THE STUDY OF BACTERIAL FLORA OF DIFFERENT TYPES IN HOSPITAL WASTE: EVALUATION OF WASTE TREATMENT AT AIIMS HOSPITAL, NEW DELHI

Savita Saini[1], Bimal K Das[2], Arti Kapil[2], Shyama S Nagarajan[3] and RK Sarma[3]

[1]Department of Pediatrics, [2]Department of Microbiology, [3]Department of Hospital Administration, All India Institute of Medical Sciences, New Delhi, India

Abstract. Bio-medical waste management rules were formulated in response to the worldwide public concern over medical waste. The practice of separation into different types of waste in health care institutes should be evaluated more scientifically. Due to a lack of data from the Indian sub-continent, this study was initiated at a tertiary care hospital. Samples were collected from different types of waste at the hospital, at different time intervals, for microbiological evaluation. The results reveal that the microbial flora isolated from infectious waste and general waste from the hospital are similar. The samples from general waste in this study reveal many types of pathogens. The bacteria present in the waste initially was low in quantity, but they replicated rapidly over time so that significant numbers were detected by 24 hours, due to environmental factors which were favorable for growth during this period. This study strongly suggests that waste should be removed from the hospital within 24 hours of its generation to prevent environmental contamination caused by any accidental spillage of waste. General waste generated in the hospital should be treated similar to infectious waste, as it can be equally hazardous.

INTRODUCTION

Ten percent of hospital generated waste is infectious, which can be hazardous to the public (De Roos, 1974). According to Wallace *et al* (1973) and De Roos (1974), hospital solid waste can contain significantly high concentrations of pathogenic organisms (Wallace *et al*, 1973; De Roos, 1974). This effect is increased if there is inadequate handling of these wastes. Infectious waste may contain pathogens, which can infect people through a number of routes, such as punctures, abrasions, or lacerations of the skin, via the mucous membranes, by inhalation or ingestion. Hazardous waste must be packaged, transferred and disposed of properly to protect both people and the environment (Anonymous, 1995; 2000).

Public concern over the disposal of medical waste and the risk of transmitting the human immunodeficiency virus, hepatitis B virus, or other agents, has markedly increased in the past few years. The public is also concerned about the emissions from incinerators that burn medical waste, whether these emissions contain microorganisms or toxic substances (Li and Jeng, 1993). 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 potential for humans, comparable to hospital waste (Rutala and Mayhall, 1992).

The All India Institute of Medical Sciences has formulated a bio-medical waste management policy in accordance with bio-medical waste rules, 1995, issued by the Ministry of Environment and Forests, Government of India (Satpathy and Pandhi, 1998). It provides rules and regulations for the management of all potentially infectious and hazardous wastes. Waste is separated at the point of origin by generators, according to the directions provided them. Collectors carry the waste to the treatment site, where it is finally treated by autoclaving and incineration. Municipal corporation employees remove the general waste along with the ash generated by incineration.

Due to the lack of literature regarding hospital waste management on the Indian subcontinent, this study was initiated. The aims of this study were to examine the survival and multiplication of bacteria under hospital conditions and

Correspondence: Prof RK Sarma, Department of Hospital Administration, All India Institute of Medical Sciences, New Delhi- 110029, India.
Tel: 91-11-2659 4708; Fax: 91-11-2658 8663, 2658 8641

to assess the efficacy of the in-house waste treatment system.

MATERIALS AND METHODS

Evaluation of growth patterns of standard bacterial strains in solid waste in hospital environmental conditions

In order to determine the growth pattern of bacteria in the hospital environment and to check the efficacy of the incinerator and the autoclave, three standardized strains, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923), were used in this experiment. Four sterile gauze pieces were placed in sterile petri dishes and 1 ml of peptone broth containing 10^8 CFU/ml of bacteria was poured on each and left for 5 minutes to soak in completely. To confirm the baseline count, one of the gauze pieces was added to a tube containing 10 ml of nutrient broth and the other four were kept in sterile plastic bags (Zip-lock). They were kept in new black garbage bags and placed in the hospital ward after sealing properly. The peptone water with the gauze piece was homogenized for recovery of viable bacteria (Peterson and Stutzenburger, 1969). The homogenate was used to make 10-fold serial dilutions from 10^{-1} to 10^{-6}. From each dilution, 0.1 ml was spread on the surface of the blood-agar plates (Trypticase-soy-agar-base and 7% defibrinated sheep blood) and MacConkey's agar plates to calculate the total viable counts. At the end of 24 and 48 hours, one of the gauze pieces was taken out and processed similarly for viable counts.

