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Isolation And Identification Of Bacterial Strains From Musi River Hyderabad And Study Of Drug Resistance

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Abstract: Incomplete metabolism in humans has resulted in release of large amount of pharmaceutical drugs into municipal water, at the same time liberation of untreated waste of pharmaceutical industries in rivers extensively opens a way for various pathogenic microorganisms to attain resistance towards the action of antibiotics. It is a fact that all round the globe water pollution is a leading cause in developing drug resistance in numerous pathogens. Keeping in view of this, an attempt has been made to analyze the water samples of Musi river (A river located in and around the Hyderabad, Telangana) as several sewage pipelines are being continuously emptied in this river from many years. Five bacterial pathogens- E.coli, Enterobacter, Pseudomonas , Shigella and Salmonella were isolated and cultured from the water samples and tested for their response towards broad spectrum antibiotics.

Enterobacter, Pseudomonas, & Salmonella were found sensitive generally to antibiotics. Whereas E.coli and Shigella were found resistant to Ceftriazone, Cefuroxime, and Streptomycin.

INTRODUCTION:

Musi river, a tributary of Krishna river in the Deccan plateau flows through Telangana state. Hyderabad city of Telangana stands on the bank of musi river. Deposition of large amount of waste regularly into Musi water could result into various environmental hazards of which the most important is Anti Microbial Resistance. Discharge of drug and particular chemicals into the environment is also one of the most worrying health threats today[1]. At the reports launch, U.N environment execution direction Erick solheim drew particular attention to pharmaceutical faculty in Hyderabad, where testing of discharged water revealed that the concentration in the treated waste water of ciprofloxacin, a vital broad spectrum antibiotic, was strong enough to treat 44,000 people. The Guardian 05/12/2017, over use of antibiotics in farming is a major new threat to human health says U.NA 2010 study by Swedish researchers showed that seven out of eight travelers to India retuned to Sweden carrying drug-resistant bacteria in their gut. The city generate a waste water flow of approximately 850 million lit/ day, that leads to discharge of large amounts of treated sewage into musi river[3]. Due to these consequences these emerge a need to search could strongly for the better alternative pathogens and decrease the use of antibiotics. The WHO has adopted a major policy change in accepting the most developing nations would have to make use of more traditional medical practices for primary health care.

Materials and methods:

1. **Sample collection :**

Water sample were collected in duplicate in sterile air tight glass container from Musi River, Hyderabad .It was transport to Mumtaz college laboratory in a cooler box containing ice packs within 30 minutes and were processed microbiologically for the isolation of bacteria.

2. **Processing of sample:**

The sample was processed on the nutrient agar plates for isolation of bacteria using serial dilution method. The morphologically distinct bacterial culture obtain by streaking technique and further colony morphology study, Motility, gram-staining and biochemical tests including catalase, oxidase and urease test done for identification, according to Mackie & McCarty 14th edition.

Identification of bacterial strains : After incubation, based on morphology and Grams staining results ,colonies were picked and plated on selective medias like EMB,SS agar, and Cetrimide agar.

Biochemical tests : IMVIC tests were performed for biochemical analysis.

Antimicrobial Resistance assay :. Muller Hinton agar medium was used for assay .Kirby Bauer method was used to determine the antimicrobial effects of various antibiotics.

Anti microbial agents : Ampicillin Sulbactum 10 µg, Ceftazidime 30 µg, Ceftriaxone 30 µg, Cefuroxime 30 µg, Ofloxacin 05 µg, CefoperazoneSulbactum 75/10 µg, Gentamicin 10 µg, Amikacin 30µg, Streptomycin 10 µg, Nitilmicin 30 µg .

3. Antibiotic sensitivity testing:

The Muller-Hinton medium was prepared as it is universally recommended culture medium for antimicrobial sensitivity testing. The Kirby-Bauer method was to determine the susceptibility of bacteria. The Bacterial inoculums were prepared and compare turbidity with 0.1ml McFarland standard. Sterile solidified Muller Hinton agar plates were inoculated and disks impregnated with specific amount of antibiotic were placed on the surface of agar and incubated at ambient temperature for 18-24 hours. The diameters for zone of inhibition were measured.

Results and Discussion :

S.No	Gram Staining	Motility	Colony Morphology	Indole test	Methyl red	Voges prosker	Citrate utilization	catalase	oxidase	urease	Identified organism
1	Gram negative	peritrichous	Greyish white smooth colony	+	+	-	-	+	-	-	E.coli
2	Gram negative	M otile	Mucoid colony	-	-	+	+	+	-	-	Enterobacter
3	Gram negative	Unipolar	Lagre opaque irregular colony	-	-	-	+	+	+	-	Pseudomonas
4	Gram negative	Non motile	tanslucent	-	+	-	-	+	+	-	Shigella

5	Gram negative	motile	smooth	-	+	-	+	+	-	-	Salmonella

Bacterial strains	Ampicillin + Sulbactum	Ceftazidine	Ceftriaxone	Cefuroxime	Oflaxacin	Cefoperazone	Gentamicin	Amikacin	Streptomycin	Netilimicin
E.Coli	15	20	**18**	**13**	18	23	17	15	**10**	18
Enterobacter	20	28	24	14	22	22	19	18	13	20
Pseudomonas	18	22	21	15	20	24	18	17	12	21
Shigella	17	30	**14**	**15**	21	30	18	16	**10**	17
Salmonella	14	27	40	14	25	21	22	18	12	20

Five bacterial strains were isolated from Musi river water sample. The results for microscopic examination, colony morphology, motility , biochemical analysis (Table 1) confirms the presence of pathogenic bacteria. Antibiotic sensitivity assay (Table 2) reveals that most of the bacteria are sensitive towards antibiotic effect, but few were found to be resistant. E.coli and Shigella resistant to Ceftriazone, Cefuroxime, and Streptomycin. This clearly indicates that this river water is highly contaminated and an open window for generation and propogation of drug resistant superbugs. Improper management of waste disposal has thus created a highly polluted environment to all forms of life in general and to human in particular. Apart from this work, many reports indicate the presence of drug resistant bacteria in drinking water and lake water as well. According to NITI Aayog medical deaths due to Antimicrobial resistance could be higher than those caused by cancer by 2050. Therefore there is a need to raise up the awareness level publically and to develop various strategies to cut the roots of water contamination atleast in the close vicinities of human colonies.

Conclusion :

Dumping of untreated municipal waste and industrial effluent impose a high risk for emergence of drug resistance genes among several pathogens. Spread of resistant strains may create serious medicinal problems in future. Applications of strict guidelines could help in eradication of Anti Microbial Resistance.

Reference:

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