

Vol. 49, Nos. 1-2 January - March. 2007 April - June. 2007 pp. 25 - 30

Pathogenic and opportunistic Gram-negative bacteria in soil, leachate and air in San Nicolás landfill at Aguascalientes, Mexico

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ABSTRACT. The occurrence of Gram-negative pathogenic and opportunistic species, was studied for two years on air, soil and leachate from the San Nicolás Landfill, Aguascalientes, Mexico. For soil and leachate four samplings were done, two during the dry season and two during the rainy season. For soil there were 15 sampling points, the leachate samples were taken on the leachate tank. For air, twelve sampling were done in three points of the landfill. Twenty pathogenic and/or opportunistic bacteria were identified from air, twenty from soil and eleven from leachate. Most of them were enteric; however respiratory tract pathogenic bacteria were also identified. Pasteurella haemolytica were isolated in all air samples. Nine species were found in the a half of the soil samples. The most frequent species in leachate were Acinetobacter baumanii, Bordetella sp, Brucella sp, and Escherichia coli var II. The occurrence of pathogenic and opportunistic species points out to the nosocomial and domestic clinical wastes discharged in the landfill as a potential risk for public and occupational health.

ORIGINAL ARTICLE

Key words: Landfill, pathogenic bacteria, opportunistic bacteria, airborne bacteria, leachates.

INTRODUCTION

Mexico is faced with serious environmental and administrative challenges regarding solid waste management. Like many developing countries, Mexico's public sanitation system lacks adequate planning, and a sustainable solid waste management program. Mexico's ten largest cities concentrated 70% of the population, resulting in changes in the population's consumption patterns, leading to a more heterogeneous composition of solid waste, and an important increment in its generation rate.⁶

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First version received: February 26, 2007; first version revised April 30, and June 6, 2007; second version received: August 06, 2007; Accepted: October 06, 2007.

RESUMEN. Para detectar la presencia de microorganismos potencialmente patógenos, durante dos años se realizó un estudio microbiológico en muestras de aire, suelo y lixiviados provenientes del relleno sanitario San Nicolás, ubicado al noreste de la Ciudad de Aguascalientes. Se realizaron cuatro muestreos del suelo en 15 puntos del relleno, dos en periodo de estiaje y dos en periodo de lluvias, cuatro en el tanque de lixiviados y doce del aire en tres puntos del relleno. Se identificaron 20 especies de bacterias patógenas y/o oportunistas en el aire, veinte en el suelo y once en los lixiviados, la mayor parte de ellas entéricas, aunque también se encontraron patógenas del tracto respiratorio. Pasteurella haemolytica se aisló en todos los muestreos de aire. En el suelo nueve especies se encontraron en la mitad de los muestreos. En los lixiviados Acinetobacter baumanii, Bordetella sp, Brucella sp y Escherichia coli var II fueron las especies más frecuentes. La presencia de especies patógenas y oportunistas indica que un número importante de desechos clínicos de origen doméstico y hospitalario llegan al relleno y representan un riesgo para la salud pública y ocupacional.

Palabras clave: Rellenos sanitarios, bacterias patógenas, bacterias oportunistas, bacterias del aire, lixiviados.

Landfills are the most widely used solid waste disposal method across the world. In major cities of Mexico this method is the best environmental and technical alternative, although several of them operate inadequately. Studies on landfills have been mainly devoted to waste composition, gas emission and physical parameters. Despite the importance of microorganisms in the decomposition of organic matter, knowledge on the bacterial population is still fragmentary. The microbiology of landfill ecosystems has not been thoroughly explored; they are unique anaerobic ecosystems with abundance of degradable organic carbon, and a wide range of microbial activities due to its heterogeneous composition. Therefore to characterize the microorganisms, researchers have developed several methods including: decomposition studies; sample collection; enumeration; microbial activity; and enzyme assays.²¹ Methanogenic and methanotrophic assemblages in landfills samples have been characterized by molecular techniques.4,22,35 Enumeration and characterization of cellulolytic bacteria from waste of a landfill were performed by Pourcher et al.³⁰ Molecular phylogenetic studies on the diversity of bacteria associated with leachates were published recently.²³

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Rev Latinoam Microbiol 2007; 49 (1-2): 25-30