Assessment of the efficacy of incinerator and autoclave

One of the inoculated gauze pieces of each control strain was sent to the autoclave at the end of 48 hours. After autoclaving, it was processed to calculate colony-forming units (CFU). Similarly, another gauze piece was sent to the incinerator at the end of 48 hours in a sterile steel box. The ash was tested for the presence of live microorganisms by re-suspending in nutrient broth that was filtered and then cultured on blood and MacConkey agar plates to perform viable counts. Five random samples of incinerator ash and autoclaved discard generated from hospital biomedical waste were evaluated.

Bacterial flora isolated from different types of hospital waste bags with specific color coding used for seperation

Four sites were selected from different wards in the hospital. Samples were collected at each site from three different types of bags of infectious waste (yellow), general waste (black) and plastics and sharps containing (blue) bags. All the plastic and sharps were treated in calcium hypochlorite solution before disposal. The bags were labeled and marked with colored ribbons for identification. Commercial sterile swabs (Hi-media) were used to swab the inner surface of the bags at a depth of approximately 20 inches. The swabs were inoculated in 5 ml of nutrient broth and transported to the laboratory.

Samples were collected from each bag at 0, 24, and 48 hours from the time of the generation of the waste and processed for the isolation and identification of pathogenic bacteria. Microbiological processing was done by standard laboratory methods (Colle *et al*, 1996).

RESULTS

Evaluation of growth patterns of control bacterial strains on solid waste in hospital environmental conditions

The colony forming units (CFU) of all three control strains, at different time intervals, are shown in Table1. The maximum temperature recorded during the study period varied from 35ºC to 41ºC and the minimum was from 21ºC to 27ºC. The relative humidity varied between 70 to 94%. *E. coli* (ATCC 25922) showed an increase in numbers from 1.7×10^3 to 6.9×10^7 CFU/ml by 24 hours, and to 7.9×10^9 CFU/ml by 48 hours. *Pseudomonas* (ATCC 27853) multiplied from 2.9×10^3 to 1.3×10^8 CFU/ml by 24 hours and to 1.3×10^{11} CFU/ml by 48 hours. *Staphylococcus aureus* (ATCC 25923) multiplied from 2.2×10^3 CFU/ml to 7.6×10^8CFU/ml by 24 hours and 3.2×10^{10} CFU/ml by 48 hours. All the strains had multiplication rates of one log per day.

Assessment of the efficacy of the incinerator and the autoclave

The ash samples of all three strains were found to be sterile after incineration. The autoclaved gauze pieces for all three strains had no bacterial growth on culture. Cultures of randomly collected samples of incinerator ash and auto-

Table 1
Growth patterns of bacteria in the hospital environment.

S. No.	Name of strain	0 hrs CFU/ml	24 hrs CFU/ml	48 hrs CFU/ml	After incineration	After autoclave
1	*E. coli* (ATCC 25922)	$1.7x10^3$	$6.9x10^7$	$7.8x10^9$	Sterile	Sterile
2	*Pseudomonas* species (ATCC 27853)	$2.9x10^3$	$1.3x10^8$	$1.3x10^{11}$	Sterile	Sterile
3	*Staphylococcus aureus* (ATCC 25923)	$2.2x10^3$	$7.6x10^8$	$3.2x10^{10}$	Sterile	Sterile

Table 2
Bacteria isolated from different types of waste separated at the time of generation in the hospital.

Site	Time	Infectious waste	General hospital waste	Plastic and sharps
1	0 hrs	Sterile	*Staphylococcus* sp *Klebsiella* sp	Sterile
1	24 hrs	*E. coli*	*Staphylococcus* sp *Klebsella* sp	Sterile
1	48 hrs	*Pseudomonas* sp *Staphylococcus* sp *E. coli*	*E. coli* *Klebsiella* sp *Staphylococcus* sp	*E. coli*
2	0 hrs	*Staphylococcus* sp	*Staphylococcus* sp	Sterile
2	24 hrs	*E. coli* *Staphylococcus* sp	*Pseudomonas* sp *Staphylococcus* sp	Sterile
2	48 hrs	*E. coli* *Staphylococcus* sp	*E. coli* *Pseudomonas* sp	Sterile
3	0 hrs	*Staphylococcus* sp	*Klebsiella* sp	Sterile
3	24 hrs	*E. coli* *Staphylococcus* sp	*Klebsiella* sp	
3	48 hrs	*E. coli*	*Klebsiella* sp *Pseudomonas* sp	*Staphylococcus* sp
4	0 hrs	Sterile	*Staphylococcus* sp	Sterile
4	24 hrs	*E. coli*	*E. coli* *Staphylococcus* sp	Sterile
4	48 hrs	*Staphylococcus* sp *Streptococcus feacalis* *Enterococcus* sp	*E. coli* *Klebsiella* sp *Staphylococcus* sp *Pseudomonas* sp	Sterile

claved waste were also found to be sterile.

