Isolation and Identification of Pathogenic Bacteria from Soil Mixed with Hospital Wastes

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Abstract

Objective: Hospital solid wastes when not incinerated (nor waste-waters are scientifically treated) remain as sources of a myriad of pathogenic microorganisms.

Methods: A total of 960 waste samples (soil mixed with waste) were collected from waste disposal site of the hospital. After successful growth, bacteria were sub-cultured in nutrient agar slants for preservation. Biochemical characteristics of isolated bacteria were done for identification of bacteria.

Results: A total of 150 collected waste soil samples yielded 103 bacterial colonies, as 65 single and 38 mixed colonies, while 47 samples had no microbial growth. Methicillin resistant *S. aureus* (MRSA), vancomycin resistant *enterococci* (VRE) and *Pseudomonas aeruginosa* isolates were in numbers, 28, 19 and 18, respectively. Of 103 colonies, 63 and 40 were from wastes of wards and ICUs. Invariably, MRSA strains were isolated, as single or mixed colonies. The average minimum inhibitory concentration (MIC) range against oxacillin was recorded as 16 µg/mL for MRSA, and Vancomycin 16 µg/mL for VRE and 8 µg/mL gentamicin for *P. aeruginosa*.

Conclusions: Site specific variation and seasonal variations underplay prevalence of different types of nosocomial spread of opportunistic pathogenic bacteria.

Keywords: *Hospital wastes, MRSA, VRE, Pseudomonas aeruginosa*

Introduction

Wastes are basically the source of pathogenic viruses, bacteria and fungi; especially, hospital solid wastes when not incinerated and waste-waters that are not scientifically treated, remain as sources of a myriad of pathogenic microorganisms. Overall soil-lots, leachates and hospital drains are the overwhelming sources of pathogens, since soil and flowing water are the immediate sink to wastes in dumpsites. Serious health problems of workers linked to places of waste generation and the collection till disposal at dumping sites are vulnerable to direct infections. In most developing countries, the concerned personnel hardly use protective devices for getting exposures. Particularly the indiscriminate waste disposal habits land at release mixed lots of ions, chemicals microbes detrimental to health from pollutes.[1] Due to faulty management of wastes, microbial pathogens from decomposing wastes get transmitted and cause emergence of community-acquired infections, contributing to the burden of morbidity and mortality from communicable diseases.[2] Moreover, pathogens resisting the typical climatic conditions of a place would be transmitted by direct contact with the skin or mucous membranes, through inhalation/ ingestion or by any suitable vector.[3] Often, soil-water interface provides niche that help survival of pathogens that cause infections in the next suitable season depending upon the types composition of hospital waste.[4] Indeed, about 5.3 million people die each year from waste related diseases.[5]

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Commonly identified bacterial pathogens such as, *Pseudomonas* sp., *Corynebacterium diphtheriae, Escherichia coli,* and *Staphylococcus* sp. as methicillin resistant *S. aureus* (MRSA) or methicillin sensitive *S. aureus* (MSSA) and a few more have been reported to be part of the hospital wastes and community wastewaters.[6, 7] MRSA-colonized humans shed bacteria/spores to aerosol from nose, skin and faeces as body-wastes.[8] Furthermore, *Enterococcus faecalis* and *E. faecium* inhabit the gastrointestinal tracts of humans and animals and are the major causes of nosocomial infections worldwide. Vancomycin being the antibiotic of choice for enterococci, the prevalence of vancomycin resistant enterococci (VRE) has increased in the recent decades, presumably due to the high antimicrobial pressure in hospitals from wastewaters.[9] Antimicrobial resistance is one of the most serious public health concerns of the twenty-first century.[10] Hospitals are the main environment for multidrug resistant (MDR) bacteria and play a major role in the emergence and spread of these bacteria that thrive in wastewaters.[11] Moreover, large amounts of antimicrobials are discharged into wastewaters and exert a continuous selective pressure upon the survival of MDR bacterial strains. Heavy metals and disinfectants may also favor the persistence of MDR in the wastewater microbiome, by eliminating sensitive strains.[12] As known, antimicrobial selective pressure favours the intraspecific and interspecific transfer of resistance genes.[4] Particularly, hospital waste-dumping effluent could increase the prevalence of resistant bacteria in the recipient sewage/soil by mechanisms of introduction of resistant bacteria from biomedical waste dumping-site soil.[13] Similar surveillance work being in dearth in literature, this work was initiated, locally. The objective of this study is surveillance by isolation, identification and antibiotic profiling of bacteria from hospital-waste contaminated soil. Herein, quantitative and qualitative data of MRSA are summarized; MIC data of the commonly isolated bacteria isolated from waste-soil samples against representative antibiotics of the day were recorded, which is an extension of previous work on MRSA.[14] This work is expected to help to revision of the present antibiotic stewardship program of the hospital.

Materials and Method

Collection of waste soil samples and isolation of bacteria

A total of 960 waste samples (soil mixed with waste) were collected from waste disposal site of the hospital. Samples were collected in sterile zip-lock plastic maintaining aseptic conditions. The collected samples were used for isolation of pathogenic bacteria; if necessary, samples were stored at 4° C after marking resources or specific location. Serial dilution of soil samples were done for the isolation of bacteria. In this technique sample suspension was prepared by adding soil mixed with 1 gm waste, each. From each dilution tube 0.1ml of dilution fluid was transferred into nutrient agar (NA) culture media and incubated at 37^0C for 24 hours. After successful growth of bacteria, those were sub- cultured in NA slants; for vigorous growth and were preserved. Biochemical characteristics of isolated bacteria were done, as previously performed.[15]

Results

From 150 collected samples, 103 bacterial colonies grew as 65 single and 38 mixed colonies on agar plates and no microbial growth was seen with 47 samples. MRSA, VRE and *P.aeruginosa* isolates were 28, 19 and 18, respectively (Figure 1). Of 103 bacterial colonies, 63 and 40 were from wards and ICU. Of course, there were only 65 single- bacteria were isolated, as a single colony; while the remaining 38 bacteria were isolated as mixed colonies (Table 1). Furthermore, MRSA strains, as both single and mixed colonies were isolated. The minimum inhibitory concentration (MIC) range against Oxacillin was 16 µg/mL, the MIC range, for MRSA, Vancomycin 16 µg/mL for VRE and 8 µg/mL gentamicin for *P.aeruginosa* . These MIC values confirmed the presence of all strains, as the break point for being resistant (Table 2).

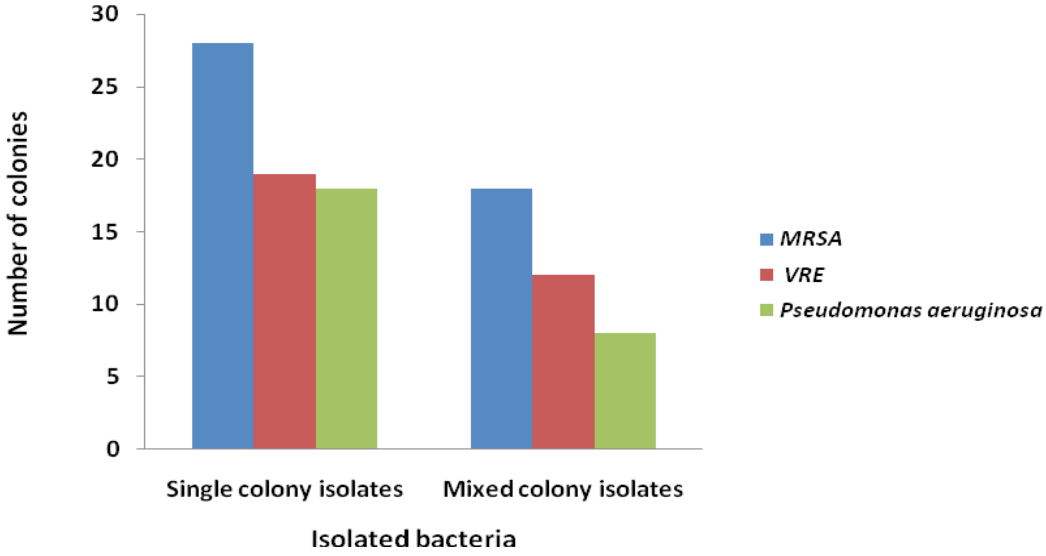

Figure 1 Growth of bacteria in cultures of waste water samples of hospital as single colony and mixed colonies. MRSA: Methicillin resistant *Staphylococcus aureus*, VRE: Vancomycin resistant *Enterococci.*

Table 1 Numbers of growing bacteria from cultures of hospital soil samples with waste.

Colonies	Wards	ICU	Total
Single colony	42	23	65
Mixed colonies	21	17	38
Total	63	40	103

Table 2 Detection of MRSA isolates based on MIC values due to oxacillin in a 12x8 micro-titre plate.

Well	Oxacillin (µg/ml)	Number of isolates MRSA = 28	Vancomycin (µg/ml)	VRE= 19	Gyntamycin (µg/ml)	P. aeruginosa = 18
1	0	28	0	19	0	18
2	≤0.25	–	≤0.25	11	≤0.25	09
3	0.5	–	0.5	17	0.5	12
4	1	–	1	–	1	11
5	2	–	2	–	2	17
6	4	–	4	–	4	18
7	8	–	8	–	8	10
8	16	08	16	11	16	–
9	32	11	32	17	32	–
10	64	19	64	19	64	–
11	128	03	128	18	128	–
12	≥ 256	07	≥ 256	17	≥ 256	–

Note: The oxacillin stock solution of 512 µg/ml was serially diluted at each successive well, from the 12th well for final concentration of 0.25 µg/ml oxacillin at the 2nd well; –, no growth; total *Staphylococcus* sp. = MRSA with123. Results of the second repeated experiment are presented.

Discussions

Since pathogenic bacteria can cause serious public health problems, which demands more research for creating public awareness. Due to irrational uses of antibiotics in last few decades, the numbers of antibiotic resistant bacteria are increasing day by day, and the condition is becoming worse in developing countries.[16] In addition, animal faecal wastes and particulate soil matter at polluted sites provide niches for pathogenic human viruses in addition to bacteria for dissemination and survival directly or as environmental vectors for the horizontal transfer pathogens and their genomes. Indeed, antibiotic resistance genes spread among bacterial consortia, which may be phylogenetically distant even.[17] The occurrence of Methicillin sensitive *S. aureus* (MSSA) and MRSA is on the rise, resulting in increased incidences of hospital-acquired and community-acquired infections worldwide, posing a major public health concern.[18] Basically, *S. aureus* is one of the most successful and adaptable human commensal-turned-pathogen, due to its proficiency in acquiring antibiotic-resistant mechanisms and pathogenic determinants, invading to nosocomial and community settings.[19] Nosocomial colonization of MSSA and MRSA can go undetected, and signs of infection may only appear months after a patient is exposed to the extent of some detectable traits of illness. In USA, MRSA and VRE were identified from a wastewater.[20] *P. aeruginosa* is ubiquitous within wastewaters, with higher concentrations in hospital than in urban wastewater. *P. aeruginosa* isolates resistant to ciprofloxacin or producing VIM-type metallo-β-lactamase, or ESBLs have been obtained from wastewater and hospital discharge sites.[21] Thus, hospital waste dumping effluent could increase the number of resistant bacteria in the recipient sewages by both mechanisms of introduction and selection of MDR bacteria isolated from biomedical waste dumping site soil. [22] Hospital wastewater, which receives high loads of antimicrobial agents and human pathogens, is considered a reservoir for antibiotic resistance and other genetic factors which promote the potential spread of AMR to the environment.

Conclusion

In this present study confirmed the presence of pathogenic bacteria in the hospital solid waste, in the different department/units of the hospital. It contains various types of nosocomial with opportunistic pathogenic bacteria were appeared in the hospitals sites variation and seasonal variation, confirmed that the prevalence types of bacteria vary based on seasonal and spatial variables.

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Conflict of Interest: The authors have no conflict of interest to declare.

Ethical Clearance: In this research no cell-lines/ animals are used. So it's not applicable for ethical clearance.

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