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Molecular detection of *Enterococcus* with special reference to *Enterococcus faecalis* from water sources and its antimicrobial resistance

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Abstract
The present study aimed at phenotypic and genotypic detection of *Enterococcus* and *E. faecalis* from different water sources and its antimicrobial resistance pattern. A total of 280 numbers of water samples were collected from five different types of sources *viz.* river, spring, stream, runoff and recreational pool in Aizawl district, Mizoram. *Enterococcus* and *E. faecalis* in water samples were detected phenotypically and genotypically by detection of *tuf* and *sodA* gene, respectively. The *E. faecalis* strains were evaluated for *in vitro* antibiotic sensitivity profile by disc diffusion and minimum inhibitory concentration assay against a panel of 14 antibiotics. The overall prevalence of *Enterococcus* was found to be 56.78% contributing to 71.66% in river, 68.33% in run-off, 63.33% in stream, 51.66% in spring and 15% in recreational pool water. The overall prevalence of *E. faecalis* was 42.14% contributing to 58.33% in river, 50% in stream, 46.66% in runoff and 41.66% in spring water. The *E. faecalis* strains were 100% sensitive to vancomycin and highest resistant to nalidixic acid (87.28%). High prevalence of *Enterococcus* and *E. faecalis* in different water sources indicates the faecal contamination of water from various animal and human wastes. Even though vancomycin resistant enterococci (VRE) is of great concern globally, present study revealed otherwise.

Keywords: *Enterococcus*, *Enterococcus faecalis*, molecular detection, water sources, antimicrobial resistance

Introduction
According to the World Health Organization (2017), contaminated drinking water is estimated to cause 485,000 diarrhoeal deaths each year. It has caused 10,738 deaths in India over five years to 2017 [39]. Contamination of water sources with pathogenic bacteria leading to illness is a major concern especially in developing countries [7]. Lack of clean water and sanitation promotes the spread of microbes and some of which can be resistant to antimicrobial treatment. The release of antibiotics into environmental water also contributes to the increasing risk of antibiotic resistant bacteria [14, 37]. However, monitoring of all pathogens in water is not possible economically and technologically, alternatively *E. coli* and enterococci have been used as faecal indicators in water [2, 12]. As enterococci are members of the intestinal microbiota of healthy humans and animals they can be released into surface water and soil by human and animal faecal material, so environmental water often contains enterococci. Though considered generally harmless, currently *Enterococcus* have become an important nosocomial pathogen, predominant species being *E. faecalis* and *E. faecium* which cause about 90% of clinical infections and they are ranked the third and fourth most prevalent human pathogens worldwide [8, 17, 34]. Enterococci are intrinsically resistant to b-lactams, cephalosporins and aminoglycosides. Furthermore, they can acquire resistance to other antibiotics including quinolones, macrolides, etc. Therefore, treatment of enterococcal infections is hindered by the development and spread of antimicrobial resistance. Resistance to antimicrobials of last resort, such as vancomycin, further impairs the control of enterococcal infections [18, 17, 34]. Rapid detection of enterococci in the environment and their antimicrobial resistance pattern is of paramount importance in reducing the spread of multi-drug resistant *Enterococcus* to human. Mizoram is the southernmost landlocked hilly state in Northeast India with heavy rainfall. The present study aimed to detect the contamination of different water environments in Aizawl, the most populous district of Mizoram, by *Enterococcus/E. faecalis* and its antimicrobial resistance pattern.

Materials and Methods
Collection of water samples
A total 280 numbers of water samples were collected from five different types of sources *viz.* river water(60), spring water (60), stream water (60), urban/ hospital runoff water (60) and recreational water (40) in Aizawl district, Mizoram for a period of one year by adopting appropriate aseptic measures.

Phenotypic detection of *Enterococcus* and *E. faecalis*
The isolation and identification of *Enterococcus* from water included three principal steps namely enrichment in Enterococcus Presumptive Broth (EPB), selective culturing on Enterococcus Confirmatory Agar (ECA) and presumptive identification by Gram's staining and biochemical tests namely negative catalase reaction, esculin hydrolysis on Bile Esculin Agar as well as growth in 6.5% NaCl as per the method described by Facklam and Collins. 1989 [18]. From the presumptive *Enterococcus* isolates, *E. faecalis* was identified based on sugar fermentation such as mannitol (positive), sorbitol (positive) and arabinose (negative).

Molecular detection of *Enterococcus* and *E. faecalis*
All the phenotypically positive *Enterococci* isolates were processed for bacterial lysate (DNA template) preparation using boiling and snap chilling method. The DNA template was used for amplification of *Enterococcus* genus specific (*tuf*) gene and *E. faecalis* species specific (*sodA*) gene by PCR assay (Ke *et al.*, 1992) [21]. The PCR confirmed Enterococci isolates were subjected to detection of *E. faecalis* by PCR using species specific *sodA* gene (Ahmed *et al.*, 2012) [4] (Table 1).

Table 1: Oligonucleotide primers used in PCR for detection of *Enterococcus* (*tuf* gene) and *E. faecalis* (*sodA* gene)

Target gene	Primer sequence [5'-3]	Product size	Reference
Tuf	F: TACTGACAAACCATTCATGATG R: AACTTCGTCACCAACGCGAAC	112bp	Ke *et al.* (1999) [21]
Soda	F: ACTTATGTGACTAACTTAACC R: TAATGGTGAATCTTGGTTTGG	360bp	Ahmed *et al.* (2012) [3]

A PCR mixture of 25 µl was preparedin 0.2 ml thin PCR tube containing PCR master mix (2X) (12.50 µl), forward primer (1 µl), reverse primer (1 µl), DNA template (4 µl) and nuclease free water (6.5 µl). The thermal cycling conditions used for amplification of DNA included initial denaturation (95 ^{0}C for 4 minutes), denaturation (95 ^{0}C for 45 seconds), annealing (55 ^{0}C for 1 minute), extension (72 ^{0}C for 1 minute) and final extension for one cycle (72 ^{0}C for 7 seconds) for 30 cycles.