Landfills contain large numbers of pathogenic and opportunistic bacteria,^{21,33} due to the presence of used disposable napkins and sanitary towels, clinical waste and domestic human origin waste as hypodermic needles and syringes. Studies about pathogenic and opportunistic bacteria in landfills are scarce. In a review published in 1992¹¹ there were 16 pathogenic species listed, the most important of them were: Acinetobacter calcoaceticus, Enterobacter cloacae, some serotypes of Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Proteus spp, Pseudomonas aeruginosa, Salmonella spp, Serratia marcescens, Staphylococcus aureus, and Yersinia enterocolitica. Rosas et al.32 isolated 13 pathogenic and opportunistic bacteria from samples of a domestic waste transfer station in Mexico City; Citrobacter, Enterobacter, Escherichia, Hafnia, Klebsiella, Salmonella, Serratia and Yersinia species were identified in several samples.

In comparison with normal operation standards for landfills in Mexico, the control and management of the San Nicolás landfill are appropriate. However, significant amounts of biologically dangerous materials (from domestic, nosocomial and mainly slaughterhouse origin) are systematically disposed in this landfill. Therefore, it is important to search for pathogens and opportunistic species that could be a sanitary risk for landfill workers, the inhabitants of the nearby districts (at less than 6 km from the landfill), and the cattle that graze and drink a few meters form the landfill. The abundant wild birds that thrive in this landfill could also be a dispersion factors for pathogenic species.

There are a wide range of pathogenic agents, including virus, bacteria, protozoa, fungi, etc. However, the goal of this study was limited to search for pathogenic and opportunistic Gram-negative bacteria in samples of air, soil and leachates from San Nicolás landfill, located outside of Aguascalientes City, Mexico, to evaluate the sanitary risk mainly for the landfill workers and frequent visitors.

MATERIALS AND METHODS

San Nicolás landfill is located at northeast of Aguascalientes city (Fig. 1), was opened in 1999 and receives 1000 tons of municipal solid waste every day. The urban limit is about 6 km away from the landfill.



Figure 1. Location of San Nicolas landfill, and the sampling sites.

Soil samples were obtained from topsoil and at 30 cm bellow the surface (clay in all this depth), from 15 sampling points distributed along the surface of the landfill (Fig.1). Four collections were done (two during dry and two during rainy seasons) during two years. For each sample 1 g of soil was diluted on 10 ml of PBS and vortexed vigorously. With this suspension, diluted series to extinction were prepared with phosphate buffer pH 7.4 (1:10-1:10⁻⁸), by triplicate.¹⁷ The last dilution of every series was grown on general and selective agar media. Standard Methods Agar (SMA) and Brain Hearth Infusion (BHI) agar were used to estimate the total counts of bacteria in the soil samples. Selective media were also used: Blood Agar for fastidious bacteria; Chocolate Agar for fastidious bacteria, especially Neisseria species; Brilliant Green Agar for selective isolation of Salmonella typhi, Proteus or Pseudomonas species and Salmonella-Shigella Agar.¹ One series was incubated al 37 °C for 48-72 h in anaerobic condition, and the other series was incubated at 37 ^oC during 48-76 h in aerobic condition. Based on its morphologic characteristics, each different kind of colony observed in the selective media used was further characterized for strain identification. First, Gram staining was done in order to test the colony purity; if necessary, the strains were isolated by spatially streaking or spreading on solid medium,¹⁷ and then they were identified using biochemical Api galleries (strips) (Biomériéux: Api 20E and Api 20NE). The results were analyzed by Api Lab System (Biomériéux). Finally, the colonies of the same kind in each selective media plate were counted and expressed as CFU/g of soil.

Leachate samples were collected from the leachate tank (Fig. 1) in sterile flasks and transferred to laboratory. They were diluted and processed as soil samples.

Air was sampled in 12 occasions, at three sites of the landfill (Fig. 1), using a portable air sampler for Agar plates (Burkard). The sampler was operated for 2 min at 20 l/min and was mounted at 2 m high tower facing into the wind. The samples were loaded with plastic Petri dishes containing 20 ml of Trypticase Soy Agar (TSA), for the cultivation of a wide variety of fastidious or no fastidious microorganisms clinical and no clinical, used for the rapid estimation of bacteriological quality.¹ TSA plates were incubated at 37 ^oC for 48 h. Colonies on each plate were counted and then transformed and expressed as CFU/m³ of air. The sampling time was short (2 min) because larger periods saturated the plates, making impossible to count and characterize the colonies. After this, the colonies were typified by their morphologic characteristics. Gram staining was done in order to test the colony purity, in the necessary cases the strains were isolated by spatially streaking or spreading on solid medium.¹⁷ The isolated colonies were transferred to Eosin Methylene Blue Agar for enterobacteria isolation, Brilliant Green Agar, Salmonella Shigella Agar, Xylose Lisine Deoxycolate Agar for enteric pathogens,¹ and incubated at 37 ^OC for 48 h. The representative colonies were isolated and identified using biochemical tests (Api 20E Biomériéux).

