

Original Research Article

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## Microbial Assessment of Bio-medical Waste from Different Health Care Units of Bikaner

Rajani Joshi, Rakesh Rao, Manohar Sain, Pankaj Mangal\* and Paramjeet Kaur

Animal biomedical waste disposal technology centre, Rajasthan University of Veterinary and Animal Sciences, Bikaner- 334001, Rajasthan, India

\*Corresponding author

### ABSTRACT

Biomedical waste has been one of the major headaches of any hospital. Eighty percent waste generated from the hospitals is general waste and the remaining twenty percent comes under the biomedical waste categories. If this waste is not properly managed, it is dangerous for people, animals and environment due to its high harmful potential. In the present investigation, aerobic bacteria like *Escherichia coli* (32%), *Staphylococcus aureus* (28%), and *Bacillus spp.* (10%) were isolated from the biomedical waste. These bacterial strains were morphologically identified and confirmed by using primary and secondary biochemical tests. Clearly there is a need of awareness to the hazards associated with improper biomedical waste disposal. Lack of apathy to the concept of waste management is a major stymie to the practice of waste disposal.

#### Keywords

Biomedical waste, Hazards, *Bacillus cereus*

#### Article Info

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### Introduction

Biomedical waste is any wastes generated during the diagnosis, treatment or immunization of animals and human beings or in research activity. Biomedical wastes are extremely hazardous type of waste and if not managed properly, can lead to serious health and environment problems (GOI, 1998). Biomedical wastes are divided into infected and non-infected wastes. Non infected wastes may be disposed by land filling as done for municipal wastes (Omran *et al.*, 2007).

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. It consists wholly or partly of human or animal tissues, blood or body fluids, excretions, drugs or other pharmaceutical products, swabs or dressings or syringes, needles or other sharp instruments, being waste which unless rendered safe may prove hazardous (including microbial (infectious), pharmacological and/or physical [e.g. sharps]

dangers to any person coming into contact with it (Jang *et al.*, 2006 and Nemathaga *et al.*, 2008) But the infected wastes require appropriate treatment processes prior to disposal. When the infectious component gets mixed with the general non-infectious waste, the entire mass becomes potentially infectious.

Infectious or hazardous hospital waste represents only a small part of the total medical waste; yet, because of ethical questions and potential health risks, it is a focal point of public interest. The pathogens present in the waste can leach out and contaminate the environment. The most typical pathogenic bacteria in bio-medical waste are of the genus *Staphylococci*, *Bacillus* and *Streptococci*, along with varying numbers of other common nosocomial pathogenic bacteria such as *Klebsiella*, *Salmonella*, *Proteus* and *Enterobacter* species (Alagoz *et al.*, 2008 and Coker *et al.*, 2009). However, the most prevalent bacteria found in clinical solid waste (CSW) are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus cereus* (Park *et al.*, 2009).

## Materials and Methods

The present study was conducted at the Animal biomedical waste disposal technology centre, Rajasthan University of Veterinary and Animal Sciences, Bikaner, India.

A total of 50 biomedical waste samples were collected from the various departments of CVAS, Bikaner viz. department of Veterinary Surgery, Veterinary Medicine, Veterinary Gynaecology and Obstetrics and department of Veterinary Pathology in sterilized yellow color coded biohazard bag as per BMW (Management and handling) rules, 2016. About 100-150 gram of biomedical waste samples were collected in pre sterilized

yellow color coded bags and brought to the Animal biomedical waste disposal technology centre. Collected biomedical wastes included the animal tissues, body parts, blood, body fluids, infectious bedding of animal and carcasses. At the time of collection all the necessary precautions were followed by wearing protective clothing (apron), hand gloves, face mask, head cover and gum boot as prescribed in the BMW (Management and handling) rules, 2016.