About 5µl of amplified PCR product was mixed with 2µl of 6x gel loading dye and analysed by agarose (1.5%) gel electrophoresis using 1x TAE buffer (pH 8.0) and ethidium bromide was added to the gel upto a final concentration of 0.5µg/ml. DNA ladder (100bp) was used as reference to compare the size of amplified products. Electrophoresis was carried out at80 V/60 mA and the gel was visualised under UV transilluminator (Alpha Imager) and documented by gel documentation system (Alpha imager).

Detection of antimicrobial resistance profile of *E. faecalis* strains
All the PCR confirmed *E. faecalis* strains were subjected to *in vitro* antibiotic sensitivity testing by disc diffusion method [6] against a panel of 14 antibiotics namely amikacin, ampicillin, ciprofloxacin, chloramphenicol, doxycycline, erythromycin, gentamicin, kanamycin, nalidixic acid, nitrofurantoin, norfloxacin, streptomycin, tetracycline and vancomycin as per Clinical Laboratory Standard Institute (CSLI) guidelines (2019) [10]. The vancomycin resistant *E. faecalis* strains in disc diffusion assay were further subjected to minimum inhibitory concentration (MIC) by agar dilution assay (CLSI, 2018) [9].

Statistical analysis
The findings of the present study were analysed by Chi square test (SPSS version 20) using the method of Snedecor and Cochran (1994) [38].

Result and Discussion
Out of the 280 samples collected from different water sources of Aizawl district, Mizoram, (India) namely river, spring, stream, urban/ hospital runoff and recreational water, 163 (58.21%) *Enterococcus* isolates were presumptively identified by phenotypic method. The presumptive *Enterococcus* was found to be highest in river water (71.66%) followed by runoff water (70.00%), stream water (63.33%), spring water (55%) and recreational pool water (17.50%). Out of 163 presumptive *Enterococcus* isolates, 159 (97.54%) *Enterococcus* strains were confirmed genotypically (*tuf* gene) (112bp) and the *Enterococcus* isolates were highest detected in river water and stream water (100%, each) followed by runoff water (97.61%), spring water (93.93%) and recreational water (85.71%). *Enterococcus* was detected lowest in recreational water (Table 2 and Figure1).

Table 2: Detection of *Enterococcus* by phenotypic and molecular method in water samples from different sources from Aizawl district, Mizoram.

Sl. No	Types of water samples	Phenotypic method				Molecular method	
		Cultural method		Biochemical method			
		No. of samples analysed	No. of samples positive for *Enterococcus*	No. of culturally positive samples analysed	No. of samples positive for *Enterococcus*	No. of phenotypically positive samples analysed for PCR	No. of samples positive for *Enterococcus* (*tuf* gene) by PCR
1	Spring water	60	61.66(37)^b	37	55. 00 (33)^a	33	93.93(31)^a
2	Stream water	60	83.33(50)^{ab}	50	63.33 (38)^a	38	100.00(38)^a
3	River water	60	86.66(52)^a	52	71.66 (43)^a	43	100.00(43)^a
4	Runoff water	60	83.33(50)^{ab}	50	70.00 (42)^a	42	97.61(41)^a
5	Recreational pool water	40	25.00(10)^c	10	17.50 (7)^b	7	85.71(6)^a
	Total	280	71.07(199)*	199	58.21(163) *	163	97.54(159) ^{NS}

From the 159 PCR positive *Enterococcus* strains, overall 58 (37.66%) and 118 (74.21%) strains were identified as *E. faecalis* in biochemical and PCR (*sodA*) assay, respectively. Detection of *E. faecalis* did not vary significantly in water from spring, stream, river and runoff water sources both in biochemical and PCR analysis. *Enterococcus faecalis* was not detected from recreational water (Table 3 and Figure2).

Table 3: Detection of *E. faecalis* by sugar fermentation test and PCR from different water sources in Aizawl district, Mizoram

Sl. No.	Types of water samples	No. of PCR positive *Enterococcus* isolates	No. of isolates positive for *E. faecalis* by sugar fermentation test	No. of isolates positive for *E. faecalis (sodA gene)* by PCR	Chi value
1	Spring water	31	40.00(12)^a	75.75(25)^a	11.33**
2	Stream water	38	41.66(15)^a	78.94(30)^a	12.26**
3	River water	43	42.86(18)^a	81.39(35)^a	14.21**
4	Runoff water	41	32.50(13)^a	68.00(28)^a	10.98**
5	Recreational pool water	6	0	0	
	Total	159	37.66(58)^{NS}	76.62(118)^{NS}	47.74**

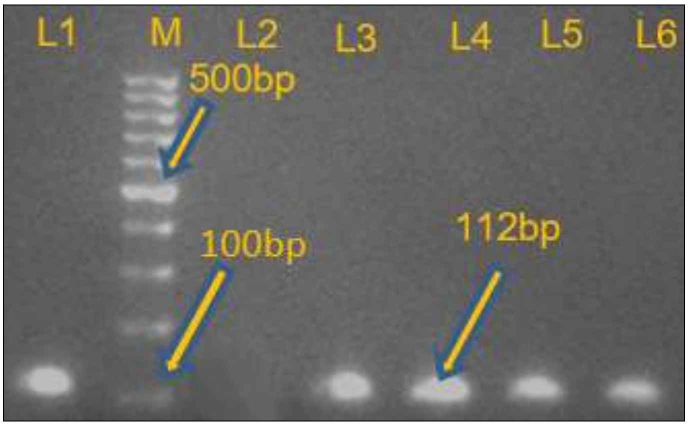

Fig 1: Agarose gel electrophoresis showing PCR amplicons of *tuf* gene (112 bp); M: 100bp ladder; L1: Positive control; L2: Negative control; L3 to L6 : Positive sample

