

Isolation and Identification of Bacteria from Sewage Water Samples and Antibiotic Sensitivity Tests of Selected Isolates

Abstract

The bacteriological status of sewage water of Jessore Medical College Hospital (surgery ward), and Shaheed Mashiur Rahman Hall of Jessore Science & Technology University was analyzed in this study. Varying with the types of sewage water, kinds and load of microorganisms may differ significantly. Hospital sewage gathers many types of microorganisms including severe pathogenic and non pathogenic origins; and the sewage water is supposed to have clinical discharges with diversified drugs and other residues. On the other hand residential hall sewage contains excreta from persons of different age and habits with their distinct physical history. The water samples were found to contain Gram negative bacteria like *Escherichia coli*, and *Enterobacter spp.* The persistent of antibiotic drugs in sewage water and some other daily leftovers can interfere with the inhabiting bacterial physiology and generate new characteristics like multi drug resistance, heavy metal tolerance and so on. This assumption was experimentally examined by performing antibiogram with four different antibiotics and result showed their sensitivity of distinct level to the two randomly selected and identified bacteria. To determine if the antibiotic resistance is of plasmid born or not, the bacterial samples has been kept into stock culture and will be taken under further experiment(s) soon.

Keywords: Sewage; Pathogen; Clinical discharges; Antibiotics; Antibiogram; Plant nutrients

Research Article

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Introduction

Sewage is the collection of untreated used water discharged from home, industry, hospital etc. Water bodies can be polluted by a wide variety of substances, including pathogenic microorganisms, putrescible organic wastes, plant nutrients, toxic chemicals, sediments, heat oil, and radioactive substances [1,2]. Among all these organic and inorganic materials, sewage is a common habitat of bacteria, which may have characteristics a little bit different from those bacteria residing other than sewage, specially the hospital sewage. The study by the Department of Health in various hospitals in Jakarta mention that the hospital waste water consists of domestic waste (86%), infectious waste (9.5%), sewage pathogens (1.5%), and 4% of hazardous waste [3].

In the developing countries, inadequate use of antibiotics and spread of antibiotic resistance is still in rise where health and environmental problems are always being overlooked. Hospital and other sewages are always being exposed with various types of hazardous and toxic chemicals and microorganisms inhabiting there are acquiring different abnormal characteristics like drug resistance, heat resistance, metal tolerance etc. These microorganisms with unusual threatening behavior is ultimately disseminating or entering into the natural environment and ultimately introducing a new level of pollution [4].

Residential hall sewage may also prone to contain many harmful microorganisms as the water bodies are catching wastes

of people from different life style. Previous research works with these types of water samples showed presence of multidrug resistant pathogens in dangerous level [5]. Taking all these research observations and environmental safety issues in concern this present study was designed to analyze the microbiological quality of two distinct types of sewage water samples of Jessore city; when determination of specific physiological characteristics of isolated bacteria was also a major focus.

Materials and Methods

Sample collection

Two water samples collected from Jessore Medical College Hospital (sewage opening of surgery ward), and from the sewage opening of Shaheed Mashiur Rahman Hall of Jessore University of Science & Technology. Samples were carried to the laboratory in insulated icebox. Certain amount of water was added to ready-to-use Pre-enrichment medium on the spot and carried to laboratory at room temperature. Samples, kept at 4°C, were analyzed without much delay to avoid any alteration.

Microbiological analysis

The two samples were diluted serially into distilled water for 1 to 10 folds using sterile apparatus. Following surface plating method microbial isolation was done. Nutrient agar (Oxoid, UK) media was used for the isolation of total aerobic bacteria and MacConkey Agar (Oxoid, UK) medium was used to isolate

coliform bacterial population. For further microbial identification confirmation selective agar media like Xylose Lysine Deoxycholate agar (Himedia, India), Thiosulfate-citrate-bile salts-sucrose agar media (Oxoid, UK), and Eosine Methylene Blue agar (Oxoid, UK) was taken into trial of this experiment.

Biochemical analysis

After isolation and identification through cultural methods and microscopic observation, two randomly selected colonies were further identified by several biochemical confirmation tests including IMViC test, catalase test, motility test, oxidase, H₂S test.

Antibiogram

Antibiotic sensitivity was performed with four different types of antibacterials following Kirby-Bauer disk diffusion method on Mueller Hinton agar (Oxoid, UK) media [6]. Here the choice of antibiotics was Streptomycine, Amoxyciline, Chloramphenicol, and Gentamycine. The zones diameter of inhibition were measured in mm sale and compared with National Committee for Clinical Laboratory Standards (NCCLS) guidelines [7].

Results

(Tables & Figures)

Table 1: Microbial analysis (Log CFU/ml) of two sewage samples.

Microorganisms	Sample Source	
	Hospital Sewage	Hall Sewage
Total aerobic bacteria	8.34	8.32
Coliform	5.3	5.11

Table 2: Physiological and morphological characteristics of two randomly selected colonies.

Names of Bacteria	Morphological/physiological Characteristics		
	Gram Staining	Shape	Motility
<i>E. coli</i>	-	Rod	+
<i>Enterobacter spp.</i>	-	Rod	+

Negative: -

Positive: +

Table 3: Characteristics of *E. coli* and *Enterobacter spp.* observed after carrying out the biochemical tests.

Names of Bacteria	Biochemical Tests							
	Indole	VP	Catalase	Citrate	H ₂ S	Urease	MR	Oxidase
<i>E. coli</i>	+	-	-	-	-	-	+	-
<i>Enterobacter spp.</i>	-	+	+	+	+	-	-	-

Positive: +

Negative: -

Table 4: Antibiotic sensitivity test result.

Antibiotics	Zone (mm diameter) of Inhibition	
	<i>E. coli</i>	<i>Enterobacter spp.</i>
Chloramphenicol (10 µg)	22	22
Gentamycine (10 µg)	14	9
Streptomycine (10 µg)	14	6
Amoxyciline (10 µg)	20	6

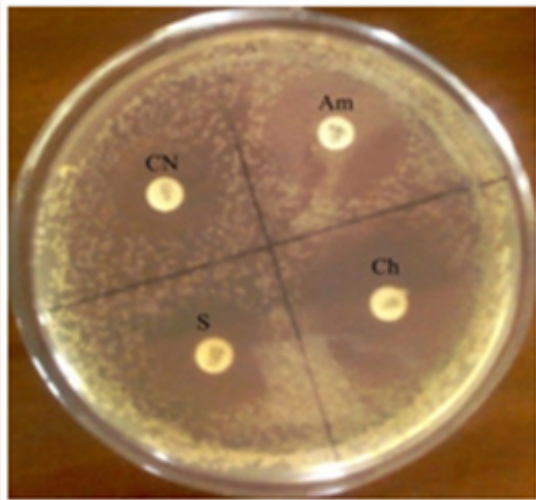


Figure 1: Antibiogram with *E. coli*.

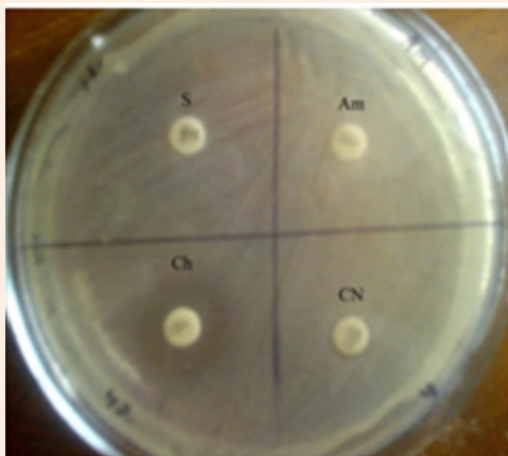


Figure 2: Antibiogram with *Enterobacter spp.*

Discussion

After microbial analysis of both water samples we see the heavy load of microorganisms in same pattern. During primary isolation, several types of colony morphology were observed and after subculture only two types of organism were isolated. Finding of the pink colony with black centre from the hospital sewage sample is a comparable matter of the two samples. Among the isolated organisms, the highest prevalence of enteric bacteria was found in the sewage sample of Jessore Medical College Hospital, surgery ward. One of the bacterium isolated from hospital sewage, *Enterobacter spp.* are responsible for urinary tract infections and hospital sepsis etc. Result showed that both of the samples contain high population of *Escherichia coli*, which cause diarrhea, *Shigella* like dysentery, hemorrhagic colitis, pyogenic infections, septicemia and also urinary tract infection. *E. coli* isolated from urinary tract infection sample showed different level of resistance

pattern against most of the commonly used antibiotics. The urinary tract is the most common site of *E. coli* infection, and more than 90% of all uncomplicated UTIs are caused by *E. coli* infection [7].

In this present experiment *E. coli* isolate was found to be sensitive against all the antimicrobial drugs under trial. But the *Enterobacter spp.* notoriously showed its resistant property against three antibacterials. Researchers had reported a high level of resistance among various *Enterobacter* strains from raw sewage and teaching hospitals, which is a close similarity with the study result being concerned. With the uncontrolled use of antibiotics and common practice and self medication there would be a selection pressure in favor of organisms possessing genes that code for resistance [8]. Inappropriate antibacterial treatment and overuse of antibiotics have contributed to the emergence of antibacterial-resistant bacteria.

Conclusion

For developing a sustainable environment the presence of resistant bacteria from water bodies should not be overlooked. Because when the carrier for these pathogens is any type open untreated water, it can run far enough to bring curse by polluting the agricultural system in addition with the surface water reservoirs [9,10]. Therefore proper waste water treatment system in association with appropriate sanitation steps must be implemented when people should also play role by following safe medical and clinical practices to eradicate antibiotic abuse.

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