DOI: 10.22270/jmpas.V12I4.4399 ISSN NO. 2320–7418

International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Research article

Isolation and characterization of multi drug resistance bacterial pathogens from Song and Suswa river water in Dehradun

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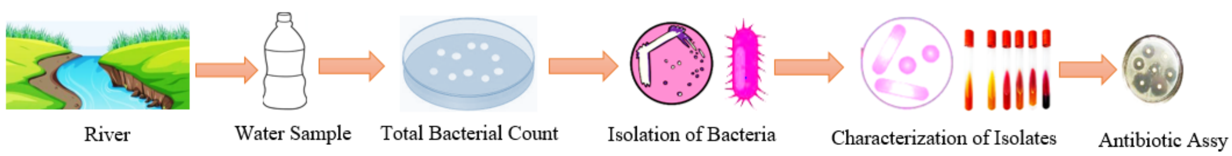
Received - 02-10-2022**, Revised** - 10-07-2023, **Accepted** - 04-08-2023 **(DD-MM-YYYY)**

Refer This Article

Rakesh Pant, Amit Gupta, Bharat Rohilla, Arsh Singh, Priya Arya, Sunidhi Shreya, Nirmal Patrick, Vijay Kumar, 2023. Isolation and characterization of multi drug resistance bacterial pathogens from Song and Suswa river water in Dehradun. Journal of medical pharmaceutical and allied sciences, V 12 - I 4, Pages - 5949 – 5954. Doi: https://doi.org/10.55522/jmpas.V12I4.4399.

ABSTRACT



Antibiotics are antimicrobial drugs that kill germs and are crucial in the fight against bacterial infections. These medications are widely used in the prevention and treatment of diseases because they may either kill or stop bacteria from growing. Microbial resistance refers to bacteria's capacity to survive antibiotics' bacteriostatic or cytotoxic effects. The samples for this study are taken from Song and Suswa River, Dehradun, Uttarakhand. The main aim of this study is to isolate several species to find out if them are resistant to antibiotics. The methodologies used in this research are isolation and characterization of bacteria, antibiotics sensitivity test. There were 17 types of antibiotics were used i.e. *Azithromycin, Amikacin, Cefaclor, Cefepime, Cefoxitin, Doxycycline Hydrochloride, Erythromycin, Gentamycin, Kanamycin, Levofloxacin, Mecillinam, Mezolocilline, Penicillin G, Vancomycin, Tetracycline, Ticarcilline, Nitrofurantoin*. The result was that species like *Salmonella, Pseudomonas, E. coli, Klebsiella* were resistant, sensitive, and intermediate against the 17 antibiotics. Antibiotic-resistant bacteria can potentially be transferred from one to another in a society. This is gradually getting frequent these days. In short, antibiotic resistance cannot be prevented or, in some cases, reversed, but we can certainly slow its progression.

Keywords: Antibiotics, Antibiotic resistance, Pathogens, Song River, Suswa River

INTRODUCTION

Human, animal, and environmental health is regarded as a global health threat due to antibiotic resistance (AR) in bacteria. Environmental settings have been shown in several studies to have positive impact on water and have a profound impact on the frequency of multidrug resistance in diverse contexts, often because AR mechanisms may emerge from environmental bacteria or because AR mechanisms may be acquired through other diseases that occur in humans and animals can pollute the environment [1, 2]. It was only a few decades ago that water contamination was only concern regarding bacterial contamination that led to diseases like cholera and typhoid fever. Many recently discovered microbial pollutants, particularly in the past two decades, pose greater risks to the safety of municipal waters, as do strains carrying antimicrobial resistance genes, too, which have been deemed to pose serious health risks to the general population. There has been a rise in antibiotic resistance which has been linked to the widespread misuse and overuse of antibiotics in human medicine, agricultural practices, and aquaculture. These have led to the build-up of metabolites in various ecosystems such as natural waterways [3, 4].

Contaminated water may cause a variety of ailments, including dysentery, cholera, diarrhea, typhoid, and in severe cases, death, especially in underdeveloped nations. Developing nations are on the front lines of waterborne illness epidemics due to scarcity of adequate medical management and a scarcity of pure drinking water. AR kills millions of people every year and is expected to become a primary global health threat in the future. 10 million deaths will be caused annually by AR by 2050. Since the discovery, manufacture, and use of antimicrobial compounds, the treatment of infectious illnesses has been transformed. The abuse of antimicrobial compounds, on the other hand, is the leading source of AR in harmful bacteria [5]. Antibiotic-resistant bacteria (ARB) are a major public health concern across the globe; also marine habitats are a known source of ARB and ARGs. Due to the widespread use of antibiotics, bacteria in sewage and agricultural runoff are selected for enriched in the aquatic system. There has traditionally been a concern about pathogens in distribution networks regarding the microbiological quality of drinking water [4, 5].

Among people who enjoy water sports, it has been discovered that ARB and other community-acquired microorganisms are related to gastrointestinal disorders as well as non-gastrointestinal disorders. ARGs are carried by bacteria in coastal waters like *E. coli*, including those carrying the CTX-M gene. This gene confers resistance to clinically relevant antibiotics and is easily organized by horizontal gene transfer (HGT), making it more common in natural settings like water treatment plants [7, 8]. Therefore, microbiological contamination of drinking water must be prevented for the sake of human health. Drinking water and other household needs are directly supplied by untreated surface water in rural areas from rivers, dams, and streams [7, 8]. ARGs and facultative pathogenic bacteria from many sources can contaminate natural waterways as they pass via treatment facilities or agriculture runoffs. In the event of direct contact and colonization, this might have a negative impact on the health of people. Due to the ability of released microbes to stay in the environment of the sea and potentially grow back to critical proportions, an environmentally beneficial treatment is advised [9].

Antimicrobial resistance has been found to be raising among enteropathogenesis especially *E. coli*, in current years occasionally leading to point-break circumstances when no antibiotic treatment choices exist. In underdeveloped nations, where enteropathogenesis are commonly found and can cause life-threatening illnesses, particularly in children, these conditions are of great concern. New Delhi: A new carbapenems has recently emerged and spread. Microbes that produce metalloid b-lactamase (NDM) are one example of a circumstance in which existing antibiotics are useless. Horizontal gene transfer (HGT) is the most popular methods for transferring antibiotic resistance genes from one microorganism to other. Plasmids are most common vectors for HGT in Enterobacteriaceae. It has been shown that resistance characteristics between Enterobacteriaceae can be transferred in vivo in an ecological system [3, 9]. Changes in natural habitats are likely to influence antibiotic resistance and, by extension, human health. ARGs have developed in microbial sources, independent of their activity in non-clinical settings. Releases of drugs and the establishment of ARGs in human-linked microflora might be the most important contributors in the future direction of antibiotic resistance in bacterial pathogens [4, 10].

MATERIALS AND METHODS

Collection of Samples

Take 250 ml of water sample (n=30 Song River and n=12 Suswa river) aseptically in a borosil bottle from several locations around the song and the Suswa River and proper labeled the sample bottle for the microbiology analysis in the lab. Sampling sites are the river Song drains the Doon Valley in Uttarakhand, India. It flows from Dhanaulti to Narendranagar, beginning as a spring-fed stream on the Mussoorie crest of the Himalayas and Suswa River the Suswa River raises in the clayey depression near the Asan's source, to the east of the Asarori-Dehradun Road. The Suswa River drains the eastern half of Dehradun and merges with the Song River before flowing into the Ganga. The rivers Song and Suswa are two of the Ganga's principal tributaries.

Total Bacterial Count

A tenfold dilution was used to count the viable cells. Bacterial culture was spread on the different enrichment medium plate after overnight growth. We kept the plates in an incubator at 37°C for 24 hours inverted and incubated [11-13].

Isolation of bacteria from water samples

Water samples were spread out on enrichment medium and incubated under perfect circumstances for development. Each bacterial isolate was subculture for further investigation [11, 14].

Characterization of isolates from water samples

The colony morphology (shape, colour, and texture) of the acquired bacterial colonies was studied macroscopically and microscopically using Gram's staining. A single isolated colony was chosen for smear preparation and staining in order to examine the isolates' morphological characteristics. Indole, Methyl red, Voges Proskauer, Citrate utilization test (IMViC test), and Triple Sugar Iron Agar (TSI) tests were used to further biochemically characterize bacterial isolates. To distinguish between positive and "false-positive" responses, appropriate positive and negative controls were applied [15,16,17].

Antibiotic sensitivity of isolates

The bacterial isolates from water samples were tested against 17 antibiotics: Azithromycin, Amikacin, Cefaclor, Cefepime, Cefoxitin, Doxycycline Hydrochloride, Erythromycin, Gentamycin, Kanamycin, Levofloxacin, Mecillinam, Mezolocilline, Penicillin G, Vancomycin, Tetracycline, Ticarcilline, Nitrofurantione [2-4].

RESULTS

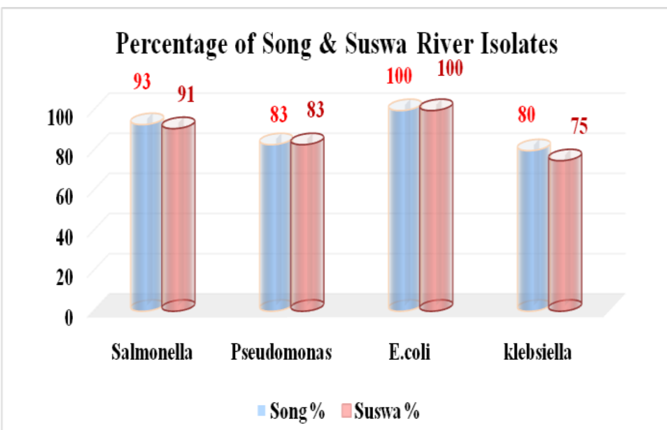
Total Bacterial Count / Standard Plate Count

On the different enrichment medium, we have found different bacterial colonies on both the Song and Suswa river. In Suswa river there were SS, Endo, *Pseudomonas* agar 7, 8, 9 colonies on 10^7 dilution plate and Mkleb, Nutrient Agar 1.2, 3.6 colonies on 10^8 dilution plate where as in Song River SS, Endo, Pseudomonas, Mkleb, Nutrient Agar 15, 8, 8, 8, 20 colonies on 10^8 dilution plate were found.

Isolation of bacteria from water samples

The bacteria's which were found in the dilution plate were inoculated in the nutrient broth and incubated the nutrient broth tubes for 37°C for 24 hours and after incubation strick the bacterial culture on enrichment medium and isolate the *Salmonella, Pseudomonas, E. coli, Klebsiella Spec.* are found in the Song and Suswa river water sample. **Figure 1.**

Figure 1: Percentage of Song and Suswa River Isolates



Characterization of isolates from water samples

The bacteria characterized based on the nature of colonies, Colour andGram's staining of the *Salmonella, Pseudomonas, E. coli, Klebsiella* Spec. are shown in different colonies and colour on the selective media in Endo agar, *E. coli* showed clear colonies and Metallic green and reddish colour. SS agar, *Salmonella spec.* showed opaque colonies and black center colour. Mkleb Agar *Klebsiella Spec*. showed Mucoid colonies and Pink, White colour. Pseudomonas Agar *Pseudomonas Spec.* showed Irregular colonies and Yellow, slightly greenish colour. On the microscopic observation all species showed negative rods and pink colour. For factors such as acid or alkali production and gas production, the biochemical tests generated varying results for different bacteria. During a citrate test, E. coli showed a negative result, a positive result for indole production, a negative result in the TSI agar, and a negative result in the MR-VP test. While producing alkaline gas in the TSI agar, Salmonella showed positive results for citrate utilization, Indole synthesis, and MR-VP tests. Despite producing no gas, *Pseudomonas* showed positive tests for citrate utilization, indole production, and MR-VP production. *Klebsiella*, meanwhile, showed positive results for citrate utilization and MR-VP, but negatively for Indole production. It also produced acid in TSI agar combined with gas.

Antibiotic sensitivity of isolates

The bacterial isolates from water samples were tested against 17 antibiotics: Azithromycin, Amikacin, Cefaclor, Cefepime, Cefoxitin, Doxycycline Hydrochloride, Erythromycin, Gentamycin, Kanamycin, Levofloxacin, Mecillinam, Mezolocilline, Penicillin G, Vancomycin, Tetracycline, Ticarcilline, Nitrofurantione. *Salmonella sp.* was found to be in resistance to 7 antibiotics, sensitive to 8 antibiotics and intermediate to 2 antibiotics; *Pseudomonas sp.* was found to be in resistance to 10 antibiotics, sensitive to 4 antibiotics, and intermediate to 2 antibiotics; *E. coli sp.* was found to be in resistance to 12 antibiotics, sensitive to 5 antibiotics. *Klebsiella sp.* was found to be resistance to 11 antibiotics, sensitive to 4 antibiotics, and intermediate to 2 antibiotics. **Table 1, Figure 2**

Salmonella sp. from Suswa River was found to be in resistance to 13 antibiotics, sensitive to 1 antibiotic, and intermediate to 3 antibiotics. *Pseudomonas sp.* was found to be in resistance to 9 antibiotics, sensitive to 6 antibiotics, and intermediate to 1 antibiotic. *E. coli sp.*was found to be in resistance to12 antibiotics, sensitive to 4 antibiotics, and intermediate to 1 antibiotic. *Klebsiella sp.*was found to be in resistance to 6 antibiotics, sensitive to 7 antibiotics, and intermediate to 4 antibiotics. **Table 1, Figure 2.**

DISCUSSION

In today's medical practice, enhanced counteraction of germs to widely administered antibiotics has become a big concern. Antibiotic resistance is a "serious hazard to public health," according to the WHO, because of the damage it presents to global public health. AR is on the rise, which means that normal illnesses will become untreatable, procedures will become risky, and healthcare costs will soar [6, 7]. In addition to serving as one of the most vital habitations for bacteria on earth, water is also a major medium of diffusion for bacteria in nature, as well as a significant source of AR. Water acts as a reservoir for resistant strains, an enhancer for genetic factors from earlier infected individuals and emitted as emissions into the atmosphere, and a bioreactor for microbial exchange of resistant genes in pathogenic and non-pathogenic bacteria as a microbial site [17, 18].

Since the golden period of penicillin manufacturing in 1941, antibiotics and antimicrobials have changed the way infectious diseases are treated. However, the rapid and rising emergence of drug-resistant has reached a critical stage. Even when we create new antibiotics, bacteria have evolved resistance against them. This issue is not limited to clinical isolates; it also affects natural isolates from aquatic environments, particularly those that are contaminated. Concerns about the possible dangers of such resistant strains to human health and wildlife have become deadly. Fears about the probable risks of such resistant strains to human and animal wellbeing have escalated to the point of becoming lethal. AR is a result of extensive use and misuse of antibiotics in medical, veterinary medicine, farming, and fisheries, according to the philosophy. Every week, roughly one million pounds of antibiotics are dumped into the environment. This has been discovered in a variety of water sources across the world. Threats to our ability to cure common infectious illnesses, increasing mortality, and treatment costs are all things to be concerned [13, 19, 20].

Acitenobacter, *Pseudomonas*, and numerous *Enterobacteriaceae*, including *Escherichia coli*, *Klebsiella*, and *Serratia*, have been added to the WHO list of twelve antibacterial drugs, water microbes that they feel pose a hazard to public health. According to some research, water-treatment techniques may boost the number of microorganism's resistant to antibiotics. However, little is known about the common frequency levels of ARBs and the genes that confer antibiotic resistance in water from the tap, despite the fact that this changes significantly across time and distance within a water system, as well as the most common kinds of ARBs.

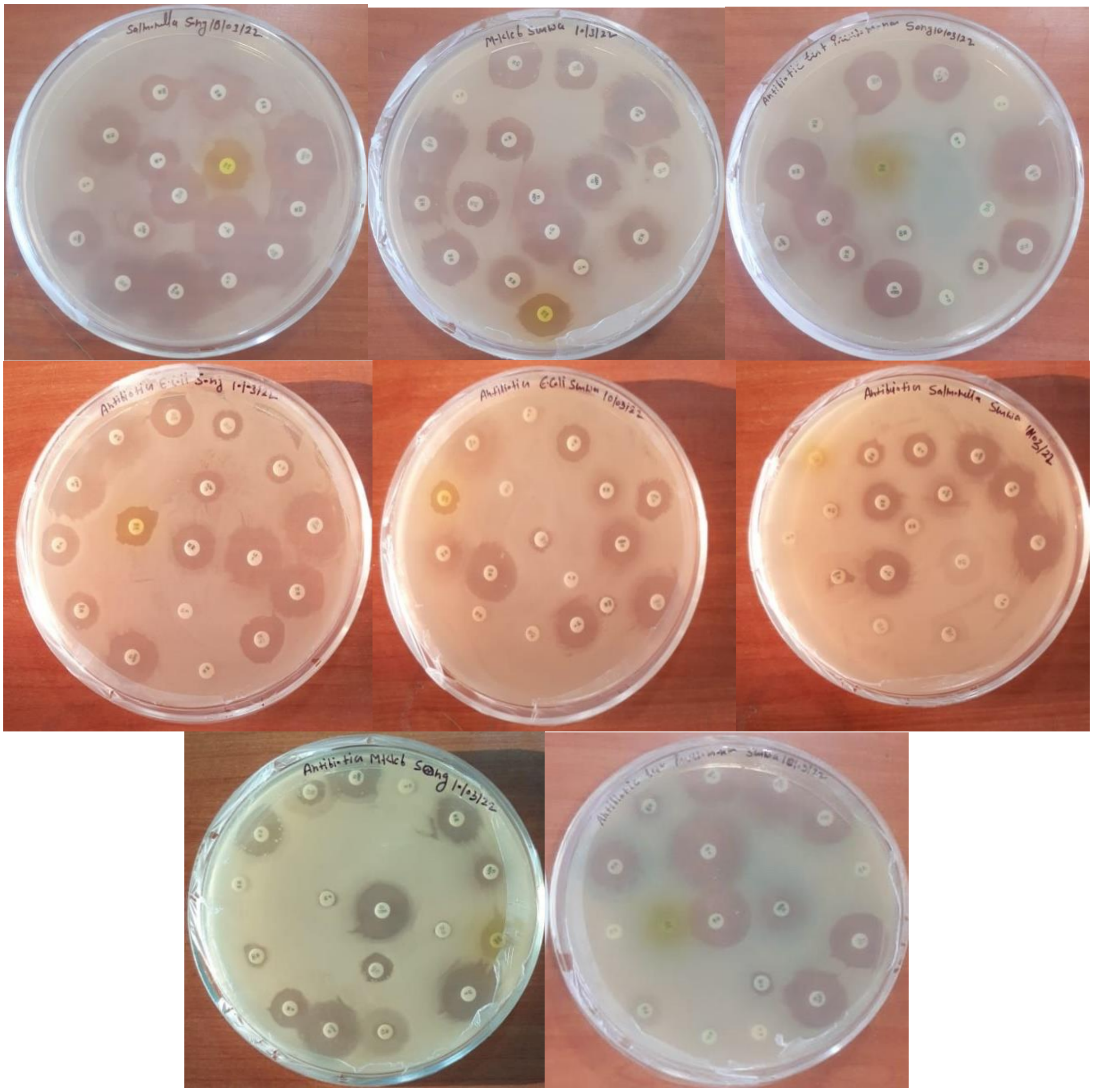
In Dehradun, Uttarakhand, this study consisted of an investigation into environmental bacteria found in surface water. Numerous reports have documented the presence of pathogenic microorganisms in surface water and the diseases associated with them. Many studies that were done in Dehradun show that diarrhea, gastroenteritis, malaria, typhoid, and cholera disease spread by the effect of water pollution [21]. Water pollution may occur due to atmospheric dissolved gases, and the decomposition of animals, vegetables, and materials. The investigation found *Salmonella*, *Pseudomonas*, *E. coli*, and *Klebsiella* in the river, which are all extremely detrimental to humans, animals, and aquatic life. Moreover, the presence of pathogenic bacteria in these species might be a serious health concern for consumers, especially those who are immunocompromised. A comparison of potable water and raw water might indicate that the treated water contains fewer microbes due to the treatment process. When water is supplied, it is important to consider the health risks posed by the bacteria in treated water [6].

The isolates were also characterized using their antibiotic resistance profiles, which was another goal of this investigation. The findings revealed that Azithromycin, Amikacin, Cefaclor, Cefepime, Cefoxitin, Doxycycline Hydrochloride, Erythromycin, Gentamycin, Kanamycin, Levofloxacin, Mecillinam, Mezolocilline, Penicillin G, Vancomycin, Tetracycline, Ticarcillin, and Nitrofurantoin were all resistant to a large proportion of the environmental isolates. Resistant may be caused by heavy pollutants such as those in sewage effluent, surface runoff, agricultural operations, wildlife, industrial pollutants, and other factors. The findings highlight the need for more research on the frequency of resistance genes in environmental isolates and the spread of resistant genes among pathogens. This would offer details on the health dangers of drinking polluted water. Heavy pollutants from sewage effluent, surface runoff, agricultural operations, wildlife, industrial pollutants, and other factors might all contribute to resistance. Overall, the findings emphasize the importance of further research into the frequency of ARGs in environmental isolates and the spread of resistant genes across pathogens. This would offer details on the health dangers of drinking polluted water [22-23].

Table 1: Antibiotics sensitivity of bacterial isolates from song and Suswa river

#	Isolates → Antibiotics ↓	**Song River**					**Suswa River**		
		Salmonella Sp.	***Pseudomonas Sp.***	***E.coli***	***Klebsiella Sp.***	***Salmonella Sp.***	***Pseudomonas Sp.***	***E.coli***	***Klebsiella Sp.***
1	**Azithromycin**	R	R	R	R	R	R	S	I
2	**Amikacin**	R	S	R	S	R	S	R	S
3	**Cefaclor**	I	R	R	R	R	R	R	R
4	**Cefepime**	S	I	S	S	I	R	R	S
5	**Cefoxitin**	I	….	S	R	R	….	S	R
6	**Doxycycline Hydrochloride**	R	R	R	R	R	S	R	S
7	**Erythromycin**	R	R	R	R	R	R	R	R
8	**Gentamycin**	S	S	S	S	R	S	I	S
9	**Kanamycin**	R	R	R	R	R	R	R	I
10	**Levofloxacin**	S	S	S	I	I	S	S	S
11	**Mecillinam**	S	S	S	S	S	S	S	S
12	**Mezolocilline**	S	I	R	R	R	R	R	R
13	**Penicillin g**	R	R	R	R	R	R	R	R
14	**Tetracycline**	S	R	R	I	I	S	R	R
15	**Vancomycin**	R	R	R	R	R	R	R	I
16	**Ticarcilline**	S	R	R	R	R	I	R	S
17	**Nitrofurantione**	S	R	R	R	R	R	R	I

Figure 2: Song and Suswa River Antibiotics sensitivity plates of bacterial isolates



CONCLUION

Antibiotic-resistant bacteria can potentially be transferred from one to another in a society. This is gradually getting frequent these days. Handwashing before and after cooking, going to the bathroom, and changing diapers are all strategies to limit the spread of germs, especially ARBs. When sneezing, or coughing cover your nose and mouth. Blowing or wiping your nose with tissues is recommended, and tissues should be disposed of carefully. If you're sick and can't handle the typical demands of your day, remain at home. If your child is sick, don't send them to day-care, preschool, or class. If antibiotics are given, finish the course – don't quit since you're getting better. Return to the doctor if you continue to feel sick. Unless your health expert advises otherwise, avoid using items that claim to include antibiotics, are antibacterial, or antimicrobial. Antibiotics may be used more wisely if clinical guidelines, direct education, and frequent antibiogram reporting are implemented. Antibiotic resistance continues to increase, posing a huge people health risk that must be addressed immediately. Antibiotic resistance cannot be prevented or, in some cases, reversed, but we can certainly slow its progression.

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