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Research Article

Identification of bacteria and fungi in the solid waste generated in hospitals of Sana'a city, Yemen

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ABSTRACT

A medical establishment such as hospitals and medical centers generate a sizable amount of hazardous waste. In Yemen, the hospitals' wastes are still largely mismanaged, mainly because the sector did not know what to do with the waste. The present study was undertaken to determine the bacterial and fungal agents present in different various of the hospitals' solid waste. The samples were collected from the different wards/departments and from the central storage rooms/dustbins of the hospitals in Sana'a city, Yemen. The presence of bacterial and fungal agents in the hospitals' waste was determined using the conventional bacteria, and fungi identification methods. The bacteria and fungi were detected in the hospitals' solid waste were *Klebsiella* spp. (9.3%), *E. coli* (12.7%), *Citrobacter* spp. (8.5), *Candida* spp. (18.6%), *Proteus* spp. (9.3%), *Cladosporium werneckii* spp. (19.5%), *Bacillus* spp. (9.3%), *Aspergillus* spp. (7.6%), *Trichothecium* spp. (0.8%), *Mucor* spp. (3.4%), and *Acinetobacter* spp. (0.8%). This study results confirmed that the prevalence types of microorganism vary based on seasonal and spatial variables.

Keywords: Hospitals waste; Healthcare waste; Pathogenic bacteria and fungi.

1. INTRODUCTION

Hospitals' wastes are a source of potentially dangerous microorganisms that can infect hospital patients, personnel, and the general public. If waste is inadequately managed, these microorganisms can be transmitted by direct contact, through the air or by a variety of vectors, and can pose a serious threat to human health and to the environment [1]. There are many different exposure routes: through injury (cut, prick), through contact with the skin or mucous membranes, through inhalation or through ingestion [2]. More specifically hospitals' waste has a high potential of carrying microorganisms that can infect people who are exposed to it, as well as the community at large if it is not properly disposed of [3]. Hospitals' solid waste may contain varieties of bacteria. The types and quantity of bacteria depend on the hospitals' solid waste compositions and its generation source. Commonly identified bacterial pathogens such as *Pseudomonas* spp., *Corynebacterium diphtheriae*, *E. coli*, and *Staphylococcus* spp., have been reported to be part of the hospitals' wastes. Hospitals' waste should be carefully con-

trolled and monitored to prevent nosocomial infection associated with the exposure to these wastes [4]. The present study was undertaken to determine the bacterial and fungal agents present in different various of the hospitals' solid waste.

2. MATERIALS AND METHODS

2.1. Sampling collection

The leachate's samples from hospitals' solid waste were collected in a sterile screw-capped bottle from the different wards/departments and from the central storage rooms/dustbins of the hospitals of (Al-Thawora, Al-Kuwait, Republic, and Military) in the summer and winter sessions, in Sana'a city. The bottles were labeled with a sample number, date, and time of collection; then immediately delivered to the National Center for Laboratories of Public Health in Sana'a. Samples collections were conducted for a period of eight months from August to March 2013.

2.2. Identification of the bacteria in hospitals' solid waste using the conventional methods

Bacteria were isolated and characterized using cultural identification, and morphological identification using gram staining reaction, sugar fermentation reaction, and biochemical tests. Fungi were isolated using the growth rate, colonial morphological features, and microscopic morphological features as described by Cheesbrough [5].

Each sample was directly inoculated onto blood agar and MacConkey agar using a standard calibrated wire loop (10 µl) for bacterial identification. Streaked culture plates were incubated at 37^{o}C overnight. On the next day, the bacterial growth on the respective media was observed, and the total colony count was done and checked for significant bacteria. Blood and MacConkey agar medium were used to culture the bacteria for gram positive and gram negative bacteria, respectively. Biochemical tests including: Kligler media, SIM, citrate, and urease test were used for the identification of pathogenic bacteria.

Each sample also was directly inoculated onto Sabouraud dextrose (SAB) agar for fungal identification. Streaked culture plates were incubated at 24^{o}C overnight. On the next day, the fungal growth on the respective media was observed, and the total colony count was done and checked for a significant of some fungal such as *Candida* spp. Additional incubation in Petri plates at 22-24 ^{o} C for 7-10 days. After 7-10 days the fungal growth on the respective media was observed, and the total colony count was done and checked for an indication others fungi. Small plaques from the edge and the center of each growing colony were transferred onto glass slides and then were examined using a compound light microscope (Olympus BX41 system microscope) to ensure the exact identification of fungi. Analysis of data was performed by the use of Excel software program. Frequencies and percentages were computed.

3. RESULTS AND DISCUSSION

This study results (Table 1) showed that the microorganisms appeared in the different sites and seasons were: *Klebsiella* spp. (9.3%), *E. coli* (12.7%), *Citrobacter* spp. (8.5%), *Candida* spp. (18.6%), *Proteus* spp. (9.3%), *Cladosporium werneckii* spp. (19.5%), *Bacillus* spp. (9.3%), *Aspergillus* spp. (7.6%), *Trichothecium* spp. (0.8%), *Mucor* spp. (3.4%), and *Acinetobacter* spp. (0.8%).

