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## Isolation and Identification of Bacterial Pathogens from Waste Water

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### Abstract

A total of 42 samples were collected from waste water source of Cuddalore, Tamil Nadu and 34 isolates of bacteria were isolated using selective media. The collected samples were processed for bacterial isolation using the enrichment broth, macconkey agar, Salmonella and Shigella agar and EMB agar. The conventional methods of swabbing and streaking were used. The optimal cultural conditions, microbiological characteristics, biochemical characteristics were studied. Among the 34 isolates 6 Salmonella spp, 6 Shigella spp, 7 E.coli, 8 Klebsiella spp, and 7 Proteus spp. From this study it was concluded that the water is commonly contaminated with microbes and this contamination may be playing a role in the transmission of potentially harmful organisms.

**Key words:** Selective media, waste water, Contamination

### INTRODUCTION

Industrial waste and its management have taken an important role in our environment. There a few factors attribute to the municipalities problem such as treatment, disposal and recycling of sewage sludge. Basically the solid waste from a municipality contains the biodegradable organic materials with a significant amount of inorganic matter [1]. The utmost challenge to the environmentalists is the eco friendly management of this waste consumed by microorganisms [2]. Industrial organic waste is consumed by the bacteria, used as nutrients by the bacteria, and is no longer present to produce odours, sludge, pollution or unsightly mess. When bacteria uptakes waste, they

convert the waste into consumable by products and in due course of this conversion they actually produce several metabolites to break down the complex waste into simple compounds. Soil microorganisms are increasingly becoming an important source in the search for industrially important molecules [3]. Bacteria are present in diverse ecological habitats. Sludge exhibits wide variations in the physical, chemical and biological properties [4]. The biological methods are usually preferred over the physicochemical in removing the majority of pollutants in wastewater treatment [5]. The role of waste water in spreading communicable diseases is much evident due to combined source of water [6]. Sewage water with faecal coliform severely affects the

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performance of humans. Salmonella, Camphylobacter spp, Staphylococcus aureus, Pseudomonas aeruginosa, Clostridium botulinum, Vibrio cholerae and Escherichia coli are the main human pathogens responsible for water contamination [7]. The aim present investigation on isolation and identification of harmful bacteria from industrial waste in cuddalore area was carried out.

## MATERIALS AND METHODS

### Collection of Sample

A total of 42 samples were collected from waste sample site of Cuddalore. Sample was collected in sterile plastic bottle maintaining aseptic conditions, stored at 4 °C and marked accordingly to their source and location. The collected samples were brought to the laboratory for isolation of bacteria.

### Dilution of Sample

Serially diluted samples were used. Then, 1ml from the sample was taking out and added into 9ml of distilled water. This step was continuously repeated until fifth dilution. 0.1 ml from each dilution bottle was added into the enrichment broth. After 24 hours the inoculum was

streaked in MacConkey agar, Eosin Methylene Blue (EMB), and SS agar by standard procedure to enhance the isolation pathogens. The plates was labelled and kept in the 37°C kept in incubator for 24 hour [8].

### Identification of Microorganism

Biochemical test were performed by standard procedure based on Bergey's manual. For the identification of these microorganisms from sludge, several biochemical identification methods such as Gram stain, spore forming, Indole, Methyl red, Citrate test, Urease Mannitol motility, catalase, Oxidase, Voges-Proskauer and Triple sugar ion test had been used [9,10].

## RESULTS

The isolation and characterization of bacterial pathogens from Cuddalore industrial waste site was undertaken in this study. Bacterial growth based on different physicochemical conditions such as media, pH, temperature, incubation period, carbon source etc. So different conditions upon which bacteria grew in natural habitat should be studied. The results for microscopic examination such as staining; motility test and cultural characters, colony morphology, Biochemical characters for samples are shown in the (Table 1).

**Table-1: Grams stain and biochemical characteristic of isolated bacteria**

Cul. No	Gram staining	Indole	Methyl red	VP	Citrate utilization	Urease	TSI	Oxidase	Catalase
SS <sub>1</sub>	(-) rods	-	+	-	-	-	k/S gas	+	-
SS <sub>2</sub>	(-) rods	-	-	+	+	+	A/A gas	-	+
SS <sub>3</sub>	(-) rods	-	+	-	-	-	k/k	+	-
SS <sub>4</sub>	(-) rods	-	+	-	-	-	y/y gas	+	-
SS <sub>5</sub>	(-) rods	-	+	-	-	-	k/S gas	+	-
SS <sub>6</sub>	(-) rods	-	+	-	-	-	k/k	+	-
SS <sub>7</sub>	(-) rods	-	-	+	+	+	A/A gas	-	+
SS <sub>8</sub>	(-) rods	-	+	-	-	-	k/S gas	+	-
SS <sub>9</sub>	(-) rods	-	-	+	+	+	A/A gas	-	+
SS <sub>10</sub>	(-) rods	-	+	-	-	-	y/y gas	+	-
SS <sub>11</sub>	(-) rods	-	+	-	-	-	k/k	+	-
SS <sub>12</sub>	(-) rods	-	+	-	-	-	y/y gas	+	-

