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Isolation and identification of bacteria from antibiotic industry liquid waste outlet

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ABSTRACT

Antibiotic pollution due to poor or inadequate waste management practice by manufacturing industries, hospitals, poultries and veterinary may make environmental microbes resistant against the antibiotics. Antibiotic plant waste outlet connected with pond in Bangladesh may contain active antibiotics or ingredients which cause resistance to the dweller bacteria due to selective pressure. In order to address the hypothesis bacterial total counts, resistance counts and resistance pattern of bacteria of Gonoshasthay Kendray (GK) pond was analyzed. Ampicillin was used to count total resistant bacteria because this antibiotic is produced in the GK antibiotic plant. The isolates (Pseudomonas, Staphylococcus, Streptococcus, and Alkaligens) identified on the basis of gram staining, morphology and biochemical tests. Thirty individual colonies were isolated from the resistance count plate to study the resistance pattern. Amoxicillin, Tetracycline, Streptomycin, Penicillin-G, Ciprofloxacin, Cefalexin, Azithromycin, Gentamycin were used as indicator antibiotic. The rate of resistance among the isolates for Amoxacillin, Ampicillin, Ciprofloxacin, Streptomycin and Penicillin-G were 100%, Azithromycin 12.5%, Gentamycin 12.5%. Only Tetracycline and Cephalexin were 100% sensitive. The results evident that antibiotic plant pond content multidrug resistance bacteria which indicating the plant liquid waste carry active antibiotics or ingredients.

Key words: Antibiotic resistant, bacteria, waste outlet, Bangladesh

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INTRODUCTION

Antibiotic resistance is a specific type of drug resistance when a microorganism has the ability of withstanding the effects of antibiotics. Antibiotic resistance evolves via natural selection acting upon random mutation, but it can also be engineered by applying an evolutionary stress on a population. Once such a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals) by plasmid exchange. Antibiotic resistance can also be introduced artificially into a microorganism through transformation protocols. If the resistance gene is linked with the gene to be implanted, the antibiotic can be used to kill off organisms that lack the new gene. The widespread use of antibiotics is playing a significant role in the emergence of resistant bacteria. Use of antibiotics in animal and poultry feeds, agriculture field lead to the spread of resistant strains to human populations (Iversen et al., 2002). In human medicine the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics by doctors as well as patients. Household use of antibacterial in soaps and other

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products, although not clearly contributing to resistance, is also discouraged (as not being effective at infection control). Furthermore, unsound practices in the pharmaceutical manufacturing industry can contribute towards the likelihood of creating antibiotic resistant strains (Sharpe 2003; Pathak et al., 1993). Industrialization and man's activities have partially or totally turned our environment to dumping sites for waste materials (Grabow and Prozesky, 1973. However, antibiotics exert a selection pressure on pathogenic bacteria as well as on bacteria belonging to the normal endogenous flora leading to resistance against antibacterials. In Bangladesh industrial waste outlet are connected with nearby water bodies in most cases. The present study was undertaken to investigate the bacteria load present in the water bodies connected with antibiotic manufacturing industries and evaluate the distribution of multiple drug resistance bacteria in the study area.

MATERIALS AND METHOD

Media

Nutrient broth, Motility Indole Urea (MIU) medium (HiMedia, India), Nutrient Agar (NA) medium (HiMedia, India), NaCl broth, Eosin Methylene Blue (EMB) Agar (HiMedia, India), Blood Agar (HiMedia, India), MacConkey's (MC) Agar medium (HiMedia, India), Muller-Hinton agar (HiMedia, India), (Triple Sugar Iron (TSI) Agar slant (HiMedia, India) were purchased as powdered form and prepared the media according to the manufactured instruction.

Bacterial plate count

For the count of the total bacteria and antibiotic specific resistant bacteria plate count technique was used. During inoculation, the mixture of microorganism was spread over the surface of an agar medium (with or without antibiotic) with a spreader and incubated at 37ºC for 24 hours. In absence of antibiotic all resistant bacteria was grown in the media. Whereas in presence of specific antibiotic only resistant bacteria was grown in respective plate. Colonies were counted as cfu/ml (unit) and colonies were isolated depending on different morphology.

Isolation and identification of bacteria

Samples were collected from Gonoshasthya antibiotic pond (GAP-2), Savar, Dhaka, Bangladesh. Samples were transported to the laboratory in cool conditions and processed within two hours of collection. A 100 ml aliquot of waste water of serial ten-fold dilutions for each sample was filtered through a 0.22 µm membrane filter paper (Millipore, Billerica, MA) which was then placed on plate count agar and incubated aerobically at 37°C for 24–48 hours. After incubation, based on colony morphology representative colonies were picked and sub-cultured on different selective and differential media such as blood agar, EMB, MSA, MacConkey agar. After obtaining pure colonies and recording important features such as haemolysis on blood agar the isolated organisms were identified biochemically in a systematic way following standard methods (Vandepitte et al., 1996).

Antibiotic sensitivity assay

Following isolation and identification of bacteria from each sample collected, the standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the isolates (Bauer et al., 1966). Bacterial inoculum was prepared by suspending the freshly grown bacteria in 4–5 ml sterile nutrient broth. The antimicrobial susceptibility testing was performed using Mueller-Hinton medium against azithromycin (15µg), amoxicillin (30 µg), tetracycline (30 µg), cephalexin (30 µg), penicillin-G (10 units), ciprofloxacin (5 µg), streptomycin (10 µg), Ampicillin (10 µg) and gentamycin (10 µg).

RESULTS AND DISCUSSION

The study was carried out to determine initial bacterial load between total count and resistant count (table 1). The initial total load of the non resistant bacteria was 395 cfu/100 µl (10^{-3} fold dilution) whereas the ampicillin resistant bacterial

load was 180 cfu/100 µl at the sample dilution (table 1). A total number of 30 bacterial strains were isolated from the waste water from antibiotic industry liquid waste outlet. Based on the colony morphology, staining and biochemical tests *Staphylococcus, Pseudomonas, Streptococcus, and* Alcaligens were identified (table 2). The identification of the isolates was confirmed by observing the colony characteristics of the organisms in the selected media (table 3).

Table 1
Initial bacterial load between total and resistant count

Dilution Factors	Bacterial load (cfu/100 µl)				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
No. of colonies for total count	TNTC	TNTC	395	170	75
No. of colonies for resistant count	TNTC	TNTC	180	18	0

TNTC= Too numerous to count

The result of the sensitivity test showed that all isolates were resistant to amoxicillin, ampicillin, ciprofloxacin, penicillin-G and streptomycin indicating the multidrug resistant of the tested organisms. Whereas all tested isolates were sensitive to cephalexin and tetracycline, Azithromycin was found active against *Pseudomonas, Staphylococcus* and *Streptococcus*. Gentamycin was only found to be resistant against *Staphylococcus*. However it is observed that all the strains were sensitive to the broad spectrum antibiotic. The finding of the study suggests that in waste water source like that of our study sites the gram-positive bacterial strains are predominant and these strains are most likely to be resistant to antimicrobial drugs relevant to the product of the industry. Since the isolated bacterial strains do have the survival ability in that adverse environment, there remain lots of chances to spread them outside by various means that may threat the human and animal health.

Table 2
Biochemical test for the isolated organisms

No of Samples	10	7	7	6
Oxidase	+	-	-	+
Catalase	+	+	-	+
MR	+	+	+	+
VP	-	-	-	-
TSI	Red/Red	Red/Red	Red/Red	Red/Red
Citrate	±	-	-	-
MIO	±	-	-	-
Urea	-	-	-	-
Indole	±	±	-	-
Nacl	+	+	+	+
NO_3 reduction	+	+	+	+
Mannitol Fermentation	-	-	-	-
Interpretation	*Pseudomonas*	*Staphylococcus*	*Streptococcus*	Alkaligens

Table 3
Cultural characteristic on Blood agar, EMB, MSA, MacConkey agar

Name of organism	Blood Agar	EMB	MSA	MacConkeyss
Pseudomonas	γ -haemolytic	Growth and no sheen	No growth	Growth
Staphylococcus	γ -haemolytic	Growth and no sheen	Growth	No growth
Streptococcus	γ -haemolytic	Growth and no sheen	No growth	No growth
Alkaligens	α -haemolytic	Growth and no sheen	No growth	Growth

Table 4
Sensitivity test of the isolated bacteria

Name of antibiotics	*Pseudomonas*	*Staphylococcus*	*Streptococcus*	Alcaligens
Amoxicillin	R	R	R	R
Ampicillin	R	R	R	R
Azithromycin	S	S	S	R
Cephalexin	S	S	S	S
Ciprofloxacin	R	R	R	R
Gentamycin	S	R	S	S
Penicillin-G	R	R	R	R
Streptomycin	R	R	R	R
Tetracycline	S	S	S	S

S=Sensitive R=Resistant MS=Moderately Sensitive (Intermediate)

The finding also indicates the necessity of controlling the pollution and spread of resistant microbial pathogens for pretreatment before discharging the waste in outside environment. The present study stated the current status of the contaminating bacteria and their load in the study area. However, further studies are need to better understanding their transmission potential in aquatic environment and control strategy of these pathogenic antibiotic resistant organisms.

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