These findings were observed might because that almost types of bacteria that appeared in this study have high ability to resist difficult environmental conditions under the impact of three methods of defense are: Thickness of cell wall, the ability to produce chemicals from inside the cell to oxidation and reduction of toxic substances faced the cell, and its ability to keep the food inside, which gives them a greater chance to maintain their growth for a longer period. Also, appearance of these species of fungi might due to their ability to grow and multiply for a longer period of time, because they have the ability to mutate into spores, such as *Cladosporium werneckii* spp., resist high temperatures, such as *Trichothecium* spp., its ability to branching and bifurcation such as *Aspergillus* spp., excreted goloco-protein such as *Mucor* spp., and have high resistant such as *Candida* spp.

This study results are in agreement with some findings of previous studies. A study conducted in Malaysia has shown several pathogenic bacteria in clinical solid waste such as *Klebsiella pneumonia*,

and *Acinetobacter baumannii* [6]. A study conducted in Tanzania reported that the bacteria that isolated from the biomedical waste were *Bacillus subtilis* (12%), *Klebsiella pneumonia* (6%), and *E. coli* (15%) [7]. A study conducted in India has reported that the different types of bacteria isolated from biomedical waste were *E. coli* (22.79%), *Klebsiella* sp. (8.82%), *Proteus vulgaris* (5.88%), *Citrobacter* sp. (2.20%) [8]. A study conducted in Bangladesh reported that the different fungi such as *Aspergillus* spp., and *Mucor* spp. were commonly found in the lab analysis [9], while were contrary to other studies. A study conducted in Odisha reported that the fungi species that would be identified were *Rhizopus nigricans*, *Aspergillus flavus*, *Penicillium rubrum*, *Trichothecium roseum*, and *Penicillium viricadum* [10]. A study conducted in Nigeria has mentioned that the fungi species that were isolated from soil of hospital dumpsite were, *Rhizopus nigricans*, *Penicillium rubrum*, *Trichothecium roseum*, and *Penicillium viricadum* [11].

Table 1. The types of microorganism that isolated from the samples were taken from the different sites and seasons.

No.	A*	Counts CFU/ml	B**	Counts CFU/ml	C***	Counts CFU/ml	D****	Counts CFU/ml
1	*Klebsiella* spp.	$\geq 10^5$	*Klebsiella* spp.	$\geq 10^5$	*Klebsiella* spp.	$\geq 10^5$	*Klebsiella* spp.	$\geq 10^4$
2	*E. coli*	$\geq 10^3$	*E. coli*	$\geq 10^5$	*E. coli*	$\geq 10^2$	*E. coli*	$\geq 10^3$
3	*Proteus* spp.	$\geq 10^2$	*Proteus* spp.	$\geq 10^2$	*Proteus* spp.	$\geq 10^2$		
4	*Citrobacter* spp.	$\geq 10^2$	*Citrobacter* spp.	$\geq 10^5$	*Citrobacter* spp.	$\geq 10^4$	*Citrobacter* spp.	$\geq 10^4$
5	*Candida* spp.	$\geq 10^4$	*Candida* spp.	$\geq 10^1$	*Candida* spp.	$\geq 10^1$	*Candida* spp.	$\geq 10^5$
6			*Bacillus* spp.	$\geq 10^5$			*Bacillus* spp.	$\geq 10^3$
7	*Aspergillus* spp.	$\geq 10^3$						
8	*Trichothecium* spp.	$\geq 10^1$						
9			*Cladosporium werneckii* spp.	$\geq 10^5$	*Cladosporium werneckii* spp.	$\geq 10^4$	*Cladosporium werneckii* spp.	$\geq 10^3$
10					*Acinetobacter* spp.	$\geq 10^1$		
11			*Mucor* spp.	$\geq 10^2$	*Mucor* spp.	$\geq 10^1$		

* The samples from the hospitals' dumpsites in the winter season.

** The samples from the departments' waste bags in the winter season.

*** The samples from the departments' waste bags in the summer season.

**** The samples from hospitals' dumpsites in the summer season.

The types of bacteria that were isolated in the present study are responsible for causing many diseases and risks to the community such as, pneumonia, meningitis, urinary tract infection, wound infection, respiratory infection, gastrointestinal illness (*Klebsiella* spp., *proteus* spp. and *Bacillus* spp.), diarrhea (*E. coli*), Primary bloodstream infections, pneumonia (*Acinetobacter* spp.), opportunistic, peritonitis (*Citrobacter* spp.) [9, 12]. *Acinetobacter baumannii* is a major cause of hospital-acquired infections including pneumonia, lung abscess, meningitis, septicaemia, urinary tract and wound infections [13].

The types of fungi that were isolated in the present study are responsible for causing many diseases and risks to the community, such as candidiasis [14]. Many fungi are opportunistic organisms and cause infections during extended antibiotic treatment and severe immunosuppression such as *Candida albicans* and *Aspergillus* spp. [15].

In all the samples the total bacterial and fungal counts were in the range of 10^1 to 10^5 CFU/ml (Table 1). This might because the physicochemical characteristics of the leachate such as pH, anaerobic conditions, and the presence of significant concentrations of toxic substances (phenol, aniline, and heavy metals), all that contributed to reducing the number of species.

4. CONCLUSIONS

This study confirmed the presence of pathogenic bacteria and fungi in the hospitals' solid waste, in the different department/units of the hospitals and in the dumpsite of the hospitals. The hospitals' solid waste contains various types of nosocomial and opportunistic pathogenic bacteria and fungi were appeared in the hospitals sites variation and seasonal variation, confirmed that the prevalence types of microorganism vary based on seasonal and spatial variables.

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AUTHORS' CONTRIBUTION

All authors have equally contribution in conducted studies and manuscript preparation. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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