Recovery of bacterial flora of different types from hospital waste separated into different color-coded bags

The bags containing plastics and sharps were mostly sterile after hypochlorite treatment, or had no pathogenic bacteria in hazard group 2 or 3 (Anonymous,1995) at 0 hours (Table 2). One had *Acinetobacter* species isolated at 24 hours and *E. coli* and *Acinetobacter* sp at 48 hours (Table 2). The cultures of two bags were found sterile even after 48 hours.

The samples at 0 hrs for all the infectious waste bags were sterile. At 24 hours, *E coli*, *Staphylococcus* species and *Acinetobacter* species were isolated in some samples. All the samples had two or more types of pathogenic microorgan-

isms at the end of 48 hours (Table 2).

The general waste contained many types of organisms, such as *E. coli*, *Klebsiella* species and *Staphylococcus* species, even in samples at 0 hours. All the samples at the end of 24 hours and 48 hours had multiple types of organisms, as shown in Table 2.

DISCUSSION

The Ministry of Forest and Environment, Governmen of India announced biomedical waste (management and handling) rules in 1998. The Department of Hospital Administration of the AIIMS designed a hospital waste management manual according to these rules, for proper separation and disposal of waste. This study was initially designed to differentiate between the microbial flora of different types of hospital waste. To examine the growth parameters of bacteria, control strains were used and the results of our study show that bacteria grows rapidly in the environmental conditions of the hospital (Table 1). Delhi's temperature and relative humidity during that period were also ideal for bacterial growth.

The microbial flora present in infectious and general waste generated by the hospital were similar (Table 2). General hospital waste contained more pathogens. The initial samples had fewer microorganisms compared to 24 hours and 48 hours. This indicates that bacteria are present in the waste initially in low quantities, but they replicate rapidly and can be easily detected within 24 hours. In this study, we took samples only up to 48 hours, therefore, slow growing pathogens were not detected. Garbage kept for days can lead to the multiplication of those slow growing microorganisms, and can be a health hazard for the public. This study showed that the longer the waste remains in the hospital, the higher the number of bacteria present in it.

Though quantitative measurements were not done in this study, the results of our study support previous findings that general waste contains microorganisms with pathogenic potential (Rutala and Mayhall, 1992). In the paper, the authors reviewed the literature in this area and found that general hospital waste was, on average, more contaminated than hospital waste. This has direct implications for hospital waste management.

The incinerated and autoclaved waste samples were found to be sterile, which indicates that the biomedical waste treatment technology of AIIMS is an important part of waste management for the hospital. This study strongly suggests that waste should be removed from the hospital within 24 hours of generation. Plastics and sharps should be separated to avoid injury and infection. The toxic gaseous waste generated by the burning of plastics make it necessary to be separated from all other types of waste. Bacteria detected from different types of waste should be further identified and antibiotic sensitivities detected, to understand the resistance patterns prevalent among hospital strains. General waste should be treated like infectious waste, to make a healthier environment.

Further studies should be done to evaluate viruses, fungi, and parasites in different categories of waste, to improve our knowledge of microbial flora, and to develop treatment technologies which better fight this devilish problem created by human beings.

REFERENCES

Anonymous. Categorization of biological agents according to hazard and containment. 4th ed. Second supplement, 1995. Available from: URL: http://www.hse.gov.uk.hthdir/noframes/agent/pdf

Anonymous. Guidelines for protecting the safety and health of health workers. NIOSH / Health Care Workers guidelines. National Institute for Occupational safety and Health. Washington DC: US Government office, Chap 6. 2000.

Colle JG, Marmion BP, Frasert AG, Simmons A, eds. Mackie and Mcartney. Practical medical microbiology. Edinburgh, New York: Churchill Livingston Publisher, 1996: 95-151, 853-64.

De Roos RL. Environmental concerns in hospital waste disposal. *Hospitals*, 1974; 48: 120-2.

Li C-S, Jeng F-T. Physical and chemical composition of hospital waste. *Infect Control Hosp Epidemiol*, 1993; 14: 145-50.

Peterson ML, Stutzenberger FJ. Microbiological evaluation of incinerator operations. *Appl Microbiol*, 1969; 18: 8-13.

Rutala WA, Mayhall CG. Madical waste. *Infect Control Hosp Epidemiol* 1992; 13: 38-48.

Satpathy S, Pandhi RK. Manual for hospital waste management at AIIMS Hospital. 1998.

Wallace LP, Zaltzman R, Burchinal JC. Where solid waste comes from; where it goes. *Modern Hosp* 1973; 121: 91-2