Heavy metal and antibiotic resistant bacteria isolated from Guheswori sewage treatment plant, Nepal

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Introduction

- Due to unplanned urbanization [1], wastewater contains discharges from toilets and bathrooms [2], industrial effluents [3], agricultural runoffs [2, 4], chemicals ions [4].
- Traces of heavy metal(s) are required for the survival of microbes for enzymatic reactions [2], while high concentration leads to imbalance of the microbe's electrolytes and inhibition metabolic reactions [4, 5].
- Bacteria survive in harsh environment by utilizing genes responsible for mechanisms like efflux pump, biosorption to the cell walls, entrapment in extracellular capsules, complexation and oxidation-reduction reactions [5], which might even be cause for antibiotic(s) resistance [6] and heavy metal(s) [7].

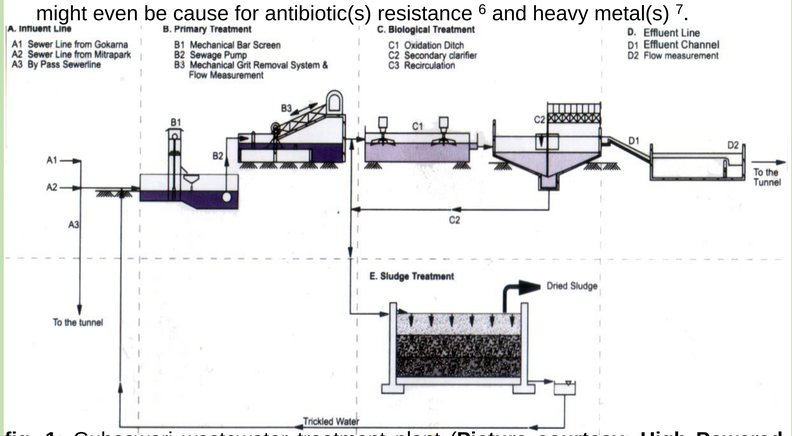


fig. 1: Guheswori wastewater treatment plant (**Picture courtesy: High Powered Committee for Integrated Development of the Bagmati Civilization (HPCIDBC), Ministry of Urban Development, Government of Nepal)**

Objectives

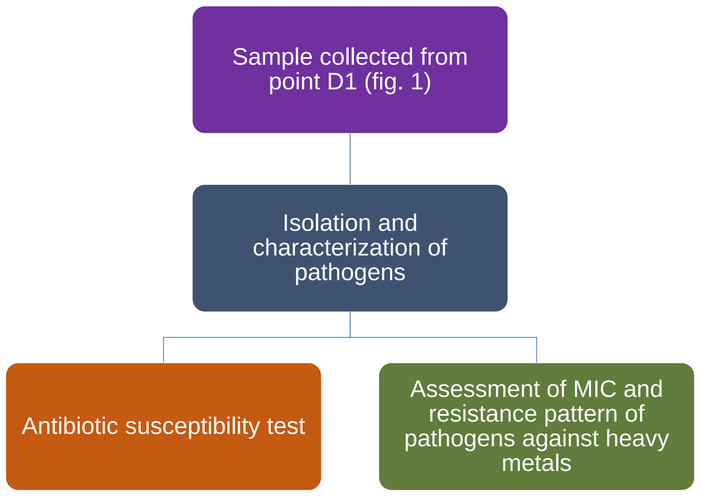
General objectives

To isolate pathogenic bacteria from the treated sewage and assess resistance pattern of the isolates against antibiotics and heavy metals.

Specific objectives

1. To isolate pathogens from treated wastewater.
2. To determine resistance pattern of pathogens against antibiotics.
3. To assess minimum inhibitory concentration (MIC) and resistance pattern of the pathogens against the heavy metals of different concentration.

Methodology



Results

- After biochemical characterization of the isolates; *S. aureus, E. faecalis, C. freundii, E. coli, E. aerogenes, P. mirabilis, P. vulgaris, Salmonella* Typhi, *P. aeruginosa* were identified.
- Antibiotic susceptibility test revealed that 42.38%, 14.52%, 1.32% were resistant to a antibiotic, 2 antibiotics, 3 antibiotics respectively while a total of 41.72% were susceptible to all 4 antibiotics.
- This data is presented in Table 1.