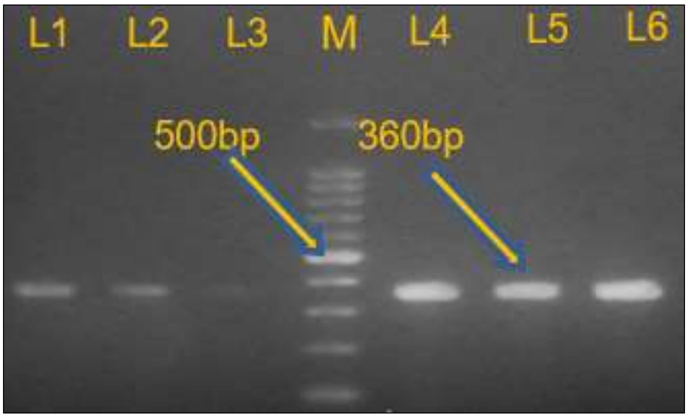

Fig 2: Agarose gel electrophoresis showing PCR amplicons of *soda* gene (360bp); M: 100bp ladder; L1: Positive control; L3: Negative control;L2, L4, L5 and L6 : Positive sample

Similar to the present finding, Iweriebor *et al*. (2015) [20] and Adeniji *et al*. (2020) [1] also detected enterococci from wastewater and beach coastal water, respectively by detecting genus-specific *tuf* gene. Lanthier *et al.* (2010 and 2011) [25, 26] genotypically detected *Enterococcus* from river, waste water and faeces of domesticated mammals, birds and wildlife by detection of genus-specific *16S r-RNA* gene. Alipour *et al*. (2014) [4] detected *E. faecalis* (68.60%) from the river and coastal water by detecting *sodA* gene whereas Veljovic *et al*. (2015)(40) detected enterococci strains from lake, rivers and springs by amplification of *16S r-RNA* gene and *E. faecalis* by detection of *sodA* gene.

Prevalence of *Enterococcus* and *E. faecalis*

The overall prevalence of *Enterococcus* was found to be 56.78% contributing to71.66% in river water, 68.33% in run-off water, 63.33% in stream water, 51.66% in spring water and 15% in recreational pool water from Aizawl district, Mizoram. The prevalence of *Enterococcus* was significantly lower ($P \leq 0.05$) in recreational water than the other water sources. The overall prevalence of *E. faecalis* from different water sources was 42.14% contributing to 58.33% in river, 50% in stream, 46.66% in run off and 41.66% in spring water (Table 4 and Figure 3).

Table 4: Prevalence of *Enterococcus* and *E. faecalis* in different water sources from Aizawl district, Mizoram.

Sl. No.	Types of water samples	No. of samples analysed	No. of samples positive for *Enterococcus* (*tuf* gene) by PCR	% of prevalence for *Enterococcus*	No. of samples positive for *E. faecalis (sodA* gene*) by PCR*	% of prevalence for *E. faecalis*
1	Spring water	60	31	51.66^a	25	41.66^a
2	Stream water	60	38	63.33^a	30	50^a
3	River water	60	43	71.66^a	35	58.33^a
4	Runoff water	60	41	68.33a	28	46.66^a
5	Recreational pool water	40	6	15^b	0	0
	Total	280	159	56.78*	118	42.14^{NS}

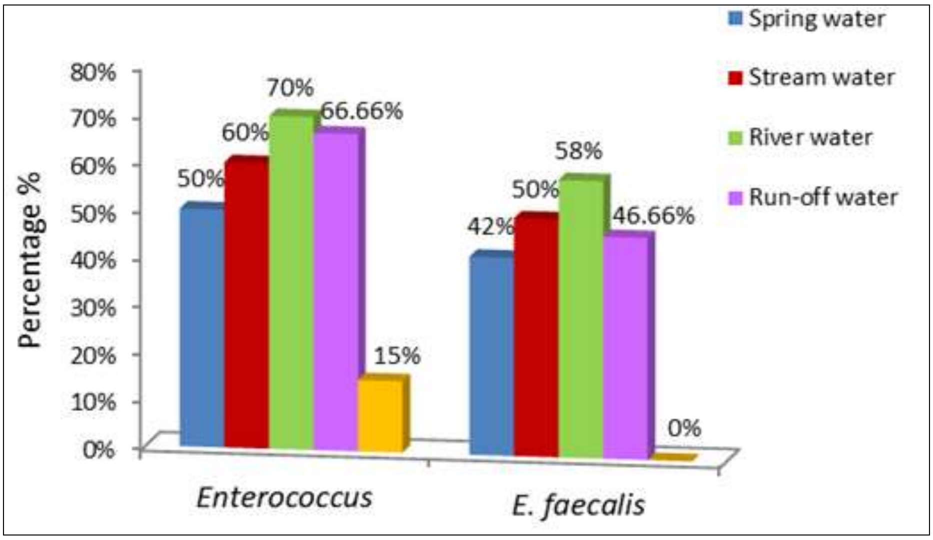

Fig 3: Prevalence of *Enterococcus* and *E. faecalis* in different water sources from Aizawl district, Mizoram

