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Research Article

EMERGENCE OF DRUG RESISTANCE IN BACTERIAL ISOLATES FROM HOSPITAL WASTEWATER: A POTENTIAL HEALTH HAZARD

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ABSTRACT

Introduction: Release of sewage effluent containing bacteria having elevated antibiotic resistance levels poses threat to environment. The antimicrobial selective pressure through indiscriminate use of antibiotics has played a significant role in enriching the MDRstrains in the hospital practice.

Materials and methods: Study was conducted at the department of agricultural microbiology, Aligarh Muslim University, Aligarh, UP (India). Wastewater samples were collected from a drain of J. N. Medical College, Aligarh. Isolation of the enterobacteria was done. All isolates were tested for their sensitivity to antimicrobial agents by means of disc diffusion method [1].

Results: In the present study, a total of 50 enterobacteria were isolated from hospital wastewater. All the isolates were tentatively identified by morphological, cultural and biochemical characteristics. All the isolates were further tested for their antibiotic susceptibility (Table 2). A high level of resistance against penicillin-G (90%) was observed in strains isolated from hospital wastewater. Resistance against cloxacillin, novobiocin, nalidixic acid and polymixin Bwas 66%, 56%, 28%, 2% respectively (Table 3). All the isolates were sensitive to erythromycin, doxycycline, gentamycin, kanamycin and chloramphenicol. Majority of isolates from wastewater were found to be resistant to multiple drug/antibiotics. Among the fifteen antibiotics/drugs tested, sevendifferent resistance patterns were observed in enterobacteria isolated from hospital wastewater.

Conclusion: Results of this study may be beneficial to design environment-friendly techniques for the removal of antibiotics from wastewater to overcome the problem of antibiotic resistance development in the aquatic environment.

KEYWORDS:Enterobacteria, Hospital, Wastewater, Resistance

INTRODUCTION

The heavy consumption of water in hospitals gives significant volumes of wastewater loaded with microorganisms, heavy metals, toxic chemicals and radioactive elements, the majority of which are pathogenic. About 85% of hospital waste is said to be non-hazardous, 10% infective/hazardous and 5% not infective in the United States of America[2].Meanwhile about 15% of hospital waste is regarded infective in most developed countries.

Several studies have evaluated the microbiological content of hospital and household waste quantitatively and qualitatively and found that general hospital waste contains microorganisms with pathogenic potentials for humans comparable to household waste [3]. *Bacillus* spp., *Staphylococcus* spp. and *Streptococcus* species are bacteria frequently encountered in hospital wastewater, varying between 5 and 10%.*Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* have also been reported along with varying numbers of other nosocomial pathogens such as *Klebsiella, Proteus* and *Enterobacter* species [4]. Most of these microorganisms have also been reported to be resistant to the commonly used antibiotics and as such have led to the outbreak of several diseases [5].

Hospital effluent could contain multidrug resistant (MDR) enterobacteria and enteric pathogens which could pose a great problem for communities. The antimicrobial selective pressure through indiscriminate use of antibiotics has played a significant role in enriching the MDR strains in the hospital practice. Therefore we have carried out antibiogram study in enterobacteria isolated from hospital waste water.

MATERIALS AND METHODS

Wastewater samples were collected from a drain of J. N. Medical College, Aligarh. Study was conducted at department of agricultural microbiology, Aligarh Muslim University, Aligarh, UP (India).Samples were collected in sterilize glass bottle container and transported to the laboratory immediately for initial processing, so that elapsed time between the sample collection and initial processing did not exceed 3 hours.
Serial dilutions of the sample were made in sterile normal saline solution. 0.1 ml from each dilution (10^{-3} to 10^{-7}) was spread on the media plates. Isolation of the Enterobacteria was done using selective and differential media such as macconkey agar, nutrient agar, eosin- methylene blue agar and *salmonella-shigella* agar. The plates were incubated at 37°Cfor 24-48 hours. After incubation, the suspected colonies were picked up, subcultured, and were then finally identified on the basis of morphological, cultural and biochemical characteristics. Isolates identified as members of the family enterobacteriaceae were selected for study.