RESULTS

Thirty nine pathogenic and opportunistic Gram-negative bacteria were isolated from samples of the San Nicolás landfill (10 were pathogenic, 17 were opportunistic and two were plant pathogens). Twenty species were isolated from soil; during dry season were observed more species (17) than in rainy season (9), and four of them were pathogenic. None species occurred in all seasons, five of them appeared in two seasons, and 13 species appeared only in one season. The mean of total bacterial count in soil, leachates and air were 3.0X10⁸ CFU/g, 1.5 X 10⁶ CFU/ml y 4.4 X10³ CFU/m³, respectively.

Microbial leachates analysis results in 12 species isolated, two of them: *Actinobacillus pleuropneumoniae* and *Bordetella sp* are pathogenic of respiratory tract (the first in pigs and the second in humans), *Escherichia coli* var II is pathogenic of the digestive and urinary tract, *Brucella sp* causes brucellosis and *Acinetobacter baumannii* is a systemic pathogen, the remainder are opportunistic. Three species: *Acinetobacter baumanii*, *Bordetella sp*, and *Brucella sp* were present in 75% of the samples.

Airborne bacteria were represented by 19 species. Only *Pasteurella haemolytica* was isolated in all samples, *Serratia plymuthica* and *Aeromonas hydroplyla* respectively were the second and third species more frequently found. Half of the species were isolated from only one sample. Some species (6) that occurred in soil and leachates are pathogenic, and a larger number are opportunistic (13).

Seven species: Aeromonas hidrophyla, Burkholderia cepacia, Enterobacter amnigenus, E. cloacae, E. sakazakii, Klebsiella pneumoniae and Serratia plymuthica were found in air and soil samples, while three species: Acinetobacter baumannii, Bordetella sp and Pasteurella multocida were observed in soil and leachates, and only one species, Ochrobactrum anthropi was isolated from air and leachates (Table 1).

aphic.com discussion

During the 2004 dry season 11 bacterial species were isolated from soil, and nine bacterial species were isolated in the 2005 dry season. By contrast, only three and six bacteria species were isolated during the 2004 and 2005 rainy seasons, respectively. This fact was probably due to migration and infiltration to lower layers caused by rain. No species were isolated in all samplings and a clear pattern was not observed for any species. Collins & Kennedy¹¹ reported 21 species found

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Species	Soil	Dry 2004 Liq*	Air	Soil	Rainy 2004 Iiq	Air	Soil	Dry 2005 Liq	Air	Rainy 2 Soil	2005 Liq	Public health remarks
Salmonella sp			33									Salmonellosis, typhoid fever, carrier
Yersinia enterocolitica Escherichia coli var II Actinobacillus pleuropneumoniae Bordetella sp	3.1 X 10⁴	5.1 X 10⁴	75 166		1.2 X 10 ⁵ 3.9 X 10 ⁴ 2.3 X 10 ⁵			1.1 X 10 ⁴ 3.2 X 10 ⁴ 2.0 X 10 ⁴		1.2 X 10⁴	2.0 X 10⁴ 1.2 X 10⁴	state, gastroentertits. ² Acute gastroenterits. ¹³ Diarrhea and urinary fract infections. ¹³ Pleuropneumonia in pigs ⁵ <i>B. pertussis</i> Whooping cough R. neranetriseis
Klebsiella pneumoniae Brucella Aeromonas tydrophyla Aeromonas salmonicida Acinetobacter baumannii	3.1 X 10⁵	1.0 X 10 ⁵ 3.0 X 10 ³	300		1.0 X 10 ⁵ 1.7 X 10 ⁵	30	3.1 X 10⁴ 6 X 10⁴	1.0 X 10⁴ 2.0 X 10⁴	60 25	2.6 X 10 ⁸		Paroxysms of coughing ^{6, 24} Paroxysms of coughing ^{6, 24} Sepsis, urinary tract infections, pneumonia ²⁸ Brucellosis ¹³ Acute diarrhea in children ¹⁸ Acute diarrhea in children ¹⁸
A. Iwoffii											2.0 X 10⁴	urinary tract infections, peritonitis, surgical site, meningitis and sinusitis ²⁵ Saprophytes, skin and throat commensals,
Burkholderia cepacia Cedecea Iapagei Citrobacter freundii Chromobacterium violaceum Chryseobacterium indologenes Chryseomonas luteola	2.0 X 10 ³ 5 X 10 ⁴			2.0 X 10 ⁴		20	2.0 X 10⁴ 5.1 X 10⁵		33	1.6 X 10⁴	1.5 X 10 ⁴	preseptal cellulits, chronic gastritis, cancer ³¹ Opportunistic, in cystic fibrosis ²⁸ Opportunistic, eritonitis ¹² Opportunistic sepsis ⁶ Opportunistic, sepsis ⁶ Opportunistic, septicemia, meningitis,
Enterobacter amnigenus	5 X 10⁴					16						endocariotis, or peritonitis* Opportunistic, pathogenic occasionally urinary tract infections, occasionally
E. cloacae E. sakazakii Entrochondre en	6.5 X 10 ⁴ 5 e V 10 ⁴					33 25				5.0 X 10⁴ 3.5 X 10⁴		meninguts and septicemia*
Erwinia sp Ewingella americana Flavimonas oryzihabitans Hafinia alvei	0.0 × 10 1.5 × 10 ³ 1.5 × 10 ³						2.0 X 10⁴		12			Plant pathogen ²¹ Opportunistic, pneumonia ³⁴ Opportunistic, bacteremia ³ Opportunistic, bilateral lung tuberculosis,
Leclercia adecarbox/ata Ochrobactrum anthropi Pantoea sp Pasteurella haemolytica P. multocida	2.0 X 10⁵		50 446		6.0 X 10 ¹	50 1,237	2.0 X 10⁴		16 50 779		4.7 X 10 ⁴	sepsis, gastroenernts ³ Opportunistic, sepsis, Opportunistic, sepsis, perumonia ³ Opportunistic, bacteremia ³ Horse and bovine pneumonia ¹³ Opportunistic, sepsis and colonizes
P. pneumotropica Proteus vulgaris				3.2 X 10⁵	2.2 X 10 ⁵		2.0 X 10⁴	6.0 X 10⁴				numan nasopinary nx. Opportunistic, pneumonia, endocarditis ¹³ Oportunistic, Bacteranda, sepsis, pneumonia,
Pseudomonas aeruginosa Ps. fluorescens						88			122		2.0 X 10⁴	unitary intecuon, our intection Oportunistic, in cystic fibrosis, immune sys tem dange, urinary infections, nosocomial infections ⁵ Plant pathogen ²⁴
Ps. putida	1.2 X 10 ⁷						3.2 X 10⁵					Opportunistic, nosocomial infections,
Rahnella aquatilis Serratia odorifera S. plymuthica		0 C < 103	122	1.6 X 10 ⁵		16 93	0 0 X 405		25 36	404 404		Opportunistic, uropathogen, laryix pathogen ¹⁵ Opportunistic, urinary infections ³ Opportunistic, urinary infections ³ Opportunistic and facultarive pathogen ²
Vibito itavians Number of species	11	2.0 A 10 ²	8	3	7	10	0.2 × 10 9	9	11	5.9 A 10	9	Opportunistic, pediatric dialifiea
*: liquid samples: leachates												

in municipal wastes of Great Britain; five of them (*Acineto-bacter* sp, *Enterobacter* spp, *Klebsiella pneumoniae*, *Pseudomonas putida* and *Serratia plymuthica*) were also isolated from the San Nicolas landfill soil samples.

The number of bacteria species isolated from the leachates (12) was less than those isolated from soil (20). The physicochemical characteristics of leachates, unfavorable for microbial grow, as high reduction conditions (redox potential of -250 mV), highly anaerobic conditions, and the presence of significant concentrations of toxic substances (phenol, aniline and heavy metals) contributed to reduce the number of species. In the 2004 dry season the number of isolated species was four, in the 2004 and 2005 rainy and in the 2005 dry season, the number of isolated species were similar (between six and seven). Five of the twelve isolated species: Acinetobacter baumanii, Actinobacillus pleuropneumoniae Bordetella sp, Brucella sp and Escherichia coli var II. were pathogenic and seven potentially pathogenic (see Table 1). The concentration for ten of the twelve species isolated was between 10⁴-10⁵ CFU/ml, this suggest a great adaptative potential for these species. Collins & Kennedy,¹¹ reported eight species isolated from leachates: Klebsiella pneumoniae, Proteus sp, Salmonella sp, Staphylococcus aureus and four species of the Streptococcus genus, in this study only Klebsiella pneumoniae was isolated.

Twenty Gram-negative airborne species were isolated, three of them were pathogenic, three caused digestive tract infections, and one caused respiratory tract infections, all remainder species were opportunistic pathogenic. In the atmosphere there are several plant, animal and human pathogenics. Dowd & Maier¹⁴ enumerate 19 pathogenic bacteria to human and domestic animals, three species from this list: *Klebsiella pneumoniae*, *Salmonella* sp. and *Yersinia enterocolitica* were isolated from the air of the San Nicolas landfill. In all air samples the total bacterial counts were in the range of 10³ to 10⁴ CFU/m³. This data agree with the results of Lis *et al.*²⁷ However, in this work only Gram negative bacteria counts were considered (see Table 1). In all samples, the Gram negative bacteria counts in air were much lower than Gram positive bacteria counts.

Lis *et al.*²⁷ found more Gram-positive bacteria than Gram-negative bacteria indoor and outdoor from landfills in Poland. In outdoor identified 24 species, eight of them were Gram-negative. Only three species: *Enterobacter amnigenus, Pasteurella haemolytica* and *Ochrobactrum* anthropii were isolated in both landfills of Poland and San Nicolas, the waste composition and environmental conditions were probably determinant factors for this poor similarity.

Rosas *et al.*³² identified 14 genera including pathogenic and opportunistic species from air samples in a domestic waste transfer station in Mexico City. Counts of bacterial colonies belonging to the genera *Enterobacter, Acinetobacter* and *Escherichia*, were the most abundant. Out of the 14 genera reported by Rosas *et* al.³² six were isolated in this study: *Enterobacter, Escherichia, Klebsiella, Pseudomonas, Salmonella* and *Serratia*, however the most abundant and more frequent bacteria species isolated from all samples in the air of the San Nicolas landfill, *Pasteurella haemolytica*, was not isolated in the Mexico City wastes.

Seven species were isolated from the air and the soil. Only three were isolated from soil and leachates, and only *Escherichia coli* var II and *Ochrobactrum anthropii* from air and leachates and none was isolated from the three environments. Perhaps *Ochrobactrum anthropii*, isolated from air and leachates samples survive in the soil too, but the number of samples was insufficient to find it.

The genus *Pasteurella* showed a great adaptive potential, similar species were capable to live in different habitats. This genus has been studied due to its ability to colonize several media.²

The occurrence of pathogenic Gram-negative bacteria isolated and identified in soil, leachate and air from the San Nicolás landfill, suggest that there is a significant sanitary risk, especially to the permanent workers of the landfill. There is a risk for the landfill workers to develop gastrointestinal and respiratory infections. However, diseases as bacteremia, meningitis, urinary tract infections, and other opportunistic infections can also occur, especially for immunosuppressed individuals.

The cattle, pigs and chicken living nearby are also in risk. There are farms very close (less than one km) to the landfill and even cattle graze and drink a few meters away from the landfill. Several strains isolated from the landfill were pathogenic for bovine, pigs and birds. These animals could also be a potential risk as pathogen dispersers. Much of the pathogenic bacteria are transmitted by infected hosts. However several pathogenic microorganisms are transmitted by environmental carriers, especially pathogenic opportunistic species.³³

The occurrence of 39 pathogenic and opportunistic bacteria in the San Nicolás landfill is a matter of public health, and the authorities must implement a program to protect the landfill workers and waste delivery drivers, to reduce the health risks. We recommend that the San Nicolás landfill authorities must implement a better control of biologically dangerous waste, mainly in the case of nosocomial and slaughterhouse residues.

ACKNOWLEDGEMENTS

We are grateful to Dr. Roberto Rico Martínez for the comments and revision to manuscript.

We are grateful to Christian Emanuel Macias Morones for his technical assistance. This study was financially supported by Fondos Mixtos-Consejo de Ciencia y Tecnología del Estado de Aguascