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A PROSPECTIVE STUDY OF WASTE WATER IN A TEACHING HOSPITAL OF SUB URBAN SETUP

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ABSTRACT

Background: The term "Wastewater" is referred to any water whose quality has been adversely changed by human or animal activities. It includes liquid waste discharged from domestic homes, hotels, hostels, agricultural, pharmaceutical, chemical, thermal power stations and other commercial sectors including hospitals. The importance of bacterial isolates from waste water environment as a reservoir of antibiotic resistance and a potential source of novel resistance genes to clinical pathogens is underestimated. This present study is framed to isolate and characterize public health important bacteria from waste water in hospital and non- hospital environments and evaluate the distribution of multi drug resistant (MDR) bacteria in this area.

Material and Methods: This was a cross-sectional study conducted from January to March 2015 at 500 bedded Rajiv Gandhi Institute of Medical Sciences (RIMS) Government General Hospital, Srikakulam, Andhra Pradesh, India. Forty samples from various outlets were aseptically collected, transported and processed within two hours using standard test procedures. The microorganisms were isolated using various media and assessed for their antimicrobial resistance pattern using 10 antimicrobial discs by Kirby-Bauer disk diffusion method.

Results: A total of 40 waste water samples were processed for the presence of drug resistant bacteria. From these 40 samples, 149 bacterial strains were recovered. Majority of bacteria 30 (75%) were from hospital environment. Most frequently isolated bacteria from both hospital environment and non-hospital environment was *Klebsiella* spp. 48 (32.21) followed by *Escherichia coli* 37 (24.84), *Staphylococcus aureus* 21 (14.08), Coagulase Negative *Staphylococci* (CoNS) 10 (6.71), *Pseudomonas aeruginosa* 14 (9.39), *Proteus* spp.,10 (6.71) *Enterococcus faecalis* 5 (3.35) in both environments. *Shigella* spp., 3 (2.01) and *Salmonella* spp., 1 (0.67) in the hospital environment, but not from non-hospital environment. Among 21 strains of *Staphylococcus aureus* isolated from both environments, 12 strains were Methicillin Resistant *Staphylococcus aureus* (MRSA) and 1 was vancomycin intermediate resistant *Staphylococcus aureus* (VRSA) and 3 were vancomycin resistant *Staphylococcus aureus* (VISA) with a trend towards superbugs.

Conclusions: In the present study, high percent of multi drug resistant bacteria (MDR) were observed in the hospital environment waste waters which may be transferred to other bacterial pathogens causing fatal infections in the community. It is therefore advised that all concerned in the healthcare sector to formulate the ways on proper liquid waste management practices in healthcare institutions to decrease the risk of disseminating pathogenic and multi drug resistant

microorganisms in the community.

KEYWORDS: Hospital environment, Microorganisms, MRSA, Multi Drug Resistant (MDR), Waste water

INTRODUCTION

The term "Wastewater" is referred to any water whose quality has been adversely changed by human or animal activities. It includes liquid waste discharged from domestic homes, hotels, hostels, agricultural, pharmaceutical, chemical, thermal power stations and other commercial sectors including hospitals.[1] In hospitals, water is consumed by various areas like hospital wards of all specialties, diagnostic laboratories, radiological units, laundries that wash linen that soaked with hospital infective material like blood and other body fluids, kitchens, administrative units and residential areas. In this process of usage, its physical, chemical, and biological quality is decreased and converted to wastewater.[2] Hospital wastewater can be hazardous to public health since it can contain many kinds of pollutants such as radioactive, chemical and pharmaceutical wastes , infective materials like blood, body fluids and also pathogenic microorganisms which may include superbugs.[3] injudicious and excessive use of antibiotics by human and animal feeds results in increase in antibiotic resistance and cause the spread of resistance genes in areas such as hospital waste water.[4] Higher numbers of resistant bacteria occur in polluted habitats like hospitals compared with unpolluted habitats like residential areas indicating that humans have contributed substantially to the increased proportion of resistant bacteria occurring in the environment.[5] Many non-metabolized drugs excreted from patients and residual chemicals enter into wastewater, which finally interacts with the microflora of hospital sewage. These microflora comprise saprophytic bacteria from the atmosphere, soil, medical devices, and water employed in the hospital practice; the pathogens are mainly released with patient excreta and other body fluids like blood and pus. These bacteria are exposed to a wide range of antimicrobials that could act as a selective pressure for the development of resistance. Due to heavy antibiotic use, hospital wastewater contains larger numbers of resistant organisms than does domestic wastewater.[6] In our semi urban area, there is no data concerning resistance profiles of microorganisms isolated from hospital or community waste waters. This present study is therefore an attempt to know the magnitude of drug resistant pathogens in hospital and non-hospital environments that may affect the public health.

MATERIALS AND METHODS

This was a cross-sectional study conducted from January to March 2015 at 500 bedded Rajivgandhi Institute of Medical Sciences (RIMS) Government General Hospital, Srikakulam, Andhra Pradesh, India. This hospital is a tertiary care teaching hospital that provides health care services to over 3 million people of Srikakulam district and neighbouring state of Odisha. Forty untreated wastewater samples were collected from RIMS General Hospital at different sites. The wastewater samples were collected at different places at different outlets of: Hospital waste water samples such as

Male and Female Surgical Wards (Sample1&2), Male and Female Medical Wards (Sample3&4), Male and Female Orthopaedic Wards (Sample5&6), Male and Female ENT Wards (Sample7&8), Male and Female Ophthalmic Wards (Sample 9&10), Male and Female Dermatology Wards (Sample11&12), Male and Female Psychiatry Wards (Sample13&14), Male and Female Pulmonology Wards (Sample15&16), Paediatric Ward (Sample 17), Obstetrics Ward (Sample 18), Gynaecology Ward (Sample 19), ICU (Sample 20), Dialysis Ward (Sample 21) Dental OP (Sample 22), Physiotherapy (Sample 23), Radio diagnosis Unit (Sample 24), Central Laboratory Pathology (Sample 25), Microbiology (Sample 26), Biochemistry (Sample 27), Casualty (Sample 28), Dept. of Microbiology (Sample29), Dept. of Pathology (Sample 30) and Non- hospital waste water samples such as Mess from Male Residents Hostel (Sample 31), Mess from

Female Residents Hostel (Sample 32), Male Residents Hostel (Sample 33), Female Residents Hostel (Sample 34), Male Senior Residents Hostel (Sample 35), Female Senior Residents Hostel (Sample 36), Teaching Staff Quarters (Sample 37), Non-Teaching Staff Quarters (Sample 38), Dean's Office (Sample 39) and Medical Superintendent's Office (Sample 40). Around 100 mL of waste water was collected from the outlet of each area in a small sterile bottle according to the method used by Nunez and Moretton.[7] The samples were transported within two hours in an ice box to the microbiology laboratory for analysis and stored in a refrigerator at 4°C until analysis. All the samples were analyzed on the day they were collected. Each sample was filtered using sterile Whatman No.1 filter paper to remove any debris and the filtrate was used for isolation of microorganisms. The samples were inoculated on nutrient agar, blood agar, MacConkey agar, Salmonella-Shigella agar, Pseudomonas agar, EMB agar obtained from Himedia, Mumbai and incubated aerobically at 37°C for 24–48 hours. The organisms were preliminarily identified based on colonial morphology, pigment production, haemolysis on blood agar, swarming and other characters. Further diagnosis was done using different biochemical reactions wherever necessary using standard testing methods.[8] The bacteria were isolated and identified in pure form from each sample collected, their antimicrobial sensitivity was tested by Kirby-Bauer disk diffusion method.[9] Bacterial inoculum was prepared by suspending the freshly grown bacteria in 5 ml of sterile nutrient broth and the turbidity was adjusted to that of a 0.5 McFarland standard. The antimicrobial susceptibility testing was performed using Mueller-Hinton agar medium obtained from Himedia, Mumbai, India using Amikacin(30 µg), Ampicillin(10 µg), Cefotaxime(30 µg), Ceftazidime(30 µg), Ceftriaxone(30 µg),Ciprofloxacin(5 µg), Gentamicin(10 µg), Lomefloxacin(10µg), Penicillin(10U), Sparfloxacin(5 µg) obtained from Himedia, Mumbai, India. The plates were incubated aerobically at 37°C for 18–24 hours. The zones of inhibition were measured and compared with Clinical and Laboratory Standards Institute (CLSI) guidelines[10] using *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC-25923 as controls. For all *Staphylococcus aureus* isolates, MRSA testing was done using cefoxitin (30 µg) disc[11] and VRSA and VISA were identified by Minimum Inhibitory Concentration (MIC) by agar dilution method.[12]

RESULTS

A total of 40 waste water samples were processed for the presence of bacterial pathogens. All the samples were positive to one or more bacterial strains. Among the total samples 149 bacterial strains were recovered. Among these samples 30 (75%) were from hospital environment and 10 (25%) were from non-hospital environment (Table -1). Most frequently isolated bacteria from both hospital environment and non-hospital environment was *Klebsiella* spp.,48 (32.21) followed by *Esch.coli* 37 (24.84), *Staphylococcus aureus* 21 (14.08), Coagulase Negative Staphylococci (CoNS) 10 (6.71), *Pseudomonas aeruginosa* 14 (9.39),*Proteus* spp.,10 (6.71) *Enterococcus faecalis* 5 (3.35) in both environments. *Shigella* spp., 3 (2.01) and Salmonella spp., 1 (0.67) in the hospital environment, but not from non-hospital environment. Gram negative bacteria predominated with 82.56% (Table 2). Antimicrobial resistance pattern was tested against 10 antimicrobials for all Gram negative and positive isolates. Majority of them were multidrug resistant. All strains in both groups were 100% resistant to ampicillin and penicillin. Majority were resistant to ciprofloxacin also. (Table-3 and 4).

DISCUSSIONS

The rate of isolation of bacterial pathogens in the hospital environment 30 (75%) was higher than the non-hospital environment10 (25%). Similar observations were reported by Feleke Moges et al.,[13] and Guardabassi et al.[14] The major factors were the injudicious use of antibiotics in human medicine, animal husbandry in the form of feeds as well as treatment and agriculture may disrupt the microbial balance in favour of resistant bacteria. The introduction of wastewater

in the environment brings about increased amount of organic matter and essential nutrients, which influence the changes in the microflora.[15] Aluyi *et al*.,[16] noted that high counts of bacterial load reflected the level of pollution in the environment. In our present study, the major isolate was *Klebsiella* spp., 48 (32.21) followed by Esch.coli 37 (24.84), *Staphylococcus aureus* 21 (14.08), Coagulase Negative *Staphylococci* (CoNS) 10 (6.71), *Pseudomonas aeruginosa* 14 (9.39), *Proteus* spp.,10 (6.71) *Enterococcus faecalis* 5 (3.35) in both environments. *Shigella* spp., 3 (2.01) and *Salmonella* spp., 1 (0.67) in the hospital environment, but not from non-hospital environment. Similar findings were observed by other researchers.[13,17] The predominant organisms in both studies were *Klebsiella* spp., followed by *Pseudomonas* spp., where as our study showed *Klebsiella* spp., followed by *Esch.coli* may be because of high content of faecal matter. Gram negative bacterial isolates were predominated than Gram positive isolates with 82.56% and 17.44% respectively. Similar findings were recorded by other workers.[7,13,17] Multi drug resistance was observed in both Gram negative and positive bacteria with 100% resistant to ampicillin and penicillin. Majority were resistant to ciprofloxacin also in both hospital and non hospital environments where as the resistance pattern was between 60 to 90% for other bacteria in Hospital environment except *Shigella* and *Salmonella* spp. because of their minimum number. In Non-hospital environment, the resistance pattern was 40 to 70%. Similar findings were observed by other workers[7,13,18] also. Among 21 strains of *Staphylococcus* aureus isolated from both environments, 12 strains were Methicillin Resistant *Staphylococococcus aureus* (MRSA) and 1 was vancomycin intermediate resistant *Staphylococcus aureus* (VRSA) and 3 were vancomycin resistant *Staphylococcus aureus* (VISA) with a trend towards superbugs. Such findings were also noted in the *Staphylococcus aureus* isolated elsewhere by other workers.[19,20,21,22] MRSA strains were isolated by other researchers in their study but not VISA and VRSA.[13,18,23,24] The resistance pattern observed for ciprofloxacin in our study was 100% in all isolates except in Hospital environment, *Shigella* spp.,(66.67%) and in Non-hospital environment, *Staphylococcus aureus* (66.67) and *Klebsiella* spp., (70%) where as Feleke et al[13] reported 12% and Islam et al[25] from Bangladesh, Chitnis et al.,[26] from India and Sharma et al.[27] from Nepal reported 100%.

