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Airborne Multiple Drug Resistant Bacteria Isolated from Concentrated Municipal Solid Waste Dumping Site of Bangalore, Karnataka, India

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Abstract

Airborne Multiple drug resistant (MDR) bacteria were isolated from municipal solid waste dumping site of Bangalore using Anderson single stage air sampler. Bacterial samples in replicate were collected and MDR were isolated by Kirby-Bauer disk diffusion method.MDR organisms like Staphylococcus aureus was recorded higher in number in the ambient air near the dump sites. Maximum populations of multi drug resistant bacteria were recorded near the dumping site. Bacterial organisms like Staphylococcus aureus and Enterococcus sp., were the most prevalent organisms recovered. The change in the distribution pattern of MDR organisms in the ambient air was statistically found to be significant (Amoxicillin, p = 0.010; Ampicillin, p = 0.011; Ciproflaxin, p = 0.005; Rifampin, p = 0.025). The percentage of MDR in organisms like streptococci and Enterococci increased near the dump sites compared to percentage of MDR recorded upwind. Higher numbers of MDR were recovered in dump site and minimum was recorded at the distance of 100m away from the dumping site. This situation may cause health effect to the villages and the agricultural lands around the dumping site.

Keywords: Dumping site, Bio aerosol, Drug resistant Bacteria, *Staphyloccoccusaureus Streptococcussp*, *Enteriococci*, Health Effects.

Introduction

Modern urbanization, municipal solid waste (MSW) creates a serious environmental problem in many developing countries. In Bangalore urban generate a great amount of municipal solid waste which is dumped on land in a uncontrolled manner. Accumulation of solid waste in unscientific way leads to leachate formation that contains a large number of xenobiotic organic compounds and contaminates the air. Soil, surface and ground waters in the vicinity of the site[1]. Pathogens found in MSW, can be viruses, bacteria, protozoa or helminthes. Municipal solid wastes Management are a large scale disposal problem in Bangalore. It has been estimated those 3600 tons per day in 2012 with the population 8,465,752. In which 1,139 tones are collected and sent to composting units such as Karnataka composting Development Corporation[2]. The remaining solid waste are collected by the municipality is dumped in open spaces or non-roadsides outsiders the city. Solid waste management failure in Bangalore during 2012 cause Solid waste dumping crisis in cities and its impacts are pathogenic Bioaerosole mission, deterioration of air and water quality. Urban waste consist of various components such as House hold, commercial and hospital waste are homogenized every street corner of Bangalore. The hazardous substance in solid waste sludge has potentially induced the expression of antibiotic resistant genes in many viable microorganisms. Unscientific disposal of solid waste are the major cause for Human exposure to the microorganisms may take place at every step near the dumping sites causes various health impacts. The present study was conducted with interest on public health of residence near the municipal solid waste dumping site in Bangalore.

Material and Methods

Study area: Study was carried out at Mandur dump site is located 14 km away from Bangalore city. The geographical location of dumping sit in Mandur dump site is 130 05”01’ N and 770 43”45’ E. About 1,800 tons of the 4,500 tons of garbage generated by the city is dumped daily in Mandur. The dump site falls within the limits of two village panchayats — Mandur and Bidarahalli. The two villages have a total population of about 20,000 to 25,000.

Sample collection: An Andersen single-stage sampler was used to collect all bacterial samples from the solid waste dumping sites. Tryptic soy agar (TSA; Hi-Media), were used to enumerate wide variety of bacterial microorganisms. Meteorological parameters (wind speed and direction) were determined by Votex flex wire Anemometer and Temperature and relative humidity were recorded using digital thermometer and hygrometer. Air samples were taken near the dumping sites and transferred to laboratory in a aseptic condition within 12hr. The sampled plates were incubated at 35°C and the colonies that developed were counted after 24 and 48 hr. After 48hr of incubation, the plates were stored at 4°C in inverted position and used for the replica platemethod[3].

Isolation: A recovered aerosolized bacterium was identified by replica plate method by culturing bacteria onto a selective medium[3]. The replica plate method was conducted using

Mannitol salt agar w for *Staphylococcus* sp., MacConkey agar for coliforms, fecal coliform agar for fecal coliforms, and selective *Streptococcus* agar for isolation of *streptococci* (Hi-Media) to confirm the bacteria. Tryptic soya agar was used as a final control and ensured that the organisms were being completely transferred to all plates and incubated at 35°C for 24 to48 hr. Further the organisms are confirmed by characteristic biochemical tests. After enumerating bacterial colonies, the plates were kept in an inverted position at 4°C until they were ready to be transferred onto Triple sugar agar slants to be used for antibiotic sensitivity test.