Furtula *et al.* (2013) [17] and Alipour *et al.* (2014) [4] had reported 100% prevalence of *Enterococcus* from surface water sites and river water in Canada and Northern Iran, respectively. From Kerela, India, Peter *et al.* (2012) [32] reported 74% prevalence of *Enterococcus* from wells, bore wells, bottled water and chlorinated hospital drinking water supply. Furtula *et al.* (2013) [17] recorded 10.71% prevalence of *E. faecalis* from intensive poultry farming area in Canada. These findings indicated that there might be faecal contamination of the water sources from animal and human sources and the level of contamination might vary depending on the rate of faecal discharge in the water sources in the hilly state. The small animal and vegetable farming system in the slope hills might be the source of the faecal contamination of water courses. The contamination of water sources might also be caused by other factors such as household and workshops waste, waste and sewage from hotels and houses which might be discharged into water. The differences in the prevalence could also be due to the different sampling sizes and geographical locations. (Alipour *et al.*, 2014) [4].

The prevalence *of Enterococcus* and *E. faecalis* was found to be highest in river water which might be because river water ultimately collects all the urban and rural wastes including agricultural and industrial wastes leading to higher level of contamination. In river water, *E. faecalis* (64%) was the most prevalent species followed by *E. faecium* (24%) in Ganga river, India as reported by Lata *et al.* (2009) [27]. Kuntz *et al.* (2003) [24] and Lanthier *et al.* (2011) [26] reported 56% and 36.40% prevalence of *E. faecalis* of all *Enterococcus* species isolated from river in Atlanta, USA and Canada, respectively. Alipour *et al.* (2014) [4] reported that most prevalent *Enterococcus* species from river water was *E. faecalis* (66.70%) followed by *E. faecium* (23%) in Northern Iran. However, the prevalence of *Enterococcus* and *E. faecalis* was significantly (P≤0.05) lower in recreational pool water which could be due to the periodic treatment of the pool water by replacement and chlorination. Wei *et al.* (2017) [42] and Xie *et al.* (2018) [43] reported 32.30% and 16.49% prevalence *E. faecalis* in spring water, respectively from China while 50% prevalence were reported from ground water in Mid-Atlantic Region, U.S by Micallef *et al.* (2013) [31]. Spring water is generally safer than other environmental water sources; however, it is likely to be contaminated through the topsoil unless the surrounding land area is protected. Irrespective of the spring originating from shallow or deep rock layers, contamination with animal excreta due to small, unorganized livestock and poultry farming in different strata of hills and extensive human activity around the springs in Mizoram might cause higher prevalence of *E. faecalis* than reported in other studies. The high rain fall in the study area (254 cm per annum) might also contribute to higher contamination of spring water.

Similar to the present finding, Sapkota *et al.* (2007) [36] reported that *E. faecalis* was the predominant (72.86%) *Enterococcus* species in stream water from Maryland, USA whereas Luczkiewicz *et al.* (2010) [28] reported *E. faecium* (68.60%) followed by *E. faecali*s (21.60%) in stream water from Poland. This is indicative of regional difference in species distribution of *Enterococci* in water sources. Varela *et al.* (2013) [40] reported higher prevalence (75.38%) of *E. faecalis* in runoff water (hospital effluent and urban waste) in Portugal than the present finding. Iweriebor *et al.* (2015) [20] reported 57% *E. faecalis* from municipal and hospital waste water in South Africa.

The present finding also indicated that *E. faecalis* is a predominant species among the *Enterococcus spp*. in different water sources as they are abundantly present in the microbiota of human and animals. In general, the spatial heterogeneity of *Enterococcus* seems to be introduced via different point and non-point sources like urban sewage, clinical and industrial discharge, agricultural runoff and stormwater route.

Antimicrobial sensitivity and resistance profile of *E. faecalis*

The *E. faecalis* strains were significantly (P≤0.05) higher sensitive to vancomycin (100%) and ampicillin (91.52%) than nitrofurantoin (85.59%) and chloramphenicol (83.90%), doxycycline (79. 66%) and tetracycline (71.18%). However, the *E. faecalis* strains showed lower sensitivity to nitrofurantoin, chloramphenicol, doxycycline and tetracycline without significant variations among them. The resistance pattern of *E. faecalis* strains did not vary significantly among nalidixic acid (87.28%), streptomycin (86.44%), amikacin and kanamycin (83. 90%, each). However, resistance of *E. faecalis* strains to these four antibiotics were significantly (P≤0.05) higher than tetracycline (16.95%), gentamicin (12.71%), ampicillin, ciprofloxacin and norfloxacin (6.78%, each), erythromycin (5.93%), doxycycline and vancomycin (0.00%) (Table 5, Figure 4).

The *E. faecalis* strains from spring and stream water were sensitive (100%) to vancomycin followed by ampicillin (92% and 92. 85%, respectively) while the strains from spring and stream water were highest resistant to amikacin (84%) and kanamycin and nalidixic acid (86.66%, each), respectively. The strains from river water were 100% sensitive to vancomycin, nitrofurantoin, tetracycline and doxycycline followed by ampicillin (94.29%), and chloramphenicol (85.71%) whereas the strains were highest resistant to streptomycin and nalidixic acid (100%, each) followed by kanamycin (94.29%) and amikacin (88.58%).The runoff water strains showed highest sensitivity to vancomycin followed by chloramphenicol and nitrofurantoin (96.43%, each), ampicillin (92.85%) and highest resistant to streptomycin (96.43%) followed by amikacin (89.29%), nalidixic acid (85.71%), kanamycin (75%) and others. However, the *E. faecalis* strains were sensitive to vancomycin in MIC even at the lowest concentration of 0.0625 µg/ml although 4.23% strains were resistant in disc diffusion assay. All the 118 *E. faecalis* strains isolated were resistant to one or more antibiotics in a range of 1 to 9 numbers of antibiotics with significantly ($P \leq 0.01$) higher resistance to 4 numbers of antibiotics than the other resistance patterns. The strains showed significant differences ($P \leq 0.05$) in sensitivity and resistance patterns among the different water sources to all the antibiotics except amikacin, ampicillin and vancomycin.