CONCLUSIONS

Multi drug resistant (MDR) bacteria were found high in our present study in the hospital environmental waste water than non-hospital environment. The contamination of water by antimicrobials or other hazardous pollutants lead to the rise in the bacterial resistance due to selection pressure. In case the resistance is transferred to bacterial pathogens causing infections in the community, where most of the currently available antimicrobials will not work properly against the infectious microorganisms in the community. Proper liquid waste management strategy is needed to ensure public health and environmental safety. It is therefore advised that all concerned in the healthcare sector to formulate the ways on proper liquid waste management practices in healthcare institutions to decrease the risk of disseminating pathogenic and multiple drug resistant microorganisms in the community.

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APPENDICES

Table 1: Distribution of Samples from Hospital and Non-Hospital Environments

Samples	Number of Samples No. (%)	Total Bacterial Isolates Recovered No. (%)
Hospital Environment	30 (75)	123 (82.55)
Non-Hospital Environment	10 (25)	26 (17.55)
Total	**40 (100)**	**149 (100)**

Table 2: Number of Bacteria Isolated from Hospital and Non-Hospital Environments

Bacterial Isolates	Hospital Environment No. (%)	Non-hospital Environment No. (%)	Total No. (%)
Klebsiella spp.	38 (25.50)	10 (6.71)	48 (32.21)
Esch. coli	31 (20.81)	06 (4.03)	37 (24.84)
S. aureus	18 (12.07)	03 (2.01)	21 (14.08)
CoNS*	09 (6.04)	01 (0.67)	10 (6.71)
Pseudomonas aeruginosa	11 (7.38)	03 (2.01)	14 (9.39)
Proteus spp.	08 (5.37)	02 (1.34)	10 (6.71)
Enterococcus faecalis	04 (2.68)	01 (0.67)	05 (3.35)
Shigella spp.	03 (2.01)	-	03 (2.01)
Salmonella spp.	01 (0.67)	-	01 (0.67)
Total	**123 (82.56)**	**26 (17.44)**	**149(100)**

*Coagulase Negative *Staphylococci*

Table 3: Antimicrobial Resistance Pattern of Bacterial Isolates from Hospital Environment

