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Research Article

# ISOLATION AND IDENTIFICATION OF BACTERIA FROM BIOMEDICAL WASTE (BMW)

**J. ANITHA* AND INDIRA A. JAYRAAJ****

**Department of Biochemistry, Kongunadu Arts and Science College, Gnanamibikai Mills (P.O), Coimbatore 641029, Tamil Nadu, India, **Email: aiproject2010@gmail.com**

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**ABSTRACT**

Present investigation was aimed to understand the microbial flora in the biomedical waste. The mean total of isolated viable aerobic and anaerobic heterotrophic bacteria population ranged from $2.7x10^7$ CFU/gm, $1.6x10^7$ CFU/gm, $2.2x10^7$ CFU/gm and $3.6x10^7$ CFU/gm. The bacteria with their frequency of isolation from the biomedical waste were *Bacillus subtilis* (12%), *Staphylococcus aureus* (9%), *Klebsiella pneumonia* (6%), and *Escherichia coli* (15%) were isolated and identified using standard protocols. Bacterial strains were morphologically identified using gram staining reaction and biochemical tests. The investigation revealed that the biomedical waste consist of bacterial community which useful for mankind and might be used for the biodegradation of 85% non-infectious hospital waste to recover the resource.

## INTRODUCTION

Biomedical wastes are extremely hazardous type of waste and if not managed properly, can lead to serious health and environment problems. Biomedical waste is any wastes generated during the diagnosis, treatment or immunization of human beings or in research activity [1]. The waste produced in the course of health care activities carries a higher potential for infection and injury than any other type of waste [2]. Biomedical wastes generated in the hospital falls under two major categories – non hazardous and bio hazardous. Constituents of non hazardous waste are non-infected plastic, cardboard, packaging material, paper etc. Bio hazardous waste again falls into two types (a) Infectious waste-sharps, non sharps, plastics disposables, liquid waste, etc. (b) Non infectious waste-radioactive waste, discarded glass, chemical waste, cytotoxic waste, incinerated waste etc Approximately 75-90% of the biomedical waste is non-hazardous and as harmless as any other municipal waste. The remaining 10-25% is hazardous and can be injurious to humans or animals and deleterious to environment. It is important to realize that if both these types are mixed together then the whole waste becomes harmful [3]. Major hospitals contribute substantially to the quantum of biomedical waste generated. Smaller hospitals, nursing homes, clinics, pathological laboratories, blood banks, etc also contribute a major chunk [4].

The pathogens present in the waste can leach out and contaminate the environment. But microorganisms play an important role in the industries for various applications. Considering these facts the aim of this study was to isolate, identify and characterize the microbial populations in the biomedical wastes.

## MATERIALS AND METHODS

The present investigation was carried out in the Department of Biochemistry, Kongunadu Arts and Science College (Autonomous), Coimbatore - 641 029. The experimental procedure adopted is described under the following heading.

### Collection of biomedical wastes samples

The samples for microbiological analysis were collected in clean polythene bags. The biomedical waste samples were collected from Rao Hospital, R.S Puram, Coimbatore – 641 002, and Parthiban Clinic, Coimbatore – 641 029 from the month of March to June, 2010.

### Isolation, Identification and Characterization of Bacteria from Biomedical Wastes

The biomedical waste samples were processed as per the method of [5]. One gram of samples was suspended in 99ml of sterile distilled water and shaken vigorously for 2 minutes. Then the suspensions were serially diluted and then the selected dilution was streaked onto Nutrient Agar (Hi Media).

Bacterial strains were morphologically identified using gram staining reaction and other biochemical tests which include; catalase, methyl red, voges proskauer (MR-VP), nitrate reduction test, starch hydrolysis, gelatin liquefaction test, coagulase, indole, motility, oxidase, urease, triple sugar iron agar (TSI) and sugar fermentation as described by [6] and [7]. Microorganisms were enumerated as described by [7] and [8].

### Statistical analysis

The result obtained in the present study statistically analysed. The result and the data were expressed as mean ± S.D from triplicate determination.

## RESULTS AND DISCUSSION

The results of the present study reveals that aerobic and anaerobic heterotrophic count of the collected hospital wastes during the study period is depicted in table 1.

**Table 1: Microbial counts and frequency of isolated bacterial strains**

| Bacterial Strains | Counts of bacteria in hospital wastes (CFU/ml) | Total Number of Isolates | Percentage (%) of appearance |
|---|---|---|---|
| *B. subtilis* | $2.7x10^7$ | 36±1.52 | 12±1.00 |
| *K. pneumonia* | $1.6x10^7$ | 22±2.00 | 6±4.93 |
| *S. aureus* | $2.2x10^7$ | 16±2.00 | 9±1.00 |
| *E. coli* | $3.6x10^7$ | 27±1.52 | 15±1.00 |

The values are expressed as mean ±SD ( n=3)

The frequency of occurrence reveals that *E. coli* (15%) has the highest prevalence followed by *Bacillus subtilis* (12%) and the least is *Klebsiella pneumonia* (6%) (Table 1). The aerobic heterotrophic counts from hospital waste were higher than anaerobic heterotrophic counts. *E. coil* was found to be the predominant species isolated in the present study. The findings is contrary with the other investigators [9] who reported that the *Bacillus* was the predominant genus found in hospital waste. *E. coli* is a harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls [10]. *Bacillus* was the

predominant genus found in hospital wastes. *B. subtilis* is a ubiquitous naturally occurring saprophytic bacterium that is commonly recovered from soil, water, air and decomposing material and represent a large number of different species as stated by [11]. All the bacterial isolates reported in this study have been reported to be associated with wastes and wastes biodegradation. The hospital wastes could have contributed immensely in the increased number of bacterial counts [12]. According to [8] 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). *E. coli*. and *P. aeruginosa* are also common along with varying numbers of other common nosocomial pathogens such as *K. proteus and* Enterobacter species. *Bacillus, E. coli, Klebsiella, and Pseudomonas* was also reported by [13]. [14] reported *E. coli*. and *Enterobacte.* [15] and [16] reported the presence of *Bacillus, E. coli, Proteus, Pseudomonas, Micrococcus, Serratia, Staphylococcus, Streptococcus* in biomedical wastes. *Pseudomonas* has been widely reported to be associated with wastes [17].

**Table 2: Biochemical characteristic of isolated bacterial strain (B. subtilis, S. aureus, K. pneumonia and E. coli)**

| Characterization | *Bacillus subtilis* | *Staphylococcus aureus* | *Klebsiella pneumonia* | E. coil. |
|---|---|---|---|---|
| Gram Staining | Gram-positive | Gram-positive | Gram-negative | Gram-negative |
| Motility | Motile | Non-motile | Motile | Motile |
| Shape | Rods | Cocci | Rods | Rods |
| Oxygen Requirement | Aerobic | Anaerobic | Anaerobic | Anaerobic |
| Colony | Yellow | Yellow | Yellow | Blue |
| Endospore | + | + | - | - |
| Catalase | + | + | + | + |
| Coagulase | - | + | - | - |
| Oxidase | + | - | - | - |
| Urease | - | - | + | - |
| Gelatinase | + | - | - | - |
| H2S production | - | - | - | - |
| Nitrate reduction | + | + | + | + |
| Indole Production | - | - | - | + |
| Methyl red test | - | + | - | + |
| Vogus Proskaur Test | + | + | + | - |
| Citrate Utilization | + | - | - | - |
| Glucose | - | + | + | + |
| Mannitol | + | + | - | - |
| Lactose | - | - | + | + |
| Sucrose | - | - | + | - |
| Fructose | + | - | - | - |
| Sorbitol | - | - | + | + |

The morphological and biochemical characters of isolated bacterial species are presented in table 2. The morphological observations in Gram's staining revealed Gram positive and gram negative bacteria. These colonies can be fluorescent or non-fluorescent under long wave ultraviolet light. From the table it is evident that the biomedical wastes does not contain virus because that viral titer tendency to decrease rapidly as the time possess. Further more the biomedical wastes have anticeptics and disinfectants which kill viruses and various types of toxic infectious bacterial strains. This is in accordance with the study of [8] who reported that through hepatitis B virus has been detected in small amount it has potential to provoke infectious has not been established.

The Gram positive and gram negative bacteria were identified as, rods and cocci. These rods are motile and cocci are non-motile in nature. The motility test is not a biochemical test since we are not looking at metabolic properties of the bacteria. Rather, this test can be used to check for the ability of bacteria to migrate away from a line of inoculation. Heterotrophic strains isolated from biomedical waste are subjected to different biochemical tests were given in table II. In our present study the increased number of colony counts was recorded by *E. coli,* which gave positive for indole production, glucose, lactose, and sorbitol. Moreover *B. subtilis* and *S. aureus* showed positive for catalase by breaking the $H_2O_2$ down into water and $O_2$, the presence of oxygen can be characterized by bubbles, vogus proskaur test and nitrate reduction. It was further observed that the catalase and nitrate reduction were for *K. pneumonia.* These results are similar to the currently isolated strains from Bergey's manual of Determinative of Bacteriology, 90% of results showed the similarity in characteristics with of isolated bacteria [18]. Similar results of sugar test for *L. acidophilus* were reported by [19]. *L. acidophilus* gave positive reactions with lactose, glucose, maltose, galactose sucrose and fructose and negative reactions with mannitol. The hospital wastes could have contributed immensely in the increase of these bacteria [12] might be due to the fact that hospital wastes is very rich in organic material as stated by [20]. Microbes will adapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream. [21] reported that truly pathogenic forms may survive in waste.

We isolated bacterial strains from biomedical wastes. These strains could used for biodegradation and therefore may be suitable for industrial applications. After isolation and identification of microorganisms, a proper disposal of biomedical waste is needed.

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