International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.2, pp 308-314, 2015

Isolation and Preliminary Characterization of Bacterial from Liquid Hospital Wastes

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Abstract: Hospital environment and its waste's accumulation are prone for spreading various infectious diseases. The major objective of the investigation is primarily planned to determine the presence of antimicrobial and other drugs in hospital liquid waste's. Further the study extended to include the microbiological investigations includes prevalence and determining antibiotic resistance. The correlation between the results of High performance liquid chromatography (HPLC) and anti microbial resistance may be a further concern. The main object of present investigation is to assess prevalence and preliminary characterization of bacterial isolates obtained from liquid hospital wastes. The samples were aseptically transferred to microbiological laboratory, bacterial isolation procedures and its conformation methodology were carried out. The results revealed the isolation of *Staphylococcus aureus, Salmonella typhi,Shigella dysentriae, Pseudomonas aeruginosa, Vibrio cholerae, Escherichia coli* and *Campylobacter* sp. Further isolates are planned to impregnate for determining the antimicrobial resistance pattern in order to compare with quantitative analysis of antibiotics by HPLC.
Keywords: Bacterial pathogens, isolation, hospital wastes.

Introduction

Waste water is considered as potent and it is referred to any water quality has been adversely abused by anthropogenic influence. The various sources of liquid waste in the hospital include outdoor and indoor departments, operation theatres, laboratories of microbiology, biochemistry, histopathology, blood bank, etc, radiology and others. The major concern is the disposal of infectious wastes such as cultures and stocks of infectious agents, wastes from infected patients, wastes contaminated with blood and its derivatives, discarded diagnostic samples, contaminated materials (swabs, bandages) and equipment (disposable medical devices, etc.)[1]. Health care waste consists of both organic and inorganic substances that enhance the growth of pathogenic microorganisms.

The untreated hospital waste possess serious health hazards to the health care workers, public and air flora on the area source of pharmaceutical products in the environment are more than just consumers expelling unabsorbed medications through excretion into septic system and waste water treatment plants. The basic principle of underlying wastewater management is the strict limit on the discharge of hazardous liquids into sewers without prior treatment[2] so that living pathogenic organisms are not introduced into the environment. Connection of hospital waste to the municipal sewage network may create problems such as public health risks and imbalance of the microbial community in the sewage systems, which in turn affect the biological treatment process[3,4]. It is very necessary to understand sources of waste that contribute pollutant to the individual waste streams and the shortcomings that will be encountered in an attempt to treat the waste.

Therefore, even if the hospitals are discharging their healthcare liquid waste into sewerage system, it is mixed with the sewage and gets in surface water without proper treatment. If the hospital effluents are not treated, concentrated forms of infectious agents and antibiotic resistant microbes are shed into communities resulting in water borne diseases such as cholera, typhoid fever, dysentery and gastroenteritis etc. Poor management of health care waste potentially exposes health care workers, waste handlers, patients and the community at large to infection, toxic effects and injuries, and risks polluting the environment. It is essential that all medical waste materials are segregated at the point of generation, appropriately treated and disposed of safely.

Untreated liquid hospital waste containing unmetabolized antibiotics in low concentration contributes largely to the development of antibiotic resistance in our natural microflora/environmental microflora. Peoples of developing countries often bear antibiotic-resistant organisms[5]. The majority of antibiotics used is only partially metabolized after administration, and are released via patient excreta into the municipal sewage system. Antibiotics used in hospitals and private households and released into effluent and municipal sewage indicates a selection pressure on bacteria[6]. Low concentrations of antibiotics in the environment may select for resistant bacteria. These resistant bacteria from environments may be transmitted to humans, in whom they cause disease that cannot be treated by conventional antibiotics[7].

Waste effluent from hospitals and clinics contain high numbers of resistant bacterial strains and residual antibiotics at a concentration to which household waste quantitatively and qualitatively and found that general hospital waste contains bacteria with pathogenic potentials for humans compared to household waste[8]. Long-term exposure of microorganisms to low concentrations of antibiotics in wastewater and surface water has the potential for the development of antibiotic resistance[9]. If the hospital effluents are not treated, concentrated forms of infectious agents and antibiotic resistant microbes are shed into communities resulting in various infectious diseases[3]. The aim of the current study is to isolate and characterize of bacterial strains from hospital liquid wastes in South India.

Experimental

This study was carried out by collecting the liquid wastes from major hospitals and reference centers of South India during the period of October 2014 to January 2015. Primary data were collected for microbiological laboratory works, for which, ten hospitals included where 100 ml of hospital effluent water specimens before discharging into the sewerage system was collected by following standard aseptic procedures. The primary data included color, texture, turbidity, and pH. Each specimen was brought to the laboratory within an hour of collection and cultured for total viable counts in Nutrient agar medium by spread plate method.