## Isolation and identification of bacteria

The Bio-medical waste samples were subjected to aerobic cultivation. Each Bio-medical waste sample was streaked on Nutrient agar, plates in primary, secondary, and tertiary fashion in order to obtain isolated colonies of bacteria. These petri plates were incubated for 24 hr at 37°C. After 24 hr incubation these isolated colonies were cultured on Mannitol salt agar (MSA), Eosine Methylene blue agar (EMB) and Mannitol egg yolk Polymyxin (MYP) agar plates respectively for isolation of *Staphylococcus*, *Escherichia*, and *Bacillus* and incubated for 24 hours at 37°C. The growth was examined for the colonial morphology and pigmentation, and in order to obtain pure culture different types of colonies were sub-cultured on separate nutrient agar plates.

## Confirmation of the isolates by using primary and secondary biochemical tests

The confirmation of the isolates such as *Staphylococcus aureus*, *Escherichia coli* and *Bacillus spp.* was done by using Gram's staining and biochemical tests provided in HiStaph™ Identification Kit ((HiMedia, Mumbai) for *Staphylococcus*, Hi25 *Enterobacteriaceae* Identification Kit (HiMedia, Mumbai) for *Escherichia coli* and HiBacillus™ Biochemical Test Kit (HiMedia, Mumbai) for *Bacillus*.

## Results and Discussion

The result of the present study reveals that frequency of isolated bacterial strains of the collected hospital wastes is depicted in (table 1; fig-1). In the present study out of 50 biomedical waste samples, 16 samples were found positive for *Escherichia coli* (32%), 14 samples for *Staphylococcus aureus* (28%) and 5 samples for *Bacillus spp.* (10%). Colonies of *Staphylococcus aureus* on Mannitol salt

agar and results of biochemical test depicted in (fig-2 and 3). *Escherichia coli* on EMB agar results of biochemical test depicted in (fig-4 and 5) and *Bacillus spp.* on MYP agar results of biochemical test depicted in (fig-6 and 7) are shown, respectively.

The detailed results of various biochemical tests performed for *Bacillus spp.*, *Escherichia coli* and *Staphylococcus aureus* are shown in (Table-2).

**Table.1** Percentage of bacteria present in bio medical waste samples

Identified bacteria	Percentage of appearance
<i>Escherichia coli</i>	32%
<i>Staphylococcus aureus</i>	28%
<i>Bacillus cereus</i>	10%

**Table.2** Biochemical characteristic of isolated bacterial strain (*Bacillus spp.*, *Staphylococcus aureus* and *Escherichia coli*)

Characterization	<i>Bacillus spp.</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
Gram staining	+	+	-
Colonies	Light pinkish	Yellow	Green
Shape	rods	cocci	rods
Catalase	+	+	+
Coagulase	-	+	-
Citrate utilization	+	-	-
Nitrate reduction	+	+	+
ONPG	-	-	+
Voges Proskauer test	+	+	-
Indole	-	-	+
Glucose	+	+	+
Sucrose	+	-	-
Lactose	-	+	+
Mannitol	+	+	-
Arabinose	+	-	-