SS <sub>13</sub>	(-) rods	-	+	-	-	-	k/S gas	+	-
SS <sub>14</sub>	(-) rods	-	-	+	+	+	A/A gas	-	+
SS <sub>15</sub>	(-) rods	-	+	-	+	+	A/A gas	+	-
SS <sub>16</sub>	(-) rods	-	+	-	-	-	k/k	+	-
SS <sub>17</sub>	(-) rods	-	+	-	-	-	y/y gas	+	-
SS <sub>18</sub>	(-) rods	-	-	+	+	+	A/A gas	-	+
SS <sub>19</sub>	(-) rods	-	+	-	-	-	k/k	+	-
SS <sub>20</sub>	(-) rods	-	+	-	+	+	A/A gas	+	-
SS <sub>21</sub>	(-) rods	-	-	+	+	+	A/A gas	-	+
SS <sub>22</sub>	(-) rods	-	+	-	-	-	y/y gas	+	-
SS <sub>23</sub>	(-) rods	-	+	-	-	-	k/S gas	+	-
SS <sub>24</sub>	(-) rods	-	+	-	-	-	k/k	+	-
SS <sub>25</sub>	(-) rods	-	-	+	+	+	A/A gas	-	+
SS <sub>26</sub>	(-) rods	-	+	-	-	-	y/y gas	+	-
SS <sub>27</sub>	(-) rods	-	-	+	+	+	A/A gas	-	+
SS <sub>28</sub>	(-) rods	-	+	-	-	-	k/S gas	+	-
SS <sub>29</sub>	(-) rods	-	+	-	-	-	k/k	+	-
SS <sub>30</sub>	(-) rods	-	+	-	+	+	A/A gas	+	-
SS <sub>31</sub>	(-) rods	-	+	-	-	-	k/S gas	+	-
SS <sub>32</sub>	(-) rods	-	+	-	-	-	y/y gas	+	-
SS <sub>33</sub>	(-) rods	-	+	-	-	-	k/S gas	+	-
SS <sub>34</sub>	(-) rods	-	-	+	+	+	A/A gas	-	+

## DISCUSSION

In our study, SS01 to SS34 all these 34 bacterial strains were isolated in culture media.

MacConkey agar, Eosin Methylene Blue (EMB), SS agar and nutrient agar were selected to determine the best suitable media for enhance rapid growth of the isolated strains. It was seen that SS agar media was suitable for maximum growth of bacteria but Salmonella sp and Shigella sp,. E.coli grows well in MacConkey agar media. Visual and microscopic observation was used to characterize the selected strains. Investigation of the colony features of the bacteria are noted (Table 1).In

sewage water contain pathogenic bacteria population is very high and it will increase the mortality and survival rate in microbes. Conventional methods were successful in enumerating Klebsiella, Staphylococcus, gram-negative enteric bacteria, and commonly used indicator organisms [12].

The results showed that the 6 Salmonella spp , 6 Shigella spp 7 E.coli, 8 Klebsiella Spp, were and 7 proteus spp characterized in use of both biochemical and selective media. Gastrointestinal infections are among the most common disease caused by bacterial pathogen in waste water. These include Salmonellosis caused by a number of Salmonella species. Dysentery like

infection has also recently been found to be caused by some strains of enteropathogenic E.coli [11]. The current report (table-1) provides the cell morphology, colony and biochemical characteristics of the isolated strains which would aid in the identification and characterization of the isolated bacterial strains in future.

## CONCLUSION

An isolation of the bacterial pathogen from industrial waste water in the present study confirmed the presence of various bacterial population such as Salmonella spp, Shigella spp, E.coli, Klebsiella spp and proteus spp. Undesirable changes of water quality caused by microorganisms and these can poses a negative impact on human health. The presence of bacterial pathogens in the industrial waste water of Cuddalore is an important health concern due to the risk of developing waterborne diseases and health risk associated with immunosuppressive peoples living in the area. Our present study evidently revealed that industrial waste water is a potential source for harmful pathogens.

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