Similarly, all specimens were cultured in MacConkey agar media, blood agar and other selective media according to the suspected bacterial strains. The selective media (Hi media) included are *Salmonella*-*Shigella* agar (*Salmonella* and *Shigella* sp), Thiosulphate citrate bile salt agar (*Vibrio* sp.), Mannitol salt agar (*Staphylococcus* sp), Eosin methelene blue agar (*Escherichia coli*), *Pseudomonas* isolation agar (*Pseudomonas* sp) and *Campylobacter* selective media (*Campylobacter* sp.). All the isolates from general and selective agar were morphologically characterized; Gram's stained and performed biochemical tests for genus and species level identification.

Results and Discussion

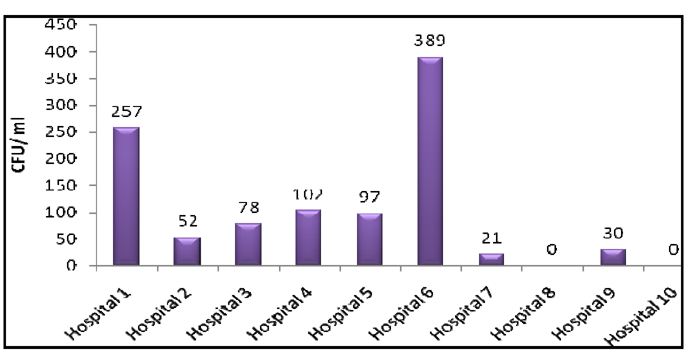

Figure 1: Hospital effluent samples vs Bacterial count

A battery of ten liquid hospital effluents was processed bacteriologically. The total viable bacterial count in Nutrient agar ranged from $21x10^6$ cfu/ ml (colony forming unit/ milliliter) to 389x 10^6 cfu/ ml. Among 10 samples two samples showed no growth. The results of the present study reveal that aerobic and anaerobic bacterial count of the collected hospital wastes during the study period is depicted in table 1.

Table 1: Bacterial counts and frequency of isolated bacterial strains

Bacterial strains	Count (cfu/ ml)	No. of samples supported
E. coli	$3.4x10^6$	4 (40)
Pseudomonas aeruginosa	$1.5x10^6$	5 (50)
Salmonella typhi	$3.2x10^6$	4 (40)
Staphylococcus aureus	$7.5x10^6$	8 (80)
Shigella dysentriae	$3.4x10^6$	4 (40)
Vibrio cholerae	$1.7x10^6$	4 (40)
Campylobacter jejuni	$1.6x10^6$	2 (60)

(Figure in parenthesis denoted percentage)

The frequency of occurrence reveals that *Staphylococcus aureus* (80%) has the highest prevalence followed by *Pseudomonas aeruginosa* (50%) and the least is *Campylobacter jejuni* (20%). The aerobic heterotrophic counts from hospital waste were higher than anaerobic heterotrophic counts. *Staphylococcus aureus* was found to be the predominant species isolated in the present study. The finding is contrary with the other investigators who reported that the *E. coli*[10] and *Bacillus*[11] was the predominant genus found in hospital waste. *Staphylococcus aureus* is a harmless and considered as common commensals, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for emergency outbreaks[12].

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. The morphological and biochemical characters of isolated bacterial species are presented in table 2. The morphological observation in Gram's staining revealed Gram positive and gram negative bacteria and further 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[10]. Heterotrophic strains isolated from biomedical waste are subjected to different biochemical tests.

In our present study the increased number of colony counts was recorded by *Staphylococcus 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, positive to coagulase and some strains showed coagulase negative. 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[13]. The hospital wastes could have contributed immensely in the increase of these bacteria[14] might be due to the fact that hospital wastes are very rich in organic material[15]. 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 and other studies also reported that truly pathogenic forms may survive in waste[16].

The incidence of antibiotic resistant bacteria in the aquatic environment has been widely recognized as a phenomenon with possible implications for public health care[17]. The pattern was almost the same for the diverse species (*E. coli*, *Vibrio, Campylobacter, Salmonella, Staphylococcus, Shigella* and *Pseudomonas*) grown from the effluent samples and strongly suggests prevalence of various plasmids that mediated the normal strains to pathogenic. Other studies also suggested the hospital effluent may provide the wonderful shelter for the growth, multiplication and virulence enhancement of pathogenic microbes[18].