Table 5: Antibiotic sensitivity and resistance pattern of *E. faecalis* strains isolated from different water sources in Aizawl district, Mizoram

Sl. No.	Antimicrobial agent		Spring water (n =25)	Stream water (n=30)	River water (35)	Run-off water (n=28)	Total (N=118)
1	Amikacin (AK)	S	4.00(1)	13.33 (4)	5.71 (2)	10.71 (3)	8.47(10)^{NS}
		R	84.00(21)	73.34 (22)	88.59 (31)	89.29(25)	83.89(99)^{NS}
2	Ampicillin (AMP)	S	92(23)	92.85(26)	94.28(33)	92.85(26)	91.52(108)^{NS}
		R	0	13.33(4)	5.71(2)	7.14(2)	6.78(8)^{NS}
3	Chloramphen-icol (C)	S	84.00(21)	70.00(21)	85.71 (30)	96.43 (27)	83.90(99)^{NS}
		R	8.00(2)	0	0	0	1.69 (2)^{NS}
4	Ciprofloxacin (CIP)	S	28.00(7)^{a}	36.67(11)^{a}	34.29(12)^{a}	7.14 (2)^{b}	27.12(32)*
		R	20.00(5)^{a}	10.00(3)^{a}	0^{b}	0^{b}	6.7 (8)*
5	Doxycycline (DO)	S	64.00(16)^{b}	80.00(24)^{b}	100 (35)^{a}	67.86(19)^{b}	79.66(94)*
		R	16.00(4)^{a}	0^{b}	0^{b}	3.57 (1)^{ab}	4.24(5)*
6	Erythromycin (E)	S	0	6.67 (2)	0	7.14 (2)	3.4 (4)^{NS}
		R	8.00(2)^{ab}	6.67(2)^{bc}	0^{c}	10.72 (3)^{a}	5.93 (7)*
7	Gentamicin (GEN)	S	52.00(13)^{a}	60.00(18)^{a}	31.43(11)^{b}	32.14(9)^{b}	43.22(51)*
		R	4.00(1)^{b}	23.33(7)^{a}	14.29 (5)^{ab}	7.14 (2)^{b}	12.71(15)*
8	Kanamycin (K)	S	0	6.67 (2)	0	7.14 (2)	3.4 (4)^{NS}
		R	76.00(19)	86.66(26)	94.29(33)	75.00(21)	83.89(99)^{NS}
9	Nalidixic Acid (NA)	S	0	6.67 (2)	0	0	1.69 (2)^{NS}
		R	72.00(18)^{b}	86.66(26)^{ab}	100 (35)^{a}	85.71(24)^{ab}	87.28 (103)*
10	Nitrofurantoin(NIT)	S	88.00(22)^{a}	56.67(17)^{b}	100 (35)^{a}	96.43 (27)^{a}	85.59 (101)*
		R	4.00(1)	10.00(3)	0	0	3.4 (4)^{NS}
11	Norfloxacin (NX)	S	52.00(13)	66.66(20)	62.85 (22)	64.28(18)	61.86 (73)^{NS}
		R	20.00(5)^{a}	6.67 (2)^{b}	0^{b}	3.57 (1)^{b}	6.78(8)*
12	Streptomycin (S)	S	8.00(2)	6.67 (2)	0	0	3.4 (4)^{NS}
		R	80.00(20)	66.67(20)	10 (35)	96.43(27)	86.44(10)^{NS}
13	Tetracycline (TE)	S	52.00(13)^{c}	53.33 (16)^{c}	100(35)^{a}	71.43 (20)^{b}	71.18(84)*
		R	44.00(11)^{a}	3.34(1)^{b}	0b	28.57(8)^{a}	16.96 (20)*
14	Vancomycin (VA)	S	(100)25	(96.67)29	(94.29)33	(92.86)26	(95.76)113^{NS}
		R	(0.00)0	(3.34)1	(5.71)2	(7.14)2	(4.23)5 ^{NS}

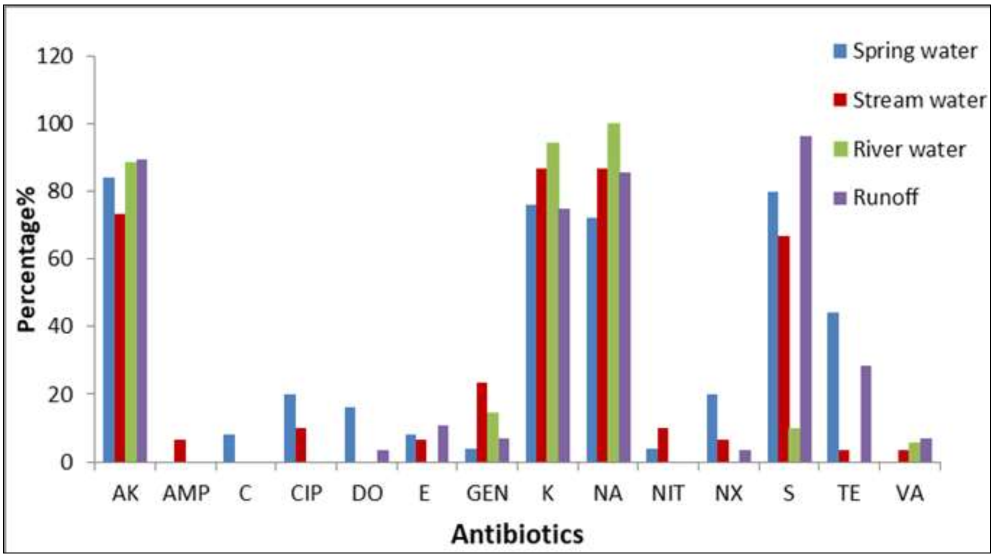

Fig 3: Antibiogram of *E. faecalis* strains isolated from different water sources