Fig.1 Percentage of bacteria present in bio medical waste samples

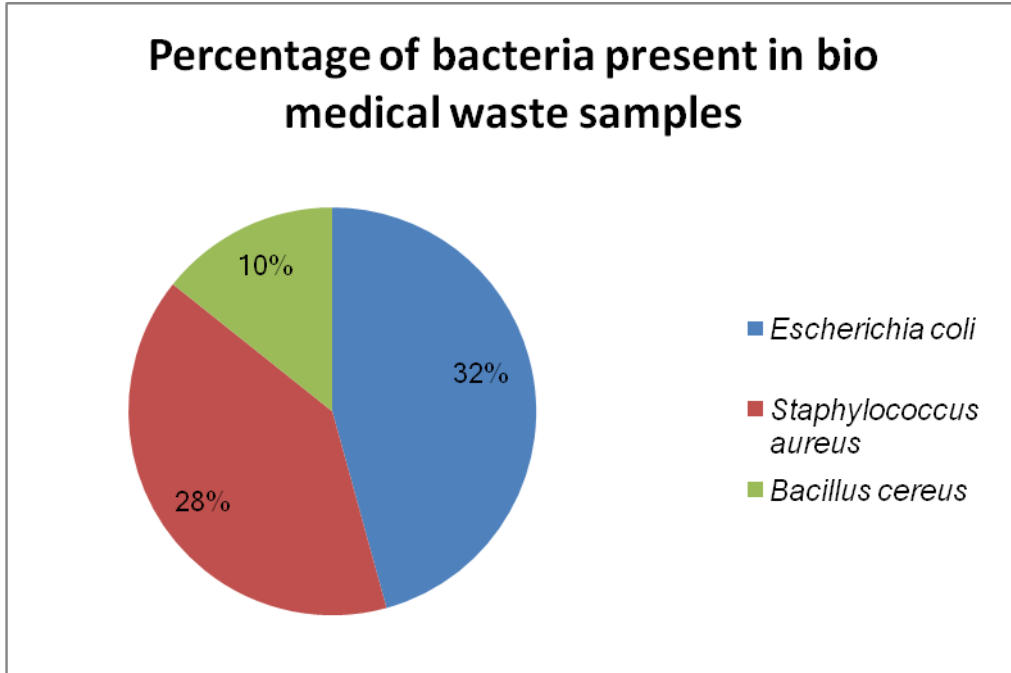
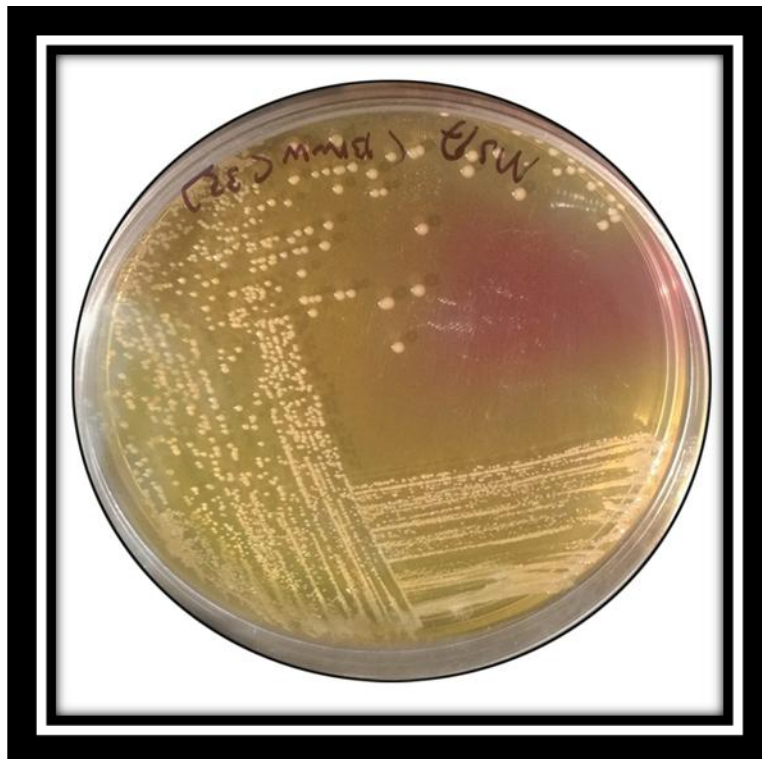