All isolates were tested for their sensitivity to antimicrobial agents by means of disc diffusion method [1]. Following antibiotics (Hi-media) were used: Amoxycillin (Am 30µg), Cloxacillin (Cx 30µg), Chloramphenicol (C 25µg), Cotrimoxazole (Co 25µg), Doxycycline (Do 30µg), Erythromycin (E 15µg), Gentamicin (G 30µg), Kanamycin (K 30µg), Methicillin (M 5µg), Novobiocin (Nv 30µg), Nalidixic acid (Na 30µg), Penicillin-G (P 10 units), Polymyxin-B (Pb 100 units), Rifampicin (R 30 µg), Tetracycline (T 30µg)

0.1 ml inoculam of the test isolates was spread on nutrient agar plates and were allowed to adsorb at room temperatureat least for 20 min. The antibiotic discs were placed and the plates were then incubated for 24 h at 37°C. Plates were recorded for resistance and sensitivity by comparing zone of inhibition with the standard chart.

RESULTS

In the present study, a total of 50 enterobacteria were isolated from hospital wastewater. All the isolates were tentatively identified by morphological, cultural and biochemical characteristics(Table 1).Out of 50 isolates, 46 were positive for indole production and 10 gave positive result towards voges-proskauer test. Citrate utilisation test was positive in most of the isolates. 40 isolates did not ferment mannitol. Out of 50 isolates, 36 were able to ferment glucose and fructose with the production of acid and gas within 48 h at 37°C.

All the isolates were further tested for their antibiotic susceptibility (Table 2). A high level of resistance against penicillin-G (90%) was observed in strains isolated from hospital wastewater. Resistance against cloxacillin, novobiocin, nalidixic acid and polymixin B was 66%, 56%, 28%, 2% respectively (Table 2). All the isolates were sensitive to erythromycin, doxycycline, gentamycin, kanamycin and chloramphenicol. Majority of isolates from wastewater were found to have resistance to multiple drug/antibiotics. Among the fifteen antibiotics/drugs tested, seven different resistance patterns were observed in *enterobacteria* isolated from hospital wastewater (Table 3). The number of antibiotics against which resistance was observed ranges from one to seven

.

Table 1:*Biochemical characterization of bacteria isolated from hospital wastewater*

S.NO.	TESTS	POSITIVE (+)	NEGATIVE (–)
1.	INDOLE PRODUCTION	46	4
2.	METHYL RED TEST	47	3
3.	VOGES-PROSKAUER TEST	10	40
4.	CITRATE UTILIZATION	32	18
5.	MANNITOL FERMENTATION	10	40
6.	OXIDASE TEST	5	46
7.	STARCH AGAR	17	33
8.	GLUCOSE FERMENTATION	ACID PRODUCTION 40	10
		GAS PRODUCTION 36	14
9.	FRUCTOSE FERMENTATION	ACID PRODUCTION 41	9
		GAS PRODUCTION 42	8
10.	SUCROSE FERMENTATION	ACID PRODUCTION 38	12
		GAS PRODUCTION 40	10
11	LACTOSE FERMENTATION	ACID PRODUCTION 39	11
		GAS PRODUCTION 40	10

Table 2:*Percent of Antibiotic Resistance Among Bacteria Isolated From Wastewater*

S.NO	ANTIBIOTICS	CONC. µg/DISC	NO OF RESISTANT ISOLATES	(%)
1.	AMOXYCILLIN	30	33	66%
2.	CHLORAMPHENICOL	25	1	2%
3.	COTRIMOXAZOLE	25	21	42%
4.	CLOXACILLIN	30	33	66%
5.	DOXYCYCLIN	30	1	2%
6.	ERYTHROMYCIN	15	3	6%
7.	GENTAMYCIN	30	0	0%
8.	KANAMYCIN	3	1	2%
9..	METHICILLIN	30	38	76%
10.	NALIDIXIC ACID	30	14	28%
11.	NOVOBIOCIN	30	28	56%
12.	PENICILLIN –G	10 UNIT	45	90%
13.	POLYMYXIN–B	100 UNIT	1	2%
14.	RIFAMPICIN	30	0	0%
15.	TETRACYCLINE	30	3	6%

Table 3:*Antibiotic resistance pattern of 50 isolates isolated from hospital wastewater*

NO. OF ANTIBIOTICS	NO OF RESISTANT ISOLATES(%)	RESISTANCE PATTERN
1	1 (2) 1 (2)	P CX
2	6(12)	NV,P
3	1(2) 1(2) 2(4) 1(2)	K,NA,NV CX,NA,P CX,NV,P NV,M,P
4	2(4) 1(2) 1(2) 1(2) 1(2)	CX,M,P,AX NV,M,P,AX CX,NV,P,AX CO,M,E,AX NA,NV,M,P
5	2(4) 6(12) 1(2) 6(12) 1(2)	NV,CO,M,P,AX CX,NA,M,,P,AX CX,T,NV,CO,M CX,CO,M,P,AX CX,CO,M,E,AX
6	1(2) 2(4) 1(2) 2(4) 3(6)	Cx,Nv,Co,M,P,Ax Cx,Co,M,P,Do-10,Ax Cx,T,Nv,Co,M,C Cx,Na,Nv,M,P,Ax Cx,T,Nv,M,P,Ax
7	3(6) 2(4) 1(2)	Cx,Na,Nv,Co,M,P,Ax Cx,Do-30,Nv,Co,M,P,Do-10 Cx,Nv,Co,M,P,Do-10,Ax

DISCUSSION

Antibiotics have been detected earlier in hospital effluent in high-income countries. Many studies have highlighted the fact that antibiotics also enter the aquatic environment through hospital effluent in low and middle-income countries. Unlike high-income countries, the situation become more problematic in low- and middle-income countries, where resource constraints might result in untreated wastewater[6].

In situations, where the wastewater is not subjected to any treatment, it is sometimes argued that antibiotics are diluted in the recipient waters in comparison with therapeutic concentrations and may not cause any harm. However, it is widely recognized that exposure to sub-therapeutic concentrations over long periods of time provides ideal conditions for the transfer of resistance genes[7].

In our study multiple antibiotic resistance was detected in isolates from hospital wastewater, and the frequency of resistance to penicillin-G, methicillin, amoxicillin, cloxacillin, novobiocin, cotrimoxazole, was very high in coliforms. Similar results have been reported in coliform isolates from sewage treatment plant[8]. Several investigation on antibiotic resistance enteric bacteria in wastewater treatment plants have led to contradictory conclusions. Some investigators reported that antibiotic resistant bacteria was removed less efficiently than antibiotic sensitive organisms. Othersconclude that elimination of total coliforms and resistant coliforms were similar [9]. As regards the presence of resistant bacteria in the wastewater treatment systems can be a way of combating this problem, as it has been shown that resistant bacteria are eliminated relatively efficiently in wastewater treatment plants.

According to previous studies, wastewater Enterococci comprise mainly the species Enterococcus faecalis, Enterococcus faecium, and Enterococcus hirae [10.11].These gram-positive cocci have slower growth and are less abundant than the enterobacteria.

It has been found that resistance among sewage bacteria during the treatment procedure can be either decreased or increased [8].Different authors have reported higher rates of resistance in hospital effluents [10,12].

It is generally agreed that the use of antimicrobials, whether for growth promotion purposes, or preventing or treating disease can select for resistant bacterial pathogens, and that these pathogens can be transmitted through food originating from sites where treated animals are processed.

In summary, results of this study may be beneficial to design environment-friendly techniques for the removal of antibiotics from wastewater to overcome the problem of antibiotic resistance development in the aquatic environment.The pretreatment of hospital effluents should be strongly encouraged by authorities and policy makers.

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