Similar to the present findings, earlier studies have reported that nalidixic acid, a quinolone, is ineffective against Gram positive bacteria including *Staphylococcus aureus* and *E. faecalis* (Bhargavi *et al*., 2010) [6]. Kimiran *et al.* (2006) [22] had reported 100% resistance of *E. faecalis* from sea water to nalidixic acid in Turkey. Bhargavi *et al.* (2010) [6] reported the 88.90% resistance to nalidixic acid in urinary enterococcal isolates in Southeast part of India. The *E. faecalis* strains showed lower resistance to fluroquinolones namely ciprofloxacin and norfloxacin while Peter *et al.* (2012) [32] reported 64% resistance of *E. faecalis* to ciprofloxacin from wells, bore wells, bottled water and chlorinated hospital drinking water in Kerela, India. Macedo *et al.* (2010) [29] and Xie *et al.* (2018) [43] reported resistance 45% and 25.50% to ciprofloxacin in *E. faecalis* strains of stream and spring water from Portugal and China, respectively. Enterococci are intrinsically resistant to low level aminoglycosides. High resistance of *E. faecalis* strains to aminoglycosides namely streptomycin, kanamycin, amikacin and gentamicin were recorded. Lata *et al.* (2009) [28] reported variable resistance of *E. faecalis* to ampicillin (20%), streptomycin (65%) and gentamicin (40%) in from Ganga river, India. Peter *et al.* (2012) [32] found 64% resistant *E. faecalis* strains to gentamicin from well water and bore well in Kerela, India and much higher resistance (97.82%) to streptomycin from Port Blair Bay, India was reported by Meena *et al*. (2015) [30]. Veljovic *et al*. (2015) [41] found *E. faecalis* strains resistant to gentamicin (55%), streptomycin (98%) and kanamycin (90%) from river and spring water from Belgrade, Serbia. However, Xie *et al.* (2018) [43] observed lower resistance in *E. faecalis* to gentamicin (19.20%), kanamycin (14.90%) and streptomycin (12.80%) from spring water in China. The resistance of *E. faecalis* strains were found to be 16.95% and 4.24% to tetracycline and doxycycline, respectively. Lata *et al.* (2009) [28] and Alipour *et al*. (2014) [5] observed variable resistance of *E. faecalis* to tetracycline accounting 14. 50% and28.60% from river in Thailand and Northern Iran, respectively while Iweriebor *et al. (*2015) [21] and Xie *et al.* (2018) [43] reported 100% and 93.60% resistant *E. faecalis* strains to tetracycline from hospital wastewater and spring water in South Africa and China, respectively. The resistance of *E. faecalis* to ampicillin was lower (6.78%) although the antibiotic is commonly used in human and animals. Comparatively higher resistance of *E. faecalis* to ampicillin was recorded by Lata *et al.* (2009) [27] and Xie *et al.* (2018) [43] from Ganga river, India (20%) and spring water (10.60%) in China, respectively. Macedo *et al.* (2010) [30] and Rathnayake *et al.* (2011) [33] found that *E. faecalis* strains from spring water in Portugal and river water in Australia were sensitive to ampicillin. However, higher resistance of *E. faecalis* to ampicillin (82.14%) was reported from well water isolates in Kerela, India by Peter *et al*. (2012) [32].

Lower resistance of *E. faecalis* strains to erythromycin (5.93%), nitrofurantoin (3.40%) and chloramphenicol (1.96%) was recorded. However, higher resistance of *E. faecalis* strains to erythromycin were recorded from Ganga river, India (Lata *et al.*, 2009) [28]. Iweriebor *et al. (*2015) [21] reported higher resistance of *E. faecalis* strains to erythromycin (80%) in hospital wastewater from South Africa. Macedo *et al.* (2010) [29] reported that *E. faecalis* strains from spring water were sensitive to nitrofurantoin in Portugal. Variable resistance pattern of *E. faecalis* strains to chloramphenicol from different drinking water sources (14.29%) and river water (34.30%) were reported from India and Northern Iran by Peter *et al.* (2012) [32] and Alipour *et al.* (2014) [5], respectively whereas Veljovic *et al*. (2015) [41] and Macedo *et al.* (2010) [29] reported that *E. faecalis* strains from spring water and river water in Belgrade, Serbia and Portugal were sensitive to chloramphenicol.

Five numbers (4.24%) of *E. faecalis* strains resistant (4.24%) to vancomycin in disc diffusion method were found to be sensitive in MIC by agar dilution assay. Similar to the present findings, Cupakova *et al.* (2003) [12] reported that all *E. faecalis* strains from wastewater in Czech Republic were found to be sensitive in MIC by microdilution method. Veljovic *et al*. (2015) [42] also reported that all the resistant strains in disc diffusion assay from river and spring water in Serbia were found to be sensitive in the micro dilution assay. Sapkota *et al.* (2007) [36] reported the 0.25-64µg/ml MIC to vancomycin in *E. faecalis* strains of surface and ground water from Maryland, USA which was below prescribed breakpoint i.e.32µg/ml by agar dilution assay. Similar to the present findings, vancomycin sensitive *E. faecalis* strains from spring water were reported by Macedo *et al.* (2010) [29] and Xie *et al*. (2018) [43] from Portugal and China, respectively. Low level of resistance to vancomycin was reported by Alipour *et al*. (2014) [4] (4.20%) from river in Northern Iran; however, Lata *et al.* (2009) [27] reported 16% resistance of *E. faecalis* strains to vancomycin in Ganga river, India.

