www.bti.org.in
ISSN 0974-1453
Research Article

PREVALENCE OF CLASS 1 AND II INTEGRONS IN MULTI-DRUG RESISTANT BACTERIA ISOLATED FROM KEHA AND SHINTA RIVERS IN GONDAR TOWN, NORTH WEST ETHIOPIA

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ABSTRACT

An integron is a gene capture and dissemination system that is found in plasmids, chromosomes and transposons. Integrons may contribute a lot in emergence and spread of antibiotic resistance and causes serious problems in the process of antimicrobial resistance gene transfer. The objective of this study was to assess the occurrence of class I and II integrons among multi–drug resistant bacteria isolated from Keha and Shinta rivers, Northwest Ethiopia. Twenty triplicate samples were collected in 2 weeks interval. Bacteria were isolated, identified and characterized on the basis of standard morphological and biochemical characteristics. Antibiotic susceptibility of the isolates was tested by disc diffusion method. Tetracycline (Te), Ciprofloxacin (Cip), Amoxicillin (Amc), Ceftriaxone (CRO), Gentamycin (Gm) and Ampicilin (Amp 10) were used for Gram negative isolates. Vancomycin (Va), Gm, Te, CRO, Cip, Amp, Erythromycin (E), (Rif), Penicillin (Pen) and Oxacillin (Oxc) were tested against Gram positive isolates. Multi-drug resistant isolates were further examined for the presence of class I and II integrons by the Polymerase Chain Reaction (PCR) using specific primers for the integrase genes *intI1* and *intI2*. A total of 91 bacteria belonging to 5 different species were isolated. Most of the isolates, 86 (94.5%) were resistant to at least one drug, and majority 69 (75.8%) of these isolates were multi–drug resistant. *IntI*1 gene was detected in 17.6% of *E. coli* (3/17) and 31.3% of *P. aeruginosa* (5/16) multi-drug resistant isolates. However, there was no *IntI2* gene in any of the isolates. The presence of *IntI1* gene in *E. coli* and *P. aeruginosa* isolates was associated with resistance to ampicin. This study indicated the prevalence of integron I from bacteria isolates from Keha and Shinta rivers and this integron might be responsible for multi-drug resistance feature of these isolates.

Keywords*:* Antibiotics, multidrug resistance, Integrons.

INTRODUCTION

During the last six decades, the ability to manage infectious diseases has greatly improved because of the availability of various antimicrobial agents. But this ability is being severely challenged by the rapidly rising antibiotic resistance pathogens (Anibal *et al*., 2010). Nowadays, multi-drug resistant pathogenic strains have become a threat for human and animal health. In developing countries, where the health sector is challenged with various constraints such as access to better or new drugs when resistance is suspected or even when the priority is to give basic health care, certainly strategies to control and prevent resistance are not at the list of priorities (Peters *et al*., 2001). The incident of microbial resistance has emerged as a result of the selective pressure exerted by the antimicrobial usage in human medicine, veterinary medicine, animal and fish farming, agriculture and food technology (Peters *et al*., 2001; Robert *et al*., 2011). Indeed, antimicrobial resistance is described as a universal ecological phenomenon where microbes and resistance genes move easily between the four ecosystems: from human being and animals to soil and water and vice versa (Emilie *et al*., 2008; Paulo *et al*., 2013**).**

The aquatic environment is an efficient natural dissemination vector of antibiotic resistant bacteria (Nardelli *et al*., 2012). The residual antibiotics discharged from domestic, hospital and agriculture sources find their way into various water bodies including rivers, ponds, *etc*., which can exert selective pressure and fecal antibiotic resistant bacteria, selected in human or animal intestines under antibiotic treatment enter the water environment mainly from treated effluents of wastewater treatment plants, field run off and direct discharge of untreated waste water (Alexandra *et al*., 2007; Osman *et al*., 2007). These fecal bacteria might then be able to transmit antibiotic resistance to autochthonous bacteria through lateral transfer when the resistance genes are carried by transferable and mobile genetic elements (Saswati *et al*., 2013).

The dissemination of drug-resistance in bacteria is complicating the treatment of infections (Anibal *et al*., 2010). Much of the problems of Multi Drug Resistance (MDR) spread are as a result of antibiotic resistance genes transferred among bacterial species via integrons, plasmids and transposons (Laura *et al*., 2005; Suman *et al*., 2013). Integrons, which are natural genetic engineering platforms, can incorporate open reading frames and convert them to functional genes, usually antibiotic resistance genes, by ensuring correct expression and thus, they contribute to cumulative bacterial resistance to multiple antibiotics (Veronique *et al*., 2007; Amy *et al*., 2010; Chung *et al*., 2011).

Integrons are classified according to the sequence of the IntI protein. The first integron discovered, the class I integron, is now known to be important in the dissemination of antibiotic resistance genes in both Gram negative and Gram positive bacteria (Xu *et al*., 2010). These integrons are typically found on plasmids or transposons, which catalyze their own mobility. Class I integrons have been found in association with dozens of different

resistance genes and have been found in environments ranging from hospitals to poultry litter (Davies and davies, 2010; Paulo *et al*., 2013). Other classes of integrons, including class II, III and unnamed class found on a *Vibrio salmonicida* plasmid, have been found on mobile elements and in association with antibiotic resistance genes (Shaohua *et al*., 2001; Diana *et al*., 2008).

Class I and II integrons are associated with carriage of antibiotic resistance genes in clinically important bacteria, and there is increasing evidence of environmental reservoirs of bacteria carrying these integron classes. The use of veterinary antibiotics selects for antibiotic resistant bacteria which, along with antibiotic residues, enter the wider environment through slurry application (Gaze *et al*., 2005; Hawkey *et al*., 2009).

Gondar is one of the towns in Ethiopia that is having relatively large scale manufacturing industries and rapidly ascending population and most of the existing industries have been discharging their wastes into the surrounding environment with little concern to the population, in particular to the nearby rivers like Keha and Shinta as a sink for disposal of domestic, agricultural and industrial wastes. As far as our knowledge goes no study has been conducted on the antimicrobial susceptibility pattern and role of integrons in bacteria isolated from these rivers. Therefore, the objective of this study was to throw light on antimicrobial susceptibility pattern and role of integrons in bacteria isolated from keha and Shinta rivers.

MATERIALS AND METHODS

Study area and period

The study region is found in the town of Gondar which is located in the North Western part of Ethiopia. It is located about 738km from Addis Ababa, capital city of Ethiopia. River Shinta which is a tributary to Angereb River that drains into Lake Tana, is located at the side of Dashen brewery and it drains from north to south and it serve as natural sewerage lines for domestic and industrial wastes; On the other hand Keha river is located near Gondar university teaching hospital in the western part of Gondar and it is highly affected by agricultural and domestic wastes. This study was conducted from September 2012 to February 2014 to assess the occurrence of class I and II integrons in multi-drug resistance bacteria isolated from Keha and Shinta rivers around Gondar town, Northwest Ethiopia.

Bacterial Isolation, identification and antimicrobial susceptibility testing

From the water sample bacteria were isolated by the spread plate method. Briefly, 1 ml of water sample was suspended in 9 ml of sterile normal saline solution and diluted serially up to 10^{-10}.This procedure was applied for all samples taken from the rivers and 0.1ml of the suspension was plated out of nutrient agar and incubated at 37^{o}C, for 24 to 48 h. Isolated colonies of different morphologies were selected and re-streaked on the nutrient agar to obtain pure cultures. The isolates were stored in tryptic soy broth containing 20% glycerol at -20^{o}C for further analysis. Pure cultures of all the isolates were subjected to Gram reaction by

Potassium hydroxide test (John, 1982). Isolates were subjected to a series of biochemical tests after cell shape and arrangement were identified by simple staining. Triple sugar iron agar (Oxoid), lysine iron agar (Oxoid), motility-indol-ornithine agar (Oxoid), Simmons citrate agar (Oxoid), urea agar (Oxoid), catalase test, haemolysis and coagulase tests were some of the tests employed for identification of each pure culture.

Antibiotic susceptibility of the bacteria isolates were assayed by Kirby-Bauer disc–diffusion method (Bauer *et al.*, 1996). All the plates were incubated for 20 min before inoculation and placed on antibiotic disc to allow excess moisture to dry. After the drying, a single loop of each isolate was inoculated into sterile normal saline and compared with 0.5 McFarland standard, the suspension was aseptically swabbed on the surface of Mueller–Hinton plates and antibiotic sensitivity disc that contains Te 30, Cip 5, Amc 30, CRO 5, Gen 10 and Amp 10 were aseptically laid on the surface of Gram negative plates. Van 30, Gen 10, Te 30, CRO 5, Cip 5, Amp 10, E 15, Rd 5, Pen 10and Oxc 1 were aseptically laid on the surface of Gram positive isolate inoculated plates. The plates were incubated at 37^{o}C for 24 h. After the incubation, zone of growth of inhibition around each disc was measured and used to classify the organisms as sensitive, intermediate or resistant to an antibiotic according to the established standard of Clinical Laboratory Standard Institute (CLSI) (2000).

DNA extraction and Integron detection

Bacterial genomic DNA was extracted from the pure cultures by Phenol-chloroform method (Amy *et al*., 2010) and the purity and concentration were analyzed by spectrophotometer at 260 and 280nm (Gueye *et al.*, 2010). The presence of class I and II integrons were detected by PCR using primers specific for the integron integrase genes *intI1*and *intI2*. *Int1*F (5'-GGGTCAAGGATCTGGATTTCG -3') and *Int*1R (5' ACA TGC GTGTAAATCATCGTC G-3') were used for amplification of class I integron region; Primers *Int*IIF (5'-CACGGATATGCGACAAAAAGGT-3') and *INT II*R (5'-GTAGCAAACGAGTGACGAAAT G- 3') were used for amplification of class II integrone region (Didier *et al*., 2000). Primer specific for 16s RNA conserved region was used as positive control to check the proper functioning of the PCR. Control F (5'- GGGAGTGCCTTCGGGAATCAGA -3') and control R (5'-TCACCGCAACATTCTGATTTG-3') were used for amplification of 16srRNA gene (Gehua *et al*., 2003). PCR amplifications for class I and II integrons were performed in a TC-412 thermocycler (Barlo world scientific Ltd.). The 25 µl reaction mixture consisted of 10 x buffer (2.5µl), 25 mM $MgCl_2$ (2µl), 10 mM dNTP (1µl), Taq DNA polymerase 0.2µl, from 25 µM (each) forward and reverse primers (0.6µl) and the remaining volume was filled by molecular grade H_2O.

Concerning the PCR conditions, the following conditions were applied; initial denaturation at 95°C for 10min, 35 cycles of denaturation at 94°C for 1 min, annealing for 1 min at 60°C for *Int* I and at 58 ^{0}C for *Int II*, extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min.

Finally amplified products were separated and visualized by electrophoresis in 1.5% agarose gels. One hundred bp DNA ladder was applied to determine the band sizes. The expected band sizes were 356 bp, 483 bp and 788 bp for 16s rRNA, class I and class II integrons PCR products, respectively (Didier *et al*., 2000; Gehua *et al*., 2003).

RESULTS

Isolation and Identification of bacteria

A total of 91 bacteria belonging to 5 different species were isolated from Keha and Shinta rivers and identified based on standard biochemical, physiological and morphological characters. The isolates identified belonged to *E. coli* (n=30), *E. aerogenes* (n=10), *P. aeruginosa* (n=20), *S. aureus* (17) and *S. pyogenes* (14).

Antibiotic susceptibility of bacterial isolates

The prevalence and antibiotic resistance profile of bacterial isolated from Keha and Shinta rivers is given in Table 1. The smallest zone of inhibition was recorded by Gen for sh79 isolate; Cip for sh89 and sh31; CRO for sh2 and sh31; Amp for k50; Amc for k1 and Te for sh 82 isolate. K61 isolate of *E. coli* showed the largest zone of inhibition diameter for Gen and Amc. Cip and Te showed the largest zone of inhibition diameters for sh9 isolate. .

Antibiotic resistance patterns of bacterial isolates

The antibiotic resistance pattern of isolated bacteria is given in Table 2. Determination of resistance patterns to 10 antibiotics for *S. aureus* and *S. pyogenes*, 6 antibiotics for Gram negative isolates revealed that 86 (94.5%) were resistant to at least one drug and the majority, 69 (75.8%) of these isolates were multi–drug resistant. Three isolates namely, *S. aureus* (k36 and k79) and *S. Pyogenes* (sh66) were resistant to eight drugs. There were two isolates in the seven drug-resistant pattern; *S. aureus* (k90) *and S. pyogene* (k60) which showed Gen, CRO, Amp, Rd, Pen, E, Oxc resistance pattern and three *P. aeruginosa* isolates (k46, sh62 and sh33) exhibited Te, Cip, CRO, Amp, Amc, Gen resistance pattern. *S. aureus* (sh86, sh6, k25, and k68) showed Te, CRO, Amp, Rd, Pen, Oxc resistance.

Isolates grouped under *S. pyogenes* (sh48 and k18) and *S. aureus* (sh70 and sh53) were resistant to: Te, CRO, Amp, Pen and Oxc. Two isolates of *P. aeruginosa* (sh4 and k19) showed Te, Cip, CRO, Amp and Amc resistance pattern. In the four drug-resistant groups, three isolates of *S. pyogene* (k42, sh14 and k59) and two isolates of *S. aureus* showed CRO, Amp, Rd and Oxc pattern of resistance. Two isolates of *E. coli* (sh23 and w100) exhibited Te, Cip, Amc and CRO pattern of resistance. Two isolates of *S. pyogene* (k12 and k13) and three isolates of *S. aureus* (k58, sh29 and k71) showed CRO, Amp, Pen and Oxc resistance pattern. Three isolates of *P. aeruginosa* exhibited Te, CRO, Amp, Amc and two isolates of *E. coli* exhibited Te, Cip, Amc, and Amp resistance pattern. Thirty one (33%) isolates showed three drug resistances with eight different patterns.

Table1. The prevalence and antibiotic resistance pattern of bacterial isolates from Keha and Shinta rivers**.**

	No. of resistant isolates (%)					
Antibiotics	*S. aureus* N=17	*S. pyogene* N=14	*E. coli* N=30	*E. arogene* N=10	*P. aeruginosa* N=20	Total N=91
Gen	1(6)	1(7)	2(7)	1(10)	4(20)	9(9.9)
Cip	2(12)	1(7)	13(43)	3(30)	10(50)	29(31.9)
CRO	16(94)	11(79)	10(33)	3(30)	16(80)	56(61.5)
Amp	15(88)	13(93)	12(40)	4(40)	15(75)	59(64.8)
Amc	N.T	N.T	15(50)	5(50)	10(50)	30(50)
Pen	15(88)	11(79)	N.T	N.T	N.T	26(83.9)
Oxc	17(100)	14(100)	N.T	N.T	N.T	31(100)
E	3(18)	2(14)	N.T	N.T	N.T	5(16.1)
Vac	1(6)	0(0)	N.T	N.T	N.T	1(3.2)
Rd	9(53)	10(31)	N.T	N.T	N.T	19(61.3)
Tet	8(47)	6(43)	14(47)	5(50)	11(55)	44(49.4)
	No. of intermediate isolates (%)					
Gen	1(6)	12(86)	3(10)	1(10)	2(10)	19(20.9)
Cip	2(12)	0(0)	2(6.7)	1(10)	4(20)	9(9.9)
CRO	1(6)	1(7)	4(13)	3(30)	1(5)	10(11)
Amp	0	0	1(3.3)	0(0)	2(10)	3(3.3)
Amc	N.T	N.T	2(6.7)	1(10)	2(10)	5(8.3)
Pen	0	0	N.T	N.T	N.T	0
Oxc	0	0	N.T	N.T	N.T	0
E	0	0	N.T	N.T	N.T	0
Vac	0	0	N.T	N.T	N.T	0
Rd	1(6)	1(7)	N.T	N.T	N.T	2(6.5)
Tet	2(12)	0(0)	1(3.3)	0(0)	5/25	8(8.8)
	No. of sensitive isolates (%)					
Gen	15(88)	1(7)	25(83)	8(80)	14(70)	63(69.2)
Cip	13(76)	13(93)	15(50)	6(60)	6(30)	53(58.2)
CRO	0(0)	2(14)	16(53)	4(40)	3(15)	25(27.5)
Amp	2(12)	1(7)	12(40)	6(60)	3(15)	24(26.4)
Amc	N.T	N.T	13(43.3)	4(40)	8(40)	25(41.7)
Pen	2(12)	3(21)	N.T	N.T	N.T	5(16.1)
Oxc	0(0)	0(0)	N.T	N.T	N.T	0
E	14(82)	12(86)	N.T	N.T	N.T	26(83.9)

Vac	16(94.1)	14(100)	N.T	N.T	N.T	30(96.8)
Rd	7(41.1)	3(21)	N.T	N.T	N.T	10(32.3)
Tet	7(41)	8(57)	15(50)	5(50)	4(20)	39(42.9)

Key:Gen, gentamicin; Cip, ciprofloxacin; CRO, ceftriaxone; Amp, ampicillin; Pen, penicillin; Oxc, oxacilin; E, erythromycin; Vac, vancomycin; Rd, rifampicin; Amc, amoxicillin; Tet, tetracycline.

Table 2. Antibiotic resistance of bacterial isolates that showed 8–3 drug resistance patterns

S.No.	Resistance Pattern	Bacteria	Isolate Code	No. of Isolate
1	**Eight-drugs**			
	Te Cip CROAmp E Rd Pen Oxc	*S. aureus*,	k36, k79	2
	Te Cip CRO Amp E Rd Pen Oxc	*S. pyogen*	sh66	1
2	**Seven –drugs**			
	Gen CRO Amp Rd Pen E Oxc	*S. aureus*	k90	1
	Gen CRO Amp Rd Pen E Oxc	*S. pyogen*	K60	1
3	**Six –drugs**			
	Te CRO Amp Rd Pen Oxc	*S. aureus*	sh86, sh6, k25, k68	4
	Te CRO Amp Rd Pen Oxc	*S. pyogen*	sh40	1
	Te Cip CRO Amp Amc Gen	*P. aeruginosa*	k46, sh 62, sh 33	1
4	**Five-drugs**			
	Te CRO Amp Pen Oxc	*S. pyogen*	sh48, k18	2
	Te Amp Rd Pen Oxc	*S. pyogen*	sh84, k63	2
	Te CRO Amp Pen Oxc	*S. aureus*	Sh70, sh53	2
	Te Cip CRO Amp Amc	*P. aeruginosa*	sh4, k19	2
5	**Four-drugs**			
	CRO Amp Rd Oxc	*S. pyogen*	k42, sh14, k59	3
	CRO Amp Rd Oxc	*S. aureus*	sh81, k73	2
	Te Cip Amp CRO	*E.coli*	Sh23, k10	2
	CRO, Amp, Pen, Oxc	*S. pyogen*	k12, k13	2
	CRO Amp Pen Oxc	*S. aureus*	k58, sh29, k71	3
	Te CRO Amp Amc	*P. aeruginosa*	K57, sh75, k72	3
	Te Cip Amc Amp	*E. coli*	sh89, k28	2
6	**Three-drugs**			
	Te Cip Amc	*E. coli*	k8, sh51, k32, k11, sh41, sh54, k52	7
	Te Cip Amc	*E. aerogenes*	K44, sh67, k16	3
	Te Cip Amc	*P. aeruginosa*	K15, sh45, k37	3

CRO Amc Amp	*E. aerogenes*	k88	1
Te Gen CRO	*E. coli*	k49, sh79	2
CRO Amp Gen	*P. aeruginosa*	k21	1
CRO Amp Gen	*E. aerogenes*	sh80	1
CRO Pen Oxc	*S. pyogen*	K43	1
CRO Pen Oxc	*S. aureus*	sh77, k39	2
TeCRO Amp	*P. aeruginosa*	k34, k38	2
Cip CRO Amc	*P. aeruginosa*	sh3, sh20	2
Cip CRO Amp	*E. coli*	sh2, k74, k50, sh31	4
Amp Pen Oxc	*S. aureus*	k78	1
Amp Pen Oxc	*S. pyogen*	sh65	1

Key: Tet, Tetracycline;Cip, Ciprofloxacin; CRO, Ceftriaxone; Amp, Ampicillin; E, Erythromycin; Rd; Rifampicin; Pen, Penicillin; Amc, Amoxicillin; Oxc, Oxacilin; Gen, Gentamicin.

Detection of class I and II integrons

About 69 (75.8%) isolates were identified as multi–drug resistant and assayed by PCR amplification for class I and class II integrase genes. The frequency of Integrons on multi drug resistance species is given in Table 3. PCR revealed the presence of class I integron only in *E. coli* and *P. aeruginosa* isolates. Class 1 integron gene was detected in17.6% of *E. coli* (3/17) and 31.3% of *P. aeruginosa* (5/16) isolates. There was no any PCR product from *E. aerogens*, *S.aureus* and *S. pyogenes* isolates, while class II integron was not detected in any of the isolates.

Table 3. Antibiotic resistance pattern of integrons positive bacteria isolated from Keha and Shinta rivers.

Organism	No. of MDR isolates	No. of isolates with indicated gene (%)		Isolate Code	Resistance pattern of integrase positive isolates
					Te, Cip, Amp, CRO
E. coli	17	*intI*1	*intI*2	Sh23	Te, Cip, Amc, Amp
				K28	Cip CRO Amp
		3(17.6)	0(0)	K50	Te, Cip, CRO, Amp,Amc, Gm
P.	16			Sh33	Te, Cip, CRO, Amp, Amc
aeruginosa		5(31.2)	0(0)	K19	Te, CRO, Amp, Amc
				Sh75	CRO, Amp, Gen
				K21	Te, CRO, Amp
				K38	

Key: Tet, Tetracycline; Cip, Ciprofloxacin; CRO, Ceftriaxone; Amp, Ampicillin; Amc, Amoxicillin; Gen, Gentamicin.

DISCUSSION

In this study, *E. coli*, *E. aerogenes*, *P. aeruginosa*, *S. aureus* and *S. pyogenes* were isolated and identified from Keha and Shinta rivers. The isolation of these human pathogens in these rivers is an indication of contamination that signals great frustration, for the surrounding vicinity that uses these rivers for different purposes. The use of these river water for drinking without treatment may facilitate widespread infections and can ultimately lead to the outbreak of epidemics. The effluent of river water, which is discharged into nearby water bodies contaminates streams, food crops on the farm and inadvertently reaches man (Khan and Malik, 2001). The isolation of these bacteria from these rivers may be due to discharging of human and animal wastes to the river.

The widespread emergence of antibiotic resistance, particularly multidrug resistance, among bacteria has become one of the most serious challenges in clinical therapy (Baker *et al*., 2009; Jose, 2012. In light of the potential health risk, many studies have focused on antibiotic-resistant bacteria from various ecosystems (Reinthaler *et al*., 2003; Mesa *et al*., 2006; Osman *et al*., 2007). In the present study, the isolated bacteria were checked for their antibiotic susceptibility and pattern of resistance to antibiotics such as: Amp, Cip, Te, CRO, Van, Amc, Rd, E, Pen, Oxc and Gen. The Shinta river has become the ultimate dumping ground of Dashen brewery, municipal, and agricultural sewage and Keha is also a reservoir of Gondar university teaching hospital, municipal and agricultural sewages thus posing significant threat to ecological balance as well as to public health. Hospital, municipal and agricultural sewages are the sources of antibiotic resistant bacteria in the aquatic environment (Martins *et al*., 2006; Emilie *et al*., 2008; Dong *et al*., 2009). In this study, the isolated bacteria displayed resistance to Amp (64.8%), CRO (61.5), Amc (50%), Te (49.4%) and Cip (31.9). Some of the isolates have shown high level of resistance for specific antibiotics. For example, all isolates of *S. aureus* and *S. pyogenes* have shown nearly 100% resistance to Oxc and 88.2% were resistant to Amp. A similar finding with 49.42% resistance to Te was reported by bacteria isolated from the Ganges river (Manisha *et al*., 2011). The occurrence of bacteria isolates with low level resistance to Gen (9.9%), this might be due to limited use of this drug in veterinary or human medicine in the study area, and 31.9% to Cip in this study is much lower than other report (Bolaji *et al*., 2011). However, Emilie *et al*., (2009) reported Gen resistance in 5.8% of *Escherichia coli* isolated from a densely populated estuary, which is lower than identified in this study.

In this study 86 (94.5%) isolates were resistant to at least one drug. Although the degrees of resistance of the bacteria were different, the majority, 69 (75.8%) of these isolates were multi–drug resistant and have shown 3-9 different resistance patterns. This is quite higher than the report of Manisha *et al*., (2011), which indicated 88.5% resistance to at least one drug and 65.51% multi–drug resistant in bacteria isolated from environment. Only 11(12.1%) isolates have shown two resistance patterns with different combinations. The relatively high

level of resistance to antimicrobial agents could be a reflection of misuse or abuse of these agents in the study area. Antibiotic prescriptions in hospitals are given without clear evidence of infection or adequate medical indication (Silva and Hoffer, 1993). Toxic broad-spectrum antibiotics are sometimes given in place of narrow-spectrum drugs as substitute for culture and sensitivity testing, with the consequent risk of the selection of drug-resistant mutants (Prescott *et al*., 1999). Recent studies have shown that antibiotics can accumulate in the environment, and even persist for up to a year (Roe *et al*., 2003).

Class I integrons are crucial elements in the worldwide problem of antibiotic resistance and are often embedded in plasmids, transposons, and chromosomes, as well, facilitating their lateral transfer into a wide range of pathogens (Alessandra, 2001; Laura *et al*., 2005; Aminov, 2011). In the present study *IntI* gene was detected in17.6% of *E. coli* (3/17) and 31.3% of *P. aeruginosa* (5/16) isolates. The high prevalence of class I integrons in *P. aeruginosa* and *E. coli* compares with 13% of *E. coli* isolates from the Rio Grande River (Roe *et al*., 2003), 3.6% of Gram-negative bacteria isolated from an estuary in the United Kingdom (Shaohua *et al*., 2001), 3% of *E. coli* isolates from tap and well water in Turkey (Osman *et al*., 2007) and 15% of bacteria in waste water from a penicillin-manufacturing plant harbored class I integrons (Dong *et al*., 2009). The frequency of class I integrons in *E. coli* isolates from Keha and Shinta rivers was in agreement with Dong *et al*., (2009) and this prevalence was lower than the report of Lin and Biyela (2005),which showed that 58% of multi drug-resistant isolates with aquatic environments contain class I integron. Class I integrons were found in 5 (31.3%) of 16 *P. aeruginosa* isolates from Keha and Shinta river water. This prevalence is in line with Henriques *et al*., (2006), who identified class I integrons in 29.6% of *Enterobacteriaceae* isolated from estuarine waters. All of the isolates that carried class I integrons were resistant to ampicillin and most of them are resistant to ciprofloxacin and tetracycline.

CONCLUSION

Keha and Shinta rivers are contaminated by bacteria of fecal origin. The finding of these fecal origin microorganisms in these rivers is an indication of field defecation and poor sanitation of the surrounding society. Out of 91bacterial isolates of the two rivers, 69 (75.8%) were resistant to multiple antimicrobial drugs. This shows that Keha and Shinta rivers are contaminated with antibiotic resistant bacteria. Only few of *E. coli* and *P. aeruginosa* multi–drug resistant bacteria isolates contain class I integrons. Eight isolates of *E. coli* and *P. aeruginosa* from 69 multi-drug resistant bacteria isolates contain class I integrons. All the class I integrons positive isolates were resistance to ampicillin. Although there are different mechanisms of resistance, the resistance of *E. coli* and *P. aeruginosa* isolates to Amp may be greatly associated with the expression of genes within class I integrons. There was no occurrence of both classes I and/or class II integrase in *E. aerogenes, S. aureus* and *S. pyogenes* microorganisms isolated from Keha and Shinta rivers.

Therefore, mechanism of drug resistance may not be associated with class I and class II integrons, and thus further study on their drug resistance pattern is warranted.

ACKNOWLEDGMENT

The authors of this paper are great full to office of the vice president for research and community service, university of Gondar for their modest financial assistances. Moreover, we are thankful to Dr. Deepak Verma Kumar for bringing us the primers for this study without which the study would have not been completed.

Conflict of Interest

The authors of this paper declare that there is no conflict of interest.

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