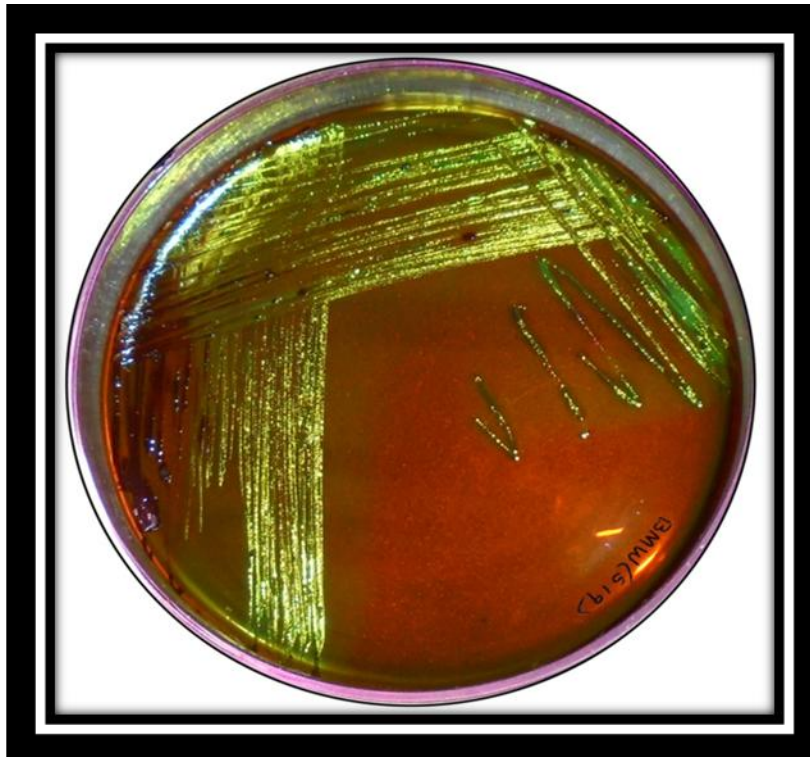
Fig.2 Isolation of *Staphylococcus aureus* on Mannitol salt agar from Biomedical Waste Sample



**Fig.3** Results of biochemical tests for *Staphylococcus aureus* using HiStaph™ commercial kits



**Fig.4** Isolation of *Escherichia coli* on EMB agar from Biomedical Waste Sample



**Fig.5** Results of biochemical tests for *Escherichia coli* obtained from HiE.coli™ commercial kits



**Fig.6** Isolation of *Bacillus spp.* on MYP agar from Biomedical Waste Sample



**Fig.7** Results of biochemical tests for *Bacillus spp.* obtained from HiBacillu<sup>STM</sup> commercial kits



In the present study out of 50 biomedical waste samples, 16 samples were found positive for *Escherichia coli* (32%), 14 samples for *Staphylococcus aureus* (28%) and 5 samples for *Bacillus spp.* (10%). *Bacillus* and *Escherichia Coli* was reported by Cook *et al.*, (1964) while Liu and Chen (1980) reported *Escherichia Coli*. Ekundayo (1977) and Gray (1967) also reported the presence of *Bacillus*, *Escherichia Coli* and *Staphylococcus* in biomedical wastes. Rastogi *et al.*, (2011) reported 18.38% *Staphylococcus aureus* and 22.29% *Escherichia coli* which is lower than present investigation. Feleke *et al.*, (2014) also isolated the lower results than present study which is *Escherichia coli* (11.5%) and *Staphylococcus aureus* (8.2%) from the 60 hospitals waste water samples. Faith *et al.*, (2010) isolated the *Escherichia Coli* (50.00%) which is higher and *Staphylococcus aureus* (14.67%) which is lower than present results from municipal solid waste. According to Oyeleke and Istifanus (2009) the live pathogens found in hospital wastes, the most predominant (80-90%) is the genus *Bacillus* with *Staphylococci* and *Streptococci* varying between 5 and 10%, where as the most common pathogens are *Staphylococcus aureus* (from 2-10 colonies

per gram of wastes). Tiku *et al.*, (2018) also identified *Staphylococcus*, *Streptococcus* and *Bacillus spp.* from solid waste of General Hospital Calabar.

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