bla_{SHV} genes, and 1 *Klebsiella* sp. carried a bla_{SHV} gene (Table 2). Two isolates did not carry either the bla_{TEM}, and bla_{SHV} or bla_{CTX-M} genes.

Twelve isolates were tetracycline resistant (Tc^r) with 7 isolates carrying a single *tet* gene. Three isolates carried the *tet*(A) gene and 1 isolate each with the *tet*(B), *tet*(D), *tet*(G) or the *tet*(M) gene. The remaining 5 isolates carried multiple *tet* genes and included 3 isolates with the *tet*(A), *tet*(C), and *tet*(G) genes, 1 isolate with both the *tet*(A) and *tet*(M) genes, and 1 isolate with both the *tet*(C) and *tet*(G) genes. Eleven isolates had erythromycin MIC >128 mg/ℓ and 5 of these isolates carried macrolide [*mef*(A)] and/or macrolide-lincosamide-streptogramin B [*erm*(B)] resistance genes, while all 11 isolates were negative for *mph*(A), *mph*(B), *mph*(C), *mph*(D), *ere*(A) and *ere*(B). Eight isolates carried the *sul1* gene and 4 of these isolates also carried the *int1* gene suggestive of an integron. One isolate was chloramphenicol resistant though the resistance gene was not determined.

Plasmids

Thirteen isolates, including 5 *E. coli*, 3 *Enterobacter* spp., 1 *Klebsiella* sp., 1 *Proteus* sp., 1 *Providencia rettgeri* sp., and 2 *Salmonella* spp., carried 1 to 3 plasmids with molecular weights of 3 to 150 kb (data not shown) while the remaining 13 antibiotic resistant isolates, had no detectable plasmid DNA using the single plasmid extraction method.

Conjugation transfer experiments

Three *E. coli*; *E. coli* 302, *E. coli* 304 , and *E. coli* 387, along with *E. cloacae* 325, *M. morganii* 390, *S. odorifera* 394, and *Citrobacter* sp. 283 and *Proteus* sp. 222 were used as donors with recipient *E. coli* HB101 and/or *E. coli* DH5α. All 3 *E. coli* donors transferred Ap^r and Tc^r at a frequency ranging from 10^{-5} to 10^{-6} /recipient. *Enterobacter cloacae* 325 carrying 2 large plasmids (>58 kb) and other donors *M. morganii* 390, *S. odorifera* 394, and *Citrobacter* sp. 283 and *Proteus* sp. 222, which had no detectable plasmid gave no transconjugants (transfer frequency $<10^{-9}$) using 4 different ratio combinations of donor to recipient (1:10, 1:50, 1:100, 1:1200) with both HB101 and DH5α as recipients in repeated experiments. All the transconjugants from the *E. coli* to *E. coli* mating were Ap^r Tc^r and carried a single large plasmid of molecular weights 58 kb, 65 kb, 75 kb for *E. coli* 304, *E. coli* 302 and *E. coli* 387 respectively (Table 3).

All transconjugants carried the bla_{TEM} conferring resistance to Ap^r, the *sul1* gene but differed in the *tet* genes that were transferred to the transconjugants. *E. coli* 302, *E. coli* 304 donors transferred *tet*(A), *tet*(C) and *tet*(G) genes, while *E. coli* 387 donor transferred only the *tet*(A) gene.

Discussion

In the current study, 26 out of 52 water-borne Ugandan Gram-negative bacteria were antibiotic resistant and 14 were multi-drug resistant. None of the isolates carried extended-spectrum β-lactamases, and 4 isolates carried genes consistent with a Class 1 integron. Ap^r was the most common resistance phenotype among the Ugandan isolates. In the *E. coli* isolates, Ap^r was associated with conjugative plasmids [58, 65, 75 kb], while the other Gram-negative isolates appeared to have chromosomally mediated Ap^r genes, which we were unable to conjugally transfer in the study. None of the antibiotic-resistant isolates were resistant to cefotaxime and ceftazidime, while clinical human African enteric bacteria are usually resistant to cephalosporins and often carry multiple β-lactamases encoded by bla_{CTX-M}, bla_{CMY}, and bla_{VIM} type genes in addition to bla_{SHV} and bla_{TEM-1} (Frank et al., 2006; Gray et al., 2006; Ktari et al., 2006; Soge et al., 2006b). Two isolates from the current Ugandan study, *Proteus* 421 and *Enterobacter* 307, were Ap^r but did not carry either bla_{SHV} , bla_{TEM-1}, bla_{CTX-M} genes. Similarly, 2 *Citrobacter* spp. 283 and 104 had erythromycin MICs > 128 mg/ℓ but did not carry any of the 8 common MLS genes examined. One SXT^r *E. coli* did not carry the *sul1* gene while all 12 Tc^r isolates carried previously characterised *tet* genes.

Three of the 8 isolates used as donors were able to conjugally transfer antibiotic resistance to the recipients. The resulting transconjugants carried a single plasmid, which carried 1-3 *tet* genes, *sul1* and bla_{TEM-1}genes. The bla_{SHV-1} was not associated with these conjugative plasmids and was not transferred in the experiments. In contrast, the remaining 5 donor isolates did not generate transconjugants.

In one Nigerian study, small differences in antibiotic susceptibility between the clinical bacteria and bacteria isolated from soil, industrial effluent, food and drinking water were found (Lateef et al., 2005), suggesting the possibility that clinical and water-borne bacteria may have the same level of antibiotic resistance. Little work on the level of antibiotic carriage in Ugandan Gram-negative bacteria is available; however, in a 1998 paper, the authors reported that 92% of the endemic *Shigella* isolated in Mbarara, Uganda were resistant to

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cotrimoxazole and 58% were resistant to ampicillin (Legros et al., 1998). Previous studies on water enteric bacteria from other African countries also showed higher rates of antibiotic resistance than those found in this study. For instance, two separate Nigerian studies have found high levels of antibiotic-resistant (93 to 94%) water-borne enteric bacteria, isolated from communal well water, and from the lower Niger Delta River (Ibiebele et al., 1989; Ogan et al., 1993). Lin et al. (2004) characterised 113 enteric bacteria, including *E. coli*, *Klebsiella* sp., *C. freundii, Enterobacter* spp., *S. marcesens*, isolated from the Mhlathuze River in South Africa and found that 94.7 % of these bacteria were resistant to $\geq$ 1 antibiotic and 75.2% of the isolates were multidrug resistant. The low level of Class 1 integrons found in the Ugandan isolates differs from recent studies of Class 1 integrons where 12% of the *E. coli* isolated from a remote community of Guarani Indians in Bolivia, and 40% of 100 multi-drug resistant Gram-negative bacteria, from the River Torsa, India, carried Class 1 integrons (Pallecchi et al., 2007; Mukherjee and Chakraborty, 2006).

Unregulated use of antibiotics in agriculture, animal husbandry, and medical therapy has been a major influence in Africa and is often cited as a major reason why there is high prevalence of multidrug resistant bacteria (Okeke et al., 2007). Why the Ugandan water bacteria in this study differ from other studies from remote areas in Africa is intriguing. However, this study does illustrate that the level of antibiotic resistance found in water-borne Gram-negative bacteria can vary among Gram-negative bacteria isolated from various remote parts of the world where exposure to western medicine and antibiotics is minimal.

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