Antimicrobial resistance occurs naturally over time, usually through genetic changes. Misuse and overuse of antimicrobials are the main drivers of antimicrobial resistance alongwith the lack of access to clean water, sanitation and hygiene for both humans and animals, poor disease prevention and control in health care facilities and animal farms, lack of awareness and enforcement of legislation. The variations in the antimicrobial resistance pattern of *E. faecalis* strains to different antibiotics might be due to the different water sources, different geographical locations, species variations of *Enterococcus*, choice of antibiotics etc. (Wei *et al*., 2017) [42]. Antibiotics are excreted from humans and animals due to poor absorption in the intestines and reduced degradation in the body and they are discharged into sewage and animal waste which plays a major role in the distribution of antibiotic resistant bacteria in the environment (Meena *et al*., 2015) [30]. Thus, not only regulation of antibiotic use but also prescribing of other broad-spectrum antimicrobials should be carried out in medical and veterinary practice to decrease the colonization with MDR *E. faecalis* in the study area.

Conclusion

The sensitivity to vancomycin and lower resistance of *E. faecalis* from different water sources against gentamicin and ampicillin seemed to be favourable from clinical point of view as the resistance against these antibiotics reduces the therapeutic possibilities in enterococcal infections in human. Detection of comparable antibiotic resistant *E. faecalis* strains from river water and runoff water indicates that a more integrated water management and monitoring system is vital for the community.

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References

1. Adeniji OO, Sibanda T, Okoh AI. Molecular detection of antibiotic resistance and virulence gene determinants of *Enterococcus* species isolated from coastal water in the Eastern Cape Province, South Africa. International Journal of Environmental Studies 2020, 1-20.
2. Ahmed W, Goonetilleke A, Gardner T. Alternative indicators for detection and quantification of faecal pollution. Water 2008;39(1):46-49.
3. Ahmed W, Sidhu JPS, Toze S. Speciation and frequency of virulence genes of *Enterococcus* spp. isolated from rainwater tank samples in Southeast Queensland, Australia. Environmental Science and Technology 2012;46(12):6843-6850
4. Alipour M, Hajiesmaili R, Talebjannat M, Yahyapour Y. Identification and antimicrobial resistance of *Enterococcus* spp. isolated from the river and coastal waters in northern Iran.The Scientific World Journal 2014. http://dx.doi.org/10.1155/2014/287458
5. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standard single disk method. American Journal of Clinical Pathology 1966;45(4):493-496.
6. Bhargavi PS, Rao GT, Mukkanti K, Kumar DB, Krishna TP. Increasing emergence of antibacterial resistance mainly in uropathogens: Southeast part of India. International Journal of Microbiology Research 2010;2(1):1.
7. Cabral JP. Water microbiology. Bacterial pathogens and water. International Journal of Environmental Research and Public Health 2010;7(10):3657-3703
8. Cho S, Jackson, CR, Frye JG. The prevalence and antimicrobial resistance phenotypes of Salmonella, *Escherichia coli* and *Enterococcus spp.* in surface water. Letters in Applied Microbiology 2020;71(1):3-25.
9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing Edn 28. CLSI supplement M100: M100-S22. Clinical and Laboratory Standards Institute, PA, USA 2018.
10. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility testing. Edn 29, Vol. 39, CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, USA, 2019.
11. Cupáková Š, Lukášová J. Agricultural and municipal waste water as a source of antibiotic-resistant enterococci. Acta Veterinaria Brno 2003;72(1):123-129.
12. Daniel DS, Lee SM, Gan HM, Dykes GA, Rahman S. Genetic diversity of Enterococcus faecalis isolated from environmental, animal and clinical sources in Malaysia. Journal of infection and Public Health 2017;10(5):617-623.
13. ECDC Annual epidemiological report 2011
14. Elmarghani EM. Enterococcal distribution and responses toenvironmental waters (Doctoral dissertation, Örebro Universitet) 2013.
15. ECDC Annual epidemiological report 2011.
16. Facklam RR, Collins MD. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. Journal of Clinical Microbiology 1989;27(4):731-734.
17. Furtula V, Jackson CR, Farrell EG, Barrett JB, Hiott LM, Chambers PA. Antimicrobial resistance in Enterococcus spp. isolated from environmental samples in an area of intensive poultry production. International Journal of Environmental Research and Public Health 2013;10(3):1020-1036.
18. Guzman-PrietoAM, van-Schaik W, Rogers MR, Coque TM, Baquero F, Corander J. Global emergence and dissemination of *Enterococci* as nosocomial pathogens: attack of the clones. Frontiers in Microbiology 2016;7:788.
19. Hammerum AM. *Enterococci* of animal origin and their significance for public health. Clinical Microbiology and Infection 2012;18(7):619-625.
20. Iweriebor BC, Gaqavu S, Obi LC, Nwodo UU, Okoh AI. Antibiotic susceptibilities of *Enterococcus* species isolated from hospital and domestic wastewater effluents in Alice, Eastern Cape Province of South Africa. International Journal of Environmental Research and Public Health 2015;12(4):4231-4246.
21. Ke D, Picard FJ, Martineau F, Ménard C, Roy PH, Ouellette M. Development of a PCR assay for rapid detection of *Enterococci*. Journal of Clinical Microbiology 1999;37(11):3497-3503.
22. Kimiran-Erdem A, Arslan EO, Yurudu NOS, Zeybek Z, Dogruoz N, Cotuk A. Isolation and identification of *Enterococci* from seawater samples: Assessment of their resistance to antibiotics and heavy metals. Environmental Monitoring and Assessment 2007;125(1-3):219-228.
23. Kinzelman J, Ng C, Jackson E, Gradus S, Bagley R. *Enterococci* as indicators of Lake Michigan recreational water quality: comparison of two methodologies and their impacts on public health regulatory events. Applied Environmental Microbiology 2003;69(1):92-96.
24. Kuntz RL, Hartel PG, Godfrey DG, McDonald JL, Gates KW, Segars WI. Targeted sampling protocol as prelude to bacterial source tracking with *Enterococcus faecalis*. Journal of Environmental Quality 2003;32(6):2311-2318.
25. Lanthier M, Scott A, Lapen DR, Zhang Y, Topp E. Frequency of virulence genes and antibiotic resistances in Enterococcus spp. isolates from wastewater and faeces of domesticated mammals and birds, and wildlife. Canadian Journal of Microbiology 2010;56(9):715-729.
26. Lanthier M, Scott A, Zhang Y, Cloutier M, Durie D, Henderson VC. Distribution of selected virulence genes and antibiotic resistance in *Enterococcus* species isolated from the South Nation River drainage basin, Ontario, Canada. Journal of Applied Microbiology 2011;110(2):407-421.
27. Lata P, Ram S, Agrawal M, Shanker R. *Enterococci* in river Ganga surface waters: propensity of species distribution, dissemination of antimicrobial-resistance and virulence-markers among species along landscape. BMC Microbiology 2009;9(1):140.
28. Łuczkiewicz A, Jankowska K, Kurlenda J, Olańczuk-Neyman K. Identification and antimicrobial resistance of *Enterococcus* spp. isolated from surface water. Water Science and Technology 2010;62(2):466-473.
29. Macedo AS, Freitas AR, Abreu C, Machado E, Peixe L, Sousa JC. Characterization of antibiotic resistant enterococci isolated from untreated waters for human consumption in Portugal. International Journal of Food Microbiology 2011;145(1):315-319.
30. Meena B, Anburajan L, Sathish T, Raghavan RV, Jha DK, Venkateshwaran P. *Enterococcus* species diversity and molecular characterization of biomarker genes in *Enterococcus faecalis* in Port Blair Bay, Andaman and