Antimicrobial susceptibility testing: Antibiotic resistant characteristic of the bacteria was determined by following Kirby-Bauer disk diffusion method[4]. Three Mueller-Hinton agar plates and three Tryptic soya agar plates were dried at room temperature for test in gantibiotic resistance. A steriled cotton swab was used totransfer several colonies of the micro organism from the slant to a sterile saline tube until thetube was the same turbidity. The bacterial density was estimated around 108cfu/mL. The Kirby Bauerdisk diffusion method was then performed in aseptic condition. The plates were checked for susceptibility after 24 hr. The zones of inhibition were recorded for all of the plates and then compared with the standard of National Committee for Clinical Laboratory Standards[5- 7]. Then determined whether the micro organism was susceptible, intermediately resistant to each antibiotic evaluated.

Results and Discussion

Ambient Microbial pollution at the dumping sites: Ambient microbial pollution was studied at Mandur dumping site. The mean number of *staphylococcus aureus* present in the air near the dumping site was 3×10^4 CFU/m3 (12%) and *Enterococcus sp*., was found to be 2.1×10^3 CFU/m3, 18 isolates (19%). The viridians groups of *streptococci* was found o be 1.1×10^2, 10 isolates (10%) and 21 isolate was identified as *Aeromonas* (23%). Nine isolates was identified as *E. coli* (10%) (table-1).

Antibiotic resistant: The *staphylococcus aureus* and *Streptococcus sp.,* developed resistant towards antibiotics like (Amoxicillin, $p = 0.010$; Ampicillin, $p = 0.011$; Ciproflaxin, $p = 0.005$; Rifampin, $p = 0.025$). Amoxicillin and Ampicillin and highest number of resistant strains were isolated near the garbage dumping site (table-1). The level of Amoxicillin – resistant *Staphylococcus aureus* in garbage dumping site found to be 36 isolates (±6.5) and 12 isolates (±4.5) % at the 100 m away from the dumping site. Ampicillin–resistant *S. aureus* in garbage dumping site found to be 28 isolates (±5.2) and 14 isolates (±3.2) at the 100m away from the dumping site. Similarly, Amoxicillin and Ampicillin resistant *Enterococcus sp.* and *Streptococcussp* were isolated inside dumping site which was found to be 24 isolates (±2.2) and 10 isolates (±3.2) at the distance of 100 away from the dumping site.

Table-1
Depicts the characterization of multi-drug resistant organisms isolated from solid waste dumpsite (NCCLS zone diameters used to categories the susceptible, intermediate, or resistant)

Name of the antibiotics	Disc Potency	Name of the species	No of Cells CFU/100ml	Zone Diameter (mm)		
				Resistant	Intermediate	Susceptible
Amoxicillin	10µg	*Staphylococcus aureus*	$1.01x10^6$	≤23	18-21	≥26
		Streptococcus sp	$1.6x10^3$	≤15	14-16	≥22
		Enteriococci	$1.12x10^4$	≤12	15-17	≥18
Ciproflaxin	10µg	*Staphylococcus aureus*	$1.01x10^6$	≤14	16-18	≥21
		Streptococcus sp	$1.6x10^3$	≤13	14-21	≥19
		Enteriococci	$1.12x10^4$	≤10	13-16	≥15
Ampicillin	10µg	*Staphylococcus aureus*	$1.01x10^6$	≤25	-	≥27
		Streptococcus sp	$1.6x10^3$	≤18	18-19	≥21
		Enteriococci	$1.12x10^4$	≤16	14-Dec	≥20
Gentamycin	10µg	*Staphylococcus aureus*	$1.01x10^6$	≤10	13-16	≥16
		Streptococcus sp	$1.6x10^3$	≤13	15-17	≥20
		Enteriococci	$1.12x10^4$	≤10	13-14	≥13
Rifampin	10µg	*Staphylococcus aureus*	$1.01x10^6$	≤14	16-18	≥16
		Streptococcus sp	$1.6x10^3$	≤14	17-18	≥19
		Enteriococci	$1.12x10^4$	≤16	17-18	≥21
Tetracyclin	10µg	*Staphylococcus aureus*	$1.01x10^6$	≤12	14-16	≥20
		Streptococcus sp	$1.6x10^3$	≤19	19-21	≥26
		Enteriococci	$1.12x10^4$	≤16	-	≥23

