Isolation and Characterization of Biofilm forming Antibiotic-Resistant Bacterial Strains from Pharmaceutical Industrial Waste

SUMMARY OF
THESIS

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Summary

The biofilm and AR significantly impact ecotoxicology and environmental sustainability. The pandemic of antibiotic pollution disrupts biogeochemical cycles (BGC) and other ecological processes. Biofilms can be beneficial for protecting our bodies from harmful agents, plant protection, wastewater treatment, and corrosion inhibition, but they can also be detrimental to our health (Mirghani et al., 2022). They can form on abiotic surfaces like medical devices and biotic surfaces like teeth, lungs, and bone. Biofilms in water systems supplying healthcare facilities can lead to life-threatening infections. They account for up to 80% of microbial infections, and can cause chronic respiratory infections, lung diseases, and chronic obstructive pulmonary disease (Boisvert et al., 2016; Gnanadhas et al., 2015; Mirghani et al., 2022).

Several research have focused on antibiotic resistant bacteria in various Wastewater industries such as sewage wastewater, drinking wastewater, pharmaceutical wastewater. The maximum work on pharmaceutical industrial wastewater has been updated to the antibiotic- resistant bacteria and their resistant gene. But their cooccurrence of biofilm formation (Phenotypic and genotypic) and antibiotic resistant in bacterial species has not been revealed so far. Therefore, the combined effect of the biofilm and antibiotic resistant generate, MDR and XDR bacteria will be a beneficial study for control the serious infections.

The study of the above hypothesis, this thesis has been composed into the following chapters; **Chapter first** has introduced the basic information on the topic of the thesis. The information related to biofilm forming antibiotic resistant bacteria in pharmaceutical wastewater and their distribution in the environment. Further, the **chapter two** also provides background of the study and comprehensive information of pharmaceutical industrial wastewater containing biofilm forming antibiotic-resistant bacteria in global and Indian prospective, as well as their harmful effects on the environment, human and animal health.

Further, **chapter three** has focused on five objectives as per topic of thesis. Chapter four has described the evaluation of toxicity of untreated and treated pharmaceutical wastewaters from wastewater treatment plants (WWTPs), on the basis of physicochemical characterization, heavy metal ,residual organic pollutants.

The **chapter four** summarized to the WWTPs, responsible for promising everyone access to clean water, sanitary conditions, and good health, are essential to achieving the UN Sustainable Development Goals (SDGs), particularly SDGs 3 and 6. In comparison to effluents of other WWTP, the PIWWTP's effluent contains high levels of BOD, COD, nitrate, phosphate, metal concentration, pharmaceutical metabolites, and so on. The inability of PIWWTPs to completely remove pharmaceutical metabolites has been widely overlooked. The amalgamation of this effluent with the recipient environmental bodies may have several detrimental effects. In addition, this residual organic pollutants of pharmaceutical wastewater after liquid-liquid extraction analysed by GC-MS to reveal the nature of pollutants present in this wastewater. The phytotoxicity and genotoxicity test was also done to assess the toxicity of HUTW, HTW, DUTW and DTW on mung bean (*Vigna radiata*) and onion (*Allium cepa*). These studies demonstrated that heavy metals, therapeutic compounds, phenols and other organic contaminants present in the pharmaceutical wastewater are primarily responsible for the cytotoxic and genotoxic consequences. It can potentially cause phytotoxicity and chromosomal aberrations and disrupt MI and other cytological processes. Due to these grave implications, immediate action is required to limit the propagation of this worldwide hazard. Public regulatory agencies must act quickly to prevent PIWWTPs from discharging hazardous effluent. The scientific community must develop more efficient, cost-effective methods for treating the PIWW.

The **chapter five** summarized to the evaluate physicochemical parameters, heavy metal concentration and organic contaminants, as well as the detection of the antibiotic in the PIWW samples, these factors play a crucial role in biofilm formation and the resistant tendency of bacteria in wastewater. This work aimed to estimate the characterization of antibiotic resistance, phenotypic and genotypic characterization of biofilm-forming MDR-identified clinical bacterial strain collected from the PIWWTPs four wastewater samples taken for the winter and summer session. This study looked into the three genes (*ompA, bap* and *pslA*), which are involved in biofilm adherence after auto-aggregation. The two foremost virulence parameters of the identified bacterial isolates are biofilm formation and MDR. The five potential identified biofilm-forming bacterial strains are *Pseudomonas aeruginosa* PTJ402 **(OM044401),** *Acinetobacter baumannii* PTJ403 **(OM044988)**, *Escherichia coli,* UTJ403 **(OM045070)**, *Klebsiella pneumoniae* UTJ404 **(OM045059)**, and *Burkholderia*

cepacia UTJ501 **(OM045288).** The finding of this research has exhibited high toxicity of wastewater, along with antibiotics, induce selective pressure upon environmental and clinical strains of bacteria to develop and propagate AR. The co-occurrence of multiple antibiotic resistance and a propensity to form biofilms increases the antibiotic resistance potential multiple times. If the biofilm-forming tendency spreads to clinical strains, recovery from infection becomes nearly impossible. The inability of WWTPs to eliminate antibiotics from wastewaters and the resulting evolution and transmission of AR, ARGs and BFGs to the receiving environment has been mostly overlooked. According to the findings of this and previous studies, WWTPs may play a substantial role in the dissemination of antibiotic resistance in the environment. As a result, immediate action is required to restrict the expansion of this global menace. Government regulators must focus on preventing WWTPs from becoming breeding grounds for antibiotic-resistant bacterial communities.

The **chapter six** summarized to investigate whether functions encoded in the metagenome could improve our ability to understand the link between microbial community structures and functions in untreated and treated wastewater . By analysing data sets from pharmaceutical wastewater treatment plants (PWWTPs), covering different configurations, operational conditions, and geographic regions, we found that wastewater metagenomic composition of the activated sludge samples. The purpose of this study was to characterize the environmental bacterial communities from the two-wastewater sample of PWWTPs around the Pharmaceutical industries in India Uttarakhand region and these are the main source of antibiotic-resistant bacteria. Here, we demonstrate that microbial community composition in a receiving water body, and it leads to proposing further studies to reduce the related ARB burden for becoming a potential health risk. This study may help to better understand the bacterial assemblages and their interrelations in pharmaceutical wastewater treatment. The sequence analysis of 16S rRNA V3–V4 hypervariable region with Illumina MiSeq platform showed 27116 and 19939. 19939 OTUs derived from pharmaceutical wastewater samples out of 405005 sequences read, respectively. The major genus and species detected in untreated and treated wastewater sample were *Hydrogenophaga (*20%*), Planctomyces* (18%), *Novosphingobium* (28%), *Hydrogenophaga* (23%)*, borealis* (31%), *pratensis* (28%), *terrae* (24%), *pratensis (41%), reducens (37%), sp. SH-PL14 (20%).*Our study evaluate that pharmaceutical wastewater 48.1% Common bacterial communities

associated with DPW and HPW were substantially different in richness, diversity, and relative abundance of taxa compared to pharmaceutical wastewater. The results of this study will be helpful in understanding the role of antibiotic-resistant bacterial communities responsible for pollution control and detoxification of complex organometallic waste and, thus, can help in designing appropriate multidrug resistant bacterial studies for eco-restoration of polluted sites.

The **chapter Seven** summarized to optimization of potential antibiotic -resistant biofilm forming strains for growth at various environmental factors (Incubation time, temperatures and pH) and the effect of carbon and nitrogen sources. In these experiments, microtiter plates were prepared similarly as in the modified method of microtiter plate assay described in screening procedure. The results of incubation period (24, 48 and 72 h), temperature (4°C, 28°C, 35°C and 45°C) and pH (4.5, 5.8, 6.5, 7.5 and 8.5) were recorded. In this study highest biofilm formation was induced in the presence of glucose (conc.1550µg/200µL) and nitrogen source it was found that Beef extract and peptone (Conc. 1900µg/200µL)

The **chapter eight** summarized to, In this study, a Antibiotic-degrading bacterial consortium containing Three consortia (C1, C2, C3) were developed with potential biofilm forming strain, C1 (*Acinetobacter baumannii* **OM044988**, *Escherichia coli* **OM045070**) C2 (*Klebsiella pneumoniae* **(OM045059)**, *Pseudomonas aeruginosa* **OM044401,** and *Burkholderia cepacia* **OM045288)** and C3 (*Pseudomonas aeruginosa* **OM044401,** *Klebsiella pneumoniae* **OM045059**) was constructed, which was capable of biodegrading within 10 days in LB medium. Tetracycline, Ampicillin respectively, which was isolated from pharmaceutical wastewater treatment plant (WWTP). Antibiotic degradation consortium developed based on the antibiotic sensitivity test perform of both antibiotics. A further experiment was carried out in 150 mL LB medium with 50 mg/L TC and AMP, pH = 7.0, the shake flasks were set on a dark shaker set at 30^0C, and 150 rpm and inoculum size, 3.5 mL (OD~0.5). consortium C3 degrade high concentration contrast to C1 and C3. and it degraded 90% and 95% of the tetracycline within 10 days. Antibiotics and its intermediates were analyzed using High Performance Liquid Chromatography (HPLC).

The **chapter nine** summarized to the findings of the thesis. This section has mentioned the brief findings of each chapter.

The **chapter ten** has compiled the cited references of thesis which are relevant to the topic and they are cited in each chapter. Each chapter has cited the recent references based on methodology and results. The available references are cited on biofilm forming antibiotic resistant bacteria, physico-chemical properties of pharmaceutical wastewater, impact of untreated and treated wastewater and biofilm forming bacteria reported at polluted sites.

The **last chapter** has listed all the scientific output based on the thesis work. There is four original research paper published in peer reviewed journal of international repute with high impact factor. One review paper also published on the international journal. A certificate of participation and oral presentation has been provided in international/national conferences.