S.No.	Antimicrobial	Disc Content	GRAM POSITIVE (n=31)			GRAM NEGATIVE (n=92)					
			S.aureus (n=18) No. (%)	Coagulase Neg.*Staph.* (n=9) No. (%)	*E.faecalis* (n=4) No. (%)	*Klebsiella* spp. (n=38) No. (%)	*Esch. coli* (n=31) No. (%)	*P.aeruginosa* (n=11) No. (%)	*Proteus* Spp. (n=8) No. (%)	*Shigella* spp. (n=3) No. (%)	*Salmonella* Spp. (n=1) No. (%)
1	Amikacin	30 µg	16(88.89)	6 (66.67)	2 (50.00)	32(84.21)	26 (83.87)	9 (81.82)	6 (75.00)	1 (33.33)	0 (0.00)
2	Ampicillin	10 µg	18 (100.00)	9 (100.00)	4 (100.00)	38 (100.00)	31 (100.00)	11(100.00)	8 (100.00)	3 (100.00)	1 (100.00)
3	Cefotaxime	30 µg	16 (88.89)	7 (77.78)	3 (75.00)	34 (89.47)	28 (90.32)	8 (72.73)	6 (75.00)	1 (33.33)	0 (0.00)
4	Ceftazidime	30 µg	17(94.44)	7 (77.78)	3 (75.00)	34 (89.47)	28 (90.32)	8 (72.73)	7 (87.5)	1 (33.33)	0 (0.00)
5	Ceftriaxone	30 µg	14(77.78)	6 (66.67)	1 (25.00)	31 (81.58)	25 (80.65)	7 ((63.64)	4 (50.00)	1 (33.33)	0 (0.00)
6	Ciprofloxacin	5 µg	18 (100.00)	9 (100.00)	4 (100.00)	38 (100.00)	31 (100.00)	11 (100.00)	8 (100.00)	2 (66.67)	1(100.00)
7	Gentamicin	10 µg	15 (83.33)	5 (55.56)	2 (50.00)	30 (78.95)	25 (80.65)	7 (63.64)	5 (62.5)	0 (0.00)	0 (0.00)
8	Lomefloxacin	10 µg	16 (88.89)	7 (77.78)	3 (75.00)	35 (92.11)	28 (90.32)	8 (72.73)	6(75.00)	2 (66.67)	1 (100.00)
9	Penicillin	10U	18 (100.00)	9 (100.00)	4 (100.00)	38 (100.00)	31 (100.00)	11(100.00)	8 (100.00)	3 (100.00)	1(100.00)
10	Sparfloxacin	5 µg	15 (83.33)	7 (77.78)	3 (75.00)	36 (94.74)	28 (90.32)	9 (81.82)	(7 87.5)	2 (66.67)	0 (0.00)

Table 4: Antimicrobial Resistance Pattern of Bacterial Isolates from Non-Hospital Environment

S. No.	Antimicrobial	Disc Content	GRAM POSITIVE (n=5)			GRAM NEGATIVE (n=21)			
			S.aureus (n=3) No. (%)	Coagulase Neg. *Staph.* (n=1) No. (%)	*E. faecalis* (n=1) No. (%)	*Klebsiella* spp. (n=10) No. (%)	*Esch. coli* (n=6) No. (%)	*P. aeruginosa* (n=3) No. (%)	*Proteus* Spp. (n=2) No. (%)
1	Amikacin	30 µg	1 (33.33)	0 (0.00)	0 (0.00)	6 (60.00)	3 (50.00)	2 (66.67)	1 (50.00)
2	Ampicillin	10 µg	3 (100.00)	1 (100.00)	1(100.00)	10 (100.00)	6 (100.00)	3 (100.00)	2 (100.00)
3	Cefotaxime	30 µg	1 (33.33)	0 (0.00)	1(100.00)	6 (60.00)	5 (83.33)	2 (66.67)	1 (50.00)
4	Ceftazidime	30 µg	2 (66.67)	0 (0.00)	1(100.00)	7 (70.00)	5 (83.33)	2 (66.67)	1 (50.00)
5	Ceftriaxone	30 µg	1 (33.33)	0 (0.00)	0 (0.00)	4 (40.00)	2 (33.33)	1 (33.33)	0 (0.00)
6	Ciprofloxacin	5 µg	2 (66.67)	1(100.00)	1(100.00)	7 (70.00)	6 (100.00)	3 (100.00)	2 (100.00)
7	Gentamicin	10 µg	1 (33.33)	0 (0.00)	0 (0.00)	4 (40.00)	2 (33.33)	1 (33.33)	0 (0.00)
8	Lomefloxacin	10 µg	2 (66.67)	0 (0.00)	1(100.00)	7 (70.00)	4 (66.67)	2 (66.67)	1 (50.00)
9	Penicillin	10U	3 (100.00)	1100.00	1(100.00)	10 (100.00)	6 (100.00)	3 (100.00)	2 (100.00)
10	Sparfloxacin	5 µg	2 (66.67)	0 (0.00)	1(100.00)	8 (80.00)	4 (66.67)	1 (33.33)	1 (50.00)