While minimum numbers of bacterial isolates were found at upwind distance of 50m from the garbage dumping site. Majority of them are *Aeromonas sp., streptococcus sp., staphylococcus sp., Enterococcussp* and *Bacillus sp*. Minimum number of drug resistant bacteria identified at upwind. The physical examination of municipal solid waste found that common household products like toothpastes, kitchen plastics, deodorant bottles, cement and paints and used syringe, expired tablets have been identified at the Mandur dumping site. More than 700 "antibacterial" domestic products have been introduced in the past five years that includes sweat socks, toothpastes, kitchen plastics, cement and paints. The more common antibacterial ingredients in these formulations are triclosan, quartenary ammonium compounds, alcohol, and bleach. Microbes resistant to each of these compounds have been documented in nature and in some human pathogens[8].

Antibiotic resistant developing agents like alcohol, alkene, and steroid based material present in the garbage may induce the drug resistant capacity in gram negative bacteria. In other hand, Organic and inorganic toxic substance in waste materials are consumed by the bacteria used as nutrients and developed the resistant character. Similar findings reported by Green *et al.,* [9]. Found that percentage of antibiotic resistant bacteria number is more near dumping sites and decreased tendency to distance. *Staphylococcus aureus* was the most prevalent organism sampled, accounting for 76% (1.4×10^4cfu/m3; SD, 8.9×10^3cfu/m3) of the bacteria recovered inside of the concentrated animal feeding site. Similarly, *staphylococci* are the third most common causes of nosocomial infections and the most common causes of nosocomial bacteria[10]. This result revealed that antibiotic resistant bacteria originated from garbage dumping site and widespread to downwind area. The presence of multidrug-resistant *staphylococci* in air significantly limits the treatment options for the patient infected by this organism. Although streptococci species normally present in the human respiratory tract, they also have been implicated as the cause of infective endocarditic and life-threatening septicemias in neutropenic patients. In addition, Streptococci have been implicated as reservoirs of erythromycin-resistance genes, possibly capable of transferring resistance determinants to more pathogenic species including *Streptococcus pneumonia* and *Streptococcus pyogenes*[11]. The study revealed that *Streptococcus sp.* and *Staphylococcus sp.* developed drug resistant character and dominated among the other airborne bacteria.

Role of meteorological parameters: Meteorological parameters like temperature, relative humidity, wind speed and direction was recorded to predict the role of meteorological parameters for dispersion and transportation of aerosolized bacteria from the origin (table-2). The average temperature (25.8^0C) and relative humidity (64%) at dumping site area is sufficient for the growth and multiplication of measophilic organisms namely, *Staphylococcus aureus, Staphylococcus sp.,* and *Enteriococcussp*. Maximum wind speed 11.2 Km/hr was recorded at dumping site which may maximize the microbialaerosolization. Similarly, the elevation of solid waste dumping site developed approximately 5 to 6 meters above the ground where the air turbulence will be high at elevation of 5 to 20 meters (figure-1). Maximum turbulence at higher elevation may influence the dispersion and transportation biological aerosol to longer distance.

Table-2
Meteorological parameters of Mandur solid waste dumping site

Meteorological parameters	Minimum	Maximum	Average
Temperature (^{0}C)	23.2	28.5	25.8
Relative Humidity (%)	56	72	64
Wind speed (Km/hr)	3.6	11.2	7.4
Wind direction	NW	NW	NW

Figure-1
View of Municipal solid waste hype at Mandur

Figure-2
Represents the medical waste (expired medicines, used syringe, Blood bags) exposed with other solid waste at Mandur dump site

Conclusion

High percentage of bacterial pollution with multidrug resistances was recovered inside dumping site of Mandur and up to100 m downwind of this dumping site at higher population than upwind. The study revealed that bacterial pollution with multidrug resistant are found within dumping site and widespread by wind. This situation could be at risk for adverse human health effects associated with exposure to large numbers of multidrug-resistant organisms for the people working in dumping site and people residing near the dumping site.

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