Table 1: Bacteria isolated from the treated wastewater along with their antibiotic resistance pattern

Sn	Isolates	Z	Antibiotics	P	G	A	T	V	C	I	Ak	Pi	Cz	Na	N
1	*S. aureus*	20	P, G, V, C	9	0	-	-	0	0	-	-	-	-	-	-
2	*E. faecalis*	14	A, T, V, N	-	-	9	1	0	-	-	-	-	-	-	0
3	*E. coli*	20	G, A, T, I	-	3	20	3	-	-	3	-	-	-	-	-
4	*E. aerogenes*	20	G, A, T, I	-	5	20	0	-	-	7	-	-	-	-	-
5	*C. freundii*	20	G, A, T, I	-	3	3	0	-	-	1	-	-	-	-	-
6	*Proteus* *P. mirabilis*	12	G, A, T, I	-	2	3	0	-	-	3	-	-	-	-	-
	Proteus *P. vulgaris*	8	G, A, T, I	-	1	2	0	-	-	2	-	-	-	-	-
7	*Salmonella* Typhi	17	G, T, I, Na	-	2	-	1	-	-	0	-	-	-	4	-
8	*P. aeruginosa*	20	I, Ak, Pi, Cz	-	-	-	-	-	-	5	2	2	1	-	-

P= Penicillin (1 unit), G= Gentamicin (10 µg), A= Ampicillin (10 µg), T= Tetracycline (30 µg), V= Vancomycin (30 µg), C=Cefoxitin (30 µg), I= Imipenem (10 µg), Ak= Amikacin (30 µg), Pi= Piperacillin (100 µg), Cz= Ceftazidime (10 µg), Na= Nalidixic acid (30 µg), N= Nitrofurantoin (300 µg), Z= sample frequency

- Result of assessment of resistance pattern of pathogenic isolates against heavy metals illuminates that with the exception of few pathogens.
- All pathogenic isolates can resist up to dilution 1250 g/L; while some isolates can resistant up to dilution 5000 g/L.
- *P. aeruginosa* isolates were able to resist up to dilution 1:1 (Figure 2c).
- This data is presented in Table 2.

Table 2: Isolates and their respective MIC dilution of different heavy metals

Sn	Heavy metal		*E. coli*	*P. aeruginosa*	*C. freundii*	*E. aerogens*	*S. aureus*	*Proteus* *P. mirabulis*	*Proteus* *P. vulgaris*	*E. faecalis*	*Salmonella* Typhi
			n= 20	n= 20	n= 20	n= 20	n= 20	n= 12	n= 8	n= 14	n= 17
1	MIC dilution of	Cd^{++}	A	B	A	A	A	A	A	B	B
2		Mn^{++}	A	A	A	A	A	A	A	A	A
3		Fe^{++}	B	*	A	B	A	A	A	A	A
4		Zn^{++}	A	A	A	A	A	A	A	A	A

***= resistant to all dilution, n= sample frequency, A= 5000 g/L (50%), B= 2500 g/L (25%)**

Pearson's chi square test shows that there is a significant association ($p < 0.001$) between isolates and antibiotic resistance pattern; isolates and heavy metal resistance pattern at dilution 10000 g/L (100%), 5000 g/L (50%), 2500 g/L (25%), 1250 g/L (12.5%).

Pearson's chi square test reveals that there is a significant association ($p < 0.001$) between antibiotic resistance pattern and resistance patterns at dilution 2500 g/L and no significant association between antibiotic resistance and heavy metal resistance pattern at dilution 100%, 50%, 12.5% ($p = 0.345, 0.86, 0.345$) respectively.

fig. 2: Respective MIC dilution for isolate

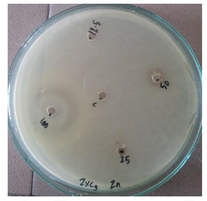


fig. 2a: Zxc_9 against Zn^{++}
The isolate is resistant against Zn^{++} of dilution 25% and 12.5%.

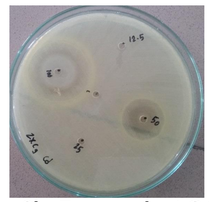


fig. 2b: Zxc_9 against Cd^{++}
The isolate is resistant against Cd^{++} of dilution 25% and 12.5%.

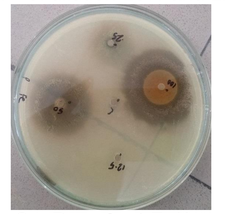


fig. 2c: P against Fe^{++}
The isolate is resistant against Fe^{++} and the mutated colony are seen in the halo zone in dilution 100%, 50% and 25%.

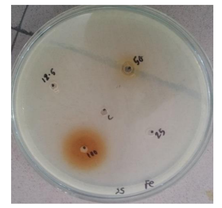


fig. 2d: SS against Fe^{++}
The isolate is resistant against Fe^{++} of dilution 25% and 12.5%.

Conclusions

The results obtained in this research can be summarized as follows:

1. Among the pathogenic isolates screened from the treated wastewater, few strains were resistant to multiple antibiotic(s) and heavy metal(s).
2. The result of this study sheds light on the fact that chemicals concentration can be lowered using heavy metal resistant organisms but heavy metal(s) might evoke antibiotic resistance.
3. It reveals that microbes exposed to pollutants have higher heavy metal resisting capacity and can be used for environmental cleanup.

References

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Acknowledgement

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