Nicobar Islands, India. Marine Pollution Bulletin 2015;94(1-2):217-227.
31. Micallef SA, Goldstein RER, George A, Ewing L, Tall BD, Boyer MS *et al*. Diversity, distribution and antibiotic resistance of *Enterococcus spp.* recovered from tomatoes, leaves, water and soil on US Mid-Atlantic farms. Food Microbiology 2013;36(2):465-474.
32. Peter A, Mathew J, Zacharia S. Antibiotic resistant *Enterococci* from drinking water sources. Asian Journal of Pharmaceutical and Clinical Research 2012;5(3):158-160.
33. Rathnayake IU, Hargreaves M, Huygens F. Antibiotic resistance and virulence traits in clinical and environmental *Enterococcus faecalis* and *Enterococcus faecium* isolates. Systemic and Applied Microbiology 2012;35(5):326-333
34. Rehman MA, Yin X, Zaheer R, Goji N, Amoako KK, McAllister T,Diarra MS. Genotypes and phenotypes of Enterococci isolated from broiler chickens. Frontiers in Sustainable Food Systems 2018;2:83.
35. Sanderson H, Ortega-Polo R, Zaheer R, Goji N, Amoako KK, Brown RS *et al*. Comparative genomics of multidrug-resistant *Enterococcus spp.* isolated from wastewater treatment plants. BMC Microbiology. 2020;20(1):20.
36. Sapkota AR, Curriero FC, Gibson KE, Schwab KJ. Antibiotic-resistant enterococci and fecal indicators in surface water and groundwater impacted by a concentrated swine feeding operation. Environmental Health Perspectives 2007;115(7):1040-1045.
37. Sirisha T, Basha PA, Kavitha B. Isolation and characterization of pathogenic bacteria from Kundu River Water of Nandyal, Kurnool, Andhra Pradesh, India. Journal of Applied Sciences 2017;17(9):475-481.
38. Snedecor GW, Cochran WG. Statistical methods, Edn 8. Lowa State University Press, USA. 1994.
39. Tripathi B. Diarrhoea took more lives than any other water-borne disease in India. India Spend, New Delhi. 2018.
40. Varela AR, Ferro G, Vredenburg J, Yanık M, Vieira L, Rizzo L, Manaia CM. Vancomycin resistant enterococci: from the hospital effluent to the urban wastewater treatment plant. Science of Total Environment 2013;450:155-161.
41. Veljović K, Popović N, Vidojević AT, Tolinački M, Mihajlović S, Jovčić B *et al*. Environmental waters as a source of antibiotic-resistant *Enterococcus* species in Belgrade, Serbia. Environmental Monitoring and Assessment 2015;187(9):599.
42. Wei L, Wu Q, Zhang J, Guo W, Chen M, XueL *et al*. Prevalence and genetic diversity of *Enterococcus faecalis* isolates from mineral water and spring water in China. Frontiers in Microbiology 2017;8:1109.
43. Xie T, Wu G, He X, Lai Z, Zhang H, Zhao J. Prevalence and molecular characterization of *Enterococcus faecalis* from spring water. Journal of Food Safety 2019;39(6):e12694.