Vol. 19 (Supplementary Issue 4), Oct-Dec 2025, ISSN 0973-8916 (Print)., 2230-7303 (Online)
10.5530/ctbp.2025.4s.10

Bacteriological Analysis of Pharmaceutical Waste Water and Surrounding Environmental Waste Samples in South India

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Abstract

To isolate and characterize the aerobic and anaerobic bacteria from South Indian pharmaceutical waste water, surface water, and ground water samples around the pharmaceutical industries. Methods: In total, 55 samples were collected from Chennai, Nellore, Hyderabad, Kerala, and Bangalore in southern India. The bacteria were isolated using various conditions, and their identification was then confirmed using biochemical characterization. VITEK 2 Compact and VITEK MS are frequently used for further identification of bacterial isolates. Results: From 55 samples, 48 isolates totaling 10 distinct bacteria were identified. The identified bacterial isolates were *Klebsiella pneumoniae* (19%), *Pseudomonas aeruginosa* (22%), *Enterobacter aerogenes* (12%), *Corynebacterium* sp. (2%), *Acinetobacter* sp. (5%), *Aeromonas punctata* (3%), *Ralstonia picketti* (2%), *Staphylococcus aureus* (28%), *Stenotrophomonas maltophillia* (3%), and *Citrobacter freundi*. Conclusion: The study's findings indicated that numerous bacterial species were present in wastewater, surface water, and groundwater samples from the pharmaceutical industries, but the spread of resistant microbes could be stopped by the effective implementation of effluent treatment plant protocols in every pharma industry. According to this study, pharmaceutical companies must have an efficient waste water treatment facility before discharging waste water into the environment in order to stop the spread of antibiotic resistance.

Keywords: Pharmaceutical effluent, resistant bacteria, isolation, identification, Antimicrobial resistance

Introduction

Antibiotic resistance (AMR) develops naturally, but it is being hastened by the widespread use of antibiotics in both humans and animals. Multi-drug-resistant and/or extremely drug-resistant microorganisms are to blame for an increasing number of infections, including pneumonia, tuberculosis, and gonorrhea[1]. As a result, medicines used to treat the infections are becoming ineffective. AMR is a significant global health problem that results in longer hospital stays, higher mortality rates, and related financial losses.

Numerous studies point to high concentrations of antibiotics, bacteria that are resistant to antibiotics, and genes associated with antibiotic resistance in a variety of global ecosystems, including those affected by sewage, agriculture, aquaculture, and pharmaceutical industrial effluent. This accumulation promotes the growth and transmission of resistant bacteria from the environment to humans or animals, as well as selection and horizontal gene transfer from commensal to pathogenic bacteria[2].

According to the World Health Organization[3], AMR is one of the top ten global health consequences affecting people globally. In this complex situation, wildlife, ecological, and human habitats are all linked.

According to a recent survey, the number of deaths caused by infections with pathogens that are resistant to antibiotics is currently estimated to be around 4.5 million per year. By the year 2050, this number is projected to rise to 10 million, with 2 million of those deaths expected to occur in India alone. The presence of pharmaceuticals, other chemicals, and antibiotics in the environment is an increasing public health concern since recent environmental monitoring initiatives have revealed that numerous antibiotics are found in soil and water. Several pharmaceutical industries emit pharmaceutical waste into the environment. It is the main environmental factor causing the emergence of germs that are resistant to antibiotics. The persistence of environmental antibiotics led to an increase in resistance among dangerous bacteria[4].

A worldwide survey of *C. striatum* human infections and nosocomial outbreaks was accomplished by the analysis of clinical, epidemiological and microbiological features of reported cases from varied countries, during a 44 year period (1976–2020) and reported the possible reasons for the increasing antimicrobial resistance. Also highlighted measures to be taken to combat AMR in China[5,6]. Out of 254 bacterial strains isolated from pharmaceutical wastewater effluents,218 (85.8%) isolates were of multidrug resistant. The microorganisms were identifiedas *Acinetobacter* sp., *Klebsiella pneumonia*, *Proteus mirabilis*, *Enterobacter* sp. and *Bacillus* sp[7].

Multiple-Drug Resistance bacterial strains isolated and identified from the effluent of Scottman Pharmaceuticals, Islamabad were of *Bacillus* sp., *Klebsiella* sp., *Staphylococcus* sp., *Enterobacter* sp., *Diplococcus* sp., *Micrococcus* sp., *Streptobacillus* sp. and *Shigella* sp. Antibiotic sensitivity test was also carried out against different antibiotics and found cefixime was the least effective medicine[8]. The impact of temperature on the variation of beta-lactamase and *mcr* genes in bacterial resistant strains in a wastewater treatment plant in Germany was reported by[9]. They isolated and confirmed co-occurrence of *mcr-1* and carbapenemase genes in a multi-drug resistant*Enterobacter doacae*and *Escherichia coli* producing extended-spectrum beta-lactamase (ESBL) by real time PCR and VITEK.

Anti-microbial resistance (AMR) profiles of three different species over a complete wastewater sample taken in the city of Sneek, Netherlands including wastewater from a nursing home, hospital, community and municipal including industrial wastewater, were tested and analyzed. A total 2886 isolates (997 *Escherichia coli,* 863 *Klebsiella* spp*.,* and 1026 *Aeromonas* spp.) were cultured from the 211 samples analyzed[10,11] found increasing antibiotic-resistant bacteria in sewage associated with the discharge of wastewater from a hospital and a pharmaceutical plant by using *Acinetobacter* species as environmental bacterial indicators.

Antibiotic resistance profile and β-lactamases production in Gram-negative bacilli isolates from hospital sewage and urban wastewater treatment plants (UWWTP) in Brazil were evaluated[12]. Background information on the ESKAPE pathogens, phenotypic and molecular methods used to detect their AMR mechanisms were summarized by[13].

The objective of the present research was to isolate and identify pathogenic and non-pathogenic bacteria that are resistant to antibiotics in samples of south Indian environment and industrial waste water. The findings of this study could highlight the necessity of adopting appropriate preventative or control measures to stop the spread of resistant bacteria in the environment.

Materials and Methods

All chemicals, media, and other reagents were supplied by HiMedia Laboratories (India). A total of 40 influent (wastewater that has not been treated) and effluent (wastewater that has been treated) samples were collected from several pharmaceutical plants that manufacture antibioticsin south Indian states like Tamil Nadu, Andhra Pradesh, Telangana, Kerala, and Karnataka and 15 Nos. of surface and ground water samples (pond water, river water, and bore well water) were also collected from the vicinity of pharmaceutical plants (Supplementary Fig. S1).Five hundred mL samples were aseptically collected, sealed, and maintained on wet ice in sterile polypropylene containers. Within 24 hours of collection, it was brought to a lab for standard microbiological analysis. Within 24-36 hours of sample collection, isolation and identification were completed by inoculating various commercial media, including nutrient agar (NA), blood agar (BA), MacConkey agar (MAC), and chocolate agar (CA). Colonies having distinctive morphological and physical characteristics were further examined after an overnight incubation[14-17]. Standard microbiological procedures such as Gram staining, coagulase test, citrate utilization test, indole test, triple sugar iron test (TSI), mannitol, oxidase, catalase, and urease tests were done to identify the species[18]. Bacterial isolates were identified using VITEK 2 Compact and VITEK MS (BioMerieux, India (P) Ltd., New Delhi) for further identification.

Results

Industrial waste discharges pose a serious risk to public health and are one of the main sources of environmental contamination that can cause disease outbreaks or the transmission of the gene responsible for antibiotic resistance through resistant bacteria. This study, which utilisedsurface water, ground water and waste water samples taken from various pharma-industries in south India, was based on the microbial community present in them. The results showed that the waste water from pharmaceutical industries and their environs had a sizable microbial population. The 55 samples that were collected included 25 influents, 15 effluents, and 15 surface and groundwater samples (5 pond water, 5 river water, and 5 bore well water) (Table 1 and Supplementary Fig. S2).

For bacterial species isolation, a total of 40 samples of industrial waste water and 15 samples of surface and ground water were treated. Gram's staining was used to further identify the 39 samples that had growth as Gram negative bacilli (31 Nos.), Gram positive cocci (16 Nos.), and Gram positive bacilli-club shaped (1). Of these 55 samples, 39 samples had growth on any one of the agars, namely Blood, MacConkey, and Chocolate agar (Table 2). The cells were found alone, in pairs, chains, and clusters.

Table 1: Waste water, Surface and ground water samples of South India

Total no. of samples	Influent	Effluent	Surface water		Ground water
			Pond water	River water	Bore well water
55	25	15	5	5	5

Table 2: Gram staining andMicrobial growth of Waste water, Surface and Ground water samples.

Total no. of samples	Total counts of microbial growth	Gram Staining		
		GNB	GPC	GPB (club shaped)
55	39	31	16	1

In this investigation, 10 different types of bacterial strains were identified from 55 samples*.* *Klebsiella pneumoniae* was found in 7 samples, *Pseudomonas aeruginosa* in 9 samples, *Enterobacter aerogenes* in 6 samples, *Corynebacterium* sp. in 1 sample, *Acinetobacter* sp. in 3 samples, *Aeromonas punctata*in 2 samples, *Ralstonia picketti* in 1 sample, *Staphylococcus aureus* was found in 16 samples, *Stenotrophomonasmaltophillia*in 2 samples, and*Citrobacter freundii*in 2 samples (Supplementary Table S1).

A bar chart displaying bacterial isolates obtained from surface water, ground water, and waste water samples of pharmaceutical industries in South India (GW- Ground water, I-Influent, E-Effluent, TN- Tamil Nadu, AP- Andhra Pradesh, TS- Telangana, KA- Karnataka, and KL- Kerala) is shown in (Fig. 1) and (Supplementary Table S2).

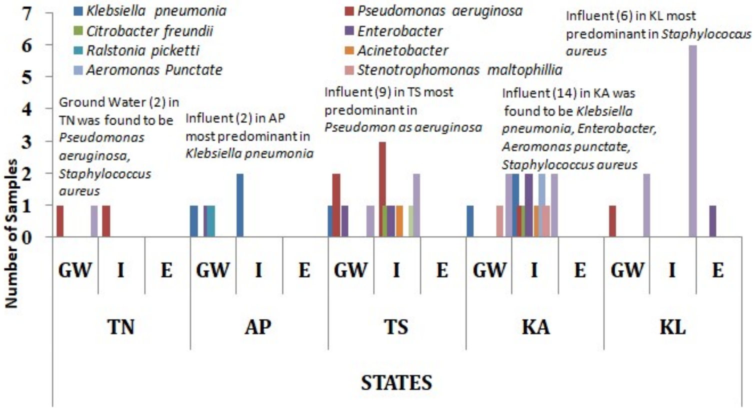
According to our findings, ten different species of bacteria, including pathogenic and nonpathogenic microbes with varying patterns of drug resistance were isolated and identified from industrial waste water, surface water, and ground water.

Three *Stenotrophomonas maltophillia*, 19 *Klebsiella pneumonia*, 22 *Pseudomonas aeruginose*, 4 *Citrobacter freundii*, 12 *Enterobacter aerogenes*, *2 Ralstonia picketti*, 5 *Acinetobacter sp*., and 3 *Aeromonas punctate* were the Gram-negative bacilli isolates.

28 *Staphylococcus aureus* (Cocci) and 2 *Corynebacterium sp.* (Gram Positive Bacilli, club-shaped) were the Gram Positive isolates (Fig. 2) and (Supplementary Table S3).

Discussion

Microbiological testing was performed on waste water, surface water, and ground water samples. Many pharmaceutical companies emit biomedical waste into the environment. It is possible that it may be the primary source of antibiotic-resistant microorganisms present in the environment. Making sure that the product is free of contaminants is a vital stage in the production of drugs. Antibiotic residues in the environment accelerated the emergence of resistance in both pathogenic and apathogenic bacteria. Biochemical characterization yielded 48 isolates of 10 different kinds of bacteria from 55 waste water, surface water, and ground water samples.Most of the bacteria that were



GW- Ground water, I-Influent, E-Effluent, TN- Tamil Nadu, AP- Andhra Pradesh, TS-Telangana, KA- Karnataka, KL- Kerala

Fig. 1: A bar chart showing bacterial isolates obtained from surface water, ground water and waste water samples of pharmaceutical industries in South India

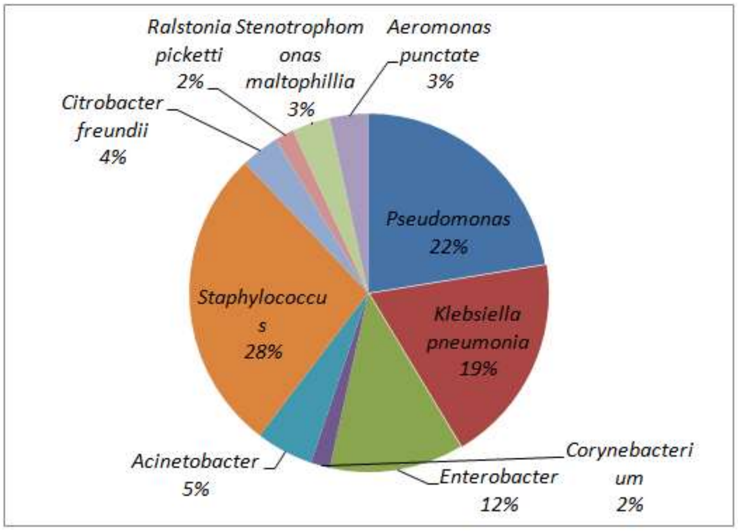


Fig. 2: A pie chart showing % bacterial isolates obtained from surface water, ground water and waste water samples of Pharmaceutical industries in South India.

isolated from the waste and other water samples were *Staphylococcus aureus* (28%), *Pseudomonas aeruginosa* (22%), *Klebsiella pneumonia* (19%), and *Enterobacter aerogenes* (12%).

It has been found, as expected, that influent samples contain microorganisms but effluent samples do not except only one isolate, identified in a Kerala wastewater sample. Hence every pharmaceutical company must conduct rigorous surveillance of its effluent treatment facilities also. Groundwater and surface water samples yielded 15 isolates, which is a significant public health risk if the isolates contain several resistance genes. This justifies inspecting any nearby pharmaceutical facilities, implementing rigorous bio-hazard waste disposal regulations, and building an effluent treatment facility to handle waste water.

Industrial waste is defined as waste generated by industrial activity and comprises any material that is rendered worthless or garbage during a production process or reaction. It can be a solid, liquid, or gas, and it can be toxic or non-hazardous. Toxic wastes that may contain bacteria include those that are toxic, poisonous, flammable, ignitable, corrosive, reactive, or radioactive. It is capable of polluting or destroying the air, land, and water supplies. Dumped heavy metal trash, pollutants, and sewage into water sources have a direct influence on the aquatic living organism community as well as the health of humans who rely on the water sources[19-24].

Infections from bacteria, parasites, and toxic chemicals are the most harmful and devastating impacts that water pollution may have on the health of humans. Numerous organisms, including bacteria, viruses, protozoa, fungi, flatworms, and roundworms, regularly contaminate waste water. Of them, bacterial infections present the greatest epidemiological risk to humans. It can lead to several disorders in individuals. One of the most important clinical infections in the world is *Staphylococcus aureus*, which exerts pressure on the healthcare system due to its drug resistance, particularly methicillin-resistant *Staphylococcus aureus* (MRSA).

The harmful use and misuse of antibiotics in both humans and animals are thought to be the cause of the global antibiotic resistance problem. But the influence of the environment made it clear where new resistance genes come from and how resistant bacteria and resistance genes are transmitted. Microorganisms that were once sensitive to antibiotics began developing multidrug resistance to various types of antibiotics because of the overuse of antibiotics. Conjugation is a common method for transferring plasmids harbouring resistance genes[25-27].

One of the main contributors to environmental pollution and a serious threat to human health is waste water released by industries. Antibiotic resistance in *S. aureus* is 80% for both ampicillin and amoxiclav, and some strains have also demonstrated resistance to chloramphenicol. *S. epidermidis* had the highest level of antibiotic resistance across all drug classes. It was resistant to every member of the beta-lactamase family of antibiotics, and some of them exhibit resistance to the drug tetracycline[28].

While medications are commonly used in developing countries, people may self-administer antibiotics, increasing the likelihood of the formation of drug-resistant strains. The conventional practice of giving low-dose antibiotic injections to animals over a long period of time to encourage growth contributes greatly to the spread of antibiotic-resistant microbes in the environment. Antibiotic use has been enormous, with global pharmaceutical industry production estimated to reach between 100,000 and 200,000 metric tonnes. The findings of this study highlight the importance of the pharmaceutical industry having an effluent treatment plant in place to avoid contaminating the environment with bacteria that may possess resistance genes.

Conclusion

A wide range of bacterial isolates were isolated from waste water, surface water, and groundwater samples collected from pharmaceutical enterprises and their surroundings. The findings demonstrated the importance of wastewater treatment plants in limiting the spread of antibiotic-resistant bacteria and antimicrobial residues to the environment, hence indirectly preventing the spread of antimicrobial resistance. The responsible authorities must conduct a periodic examination of the wastewater treatment facility to ensure the plant's effectiveness.

Acknowledgement

The authors would like thank the Department of Pharmacy & Microbiology, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai, Tamil Nadu, for their assistance.

Author’s Contribution

K.S, S.Mstudy design; K.S sampling; U.S laboratory analysis; K.S, U.S, S.M data analysis; K.S funding; K.S,S.K, S.M manuscript drafting; all authors provided helpful recommendations and comments and approved the final version of the manuscript.

Funding

This work was supported by the Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai, Tamil Nadu.

Data availability

Accession numbers for each sample are provided in supporting information (Figure S1, Tables S1, S2, S3).

Declaration

We hereby declare that this submission is entirely our own work, in own words, and that all sources used in researching it are fully acknowledged and all quotations properly identified. It has not been submitted, in whole or in part to any journal.

Conflict of Interest

The authors have no conflicts of interest to declare.

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