Table 2: Morphological and biochemical characterization of isolated bacterial strains

Characterization	*Escherichia coli*	*Salmonella typhi*	*Shigella dysentriae*	*Staphylococcus aureus*	*Campylobacter jejuni*	*Pseudomonas aeruginosa*	*Vibrio cholerae*
General characterization							
Colony morphology in NA	Circular, raised, punctiform, smooth surface, shiny and translucent	Moist, smooth and raised	Circular, convex, grayish, smooth and translucent	Circular, entire, convex, large, rough, shiny, golden yellow and opaque	-	Smooth, large, translucent, low convex and greenish blue colonies	Moist, translucent disc like colonies
Colony morphology in selective media	Metallic sheen colonies (EMB)	Black centered colonies (SSA)	Colorless colonies without balck centered (SSA)	Mannitol fermenting yellow colonies with yellow zones (MSA)	Traslucent, grey colored colonies (CBSM)	Bluish green colonies (PIA)	Yellow to green colonies (TCBS)
Gram's staining	Gram -ve	Gram -ve	Gram -ve	Gram +ve	Gram -ve	Gram -ve	Gram -ve
Motility	Motile	Motile	Non motile	Non motile	Motile	Motile	Motile
Shape	Short bacilli	Rod	Rod	Cocci	Spirilli	Rod	Comma shaped; rod
Oxygen requirement	Aerobic	Aerobic	Aerobic	Aerobic	Anaerobic	Aerobic	Aerobic
Biochemical characterization							
Catalase	Produced	Produced	Not produced	Produced	Produced	Produced	Produced
Coagulase	ND	ND	ND	Produced	ND	ND	ND
Oxidase	Not produced	Not produced	Not produced	Not produced	Produced	Produced	Produced
Urease	Reduced	Not reduced	Not reduced	ND	Produced	Not produced	Not produced
TSI test	A/A	K/A	K/A	ND	No reduction	K/K	A/A
Nitrate reduction	Reduced	Not reduced	Reduced	ND	Reduced	Reduced	Reduced
Indole production	Produced	Not produced	Produced	ND	ND	Not produced	Produced
Methyl red (acid production)	Produced	Produced	Not produced	ND	ND	Not produced	Not produced
Vogus Proskaur (acetoin production)	Not produced	Not produced	Not produced	ND	ND	Not produced	Produced
Citrate Utilization	Not utilized	Not utilized	Not utilized	ND	Not utilized	Utilized	Not utilized
Sugar fermentation							
Glucose	Utilized	Utilized	Utilized	Utilized	Not Utilized	Utilized	Utilized
Mannitol	Utilized	Utilized	Utilized	Utilized	ND	Utilized	Utilized
Lactose	Utilized	Not utilized	Not utilized	Utilized	Utilized	Not utilized	Not utilized

Sucrose	Utilized	Not utilized	Not utilized	Utilized	ND	Not utilized	Utilized
Fructose	Utilized	Not Utilized	Not utilized	Not utilized	ND	Not utilized	Utilized
Maltose	Utilized	Utilized	Not utilized	Utilized	ND	Not utilized	Utilized

[NA – Nutrient agar; EMB – Eosin Methelene blue agar; SSA – *Salmonella-Shigella* agar; MSA – Mannitol salt agar; CBSM – *Campylobacter* blood free selective medium; PIA – *Pseudomonas* Isolation agar; TCBS – Thiosulphate Citrate Bile Salt agar; ND – Not determined; TSI – Triple Sugar Iron agar; A/A – Acid slant/ Acid butt; K/A – Alkaline slant/ Acid butt]

We isolated bacterial strains from biomedical wastes. These strains could be dangerous due to the high risk of mutating itself as virulent and multidrug resistant, thus leads to environmental hazard. It is recommended that all the effluents drained were properly disinfected before reached to the central processing unit and necessary steps has to be taken by the authorities to protect the environment. 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 and reported that truly pathogenic forms may survive in waste[10].

According to the Biomedical Waste (Management and Handling) Rules 1998, liquid pathological and chemical waste should be appropriately treated before discharge into the sewer. Pathological waste must be treated with chemical disinfectants, neutralized and then flushed into the sewage system. Chemical waste should first be neutralized with appropriate reagents and then flushed into the sewer system[19].

Conclusively, it was observed that hospital wastes have negative influence on the microbiological and physiochemical parameters on the environment. The microbial load as well as the high densities of the physiochemical parameters suggests that the activities of hospital wastes in the environment is a major health and environmental threat[20], which therefore call for a proper regulatory system on disposal of hospital waste in the world, especially in the developing countries like India.

The bulk of the bacteria in the hospital effluent remain firmly adhered to solid particles, where aeration and clarification removes bulk of the bacteria by physical processes including flocculation, the treated liquid effluent still contains sizeable loads of bacteria and inactivation by chlorination and chemical procedures is required and the bacteria get concentrated in sludge and a greater concentration of chlorine is required for decontamination than treated water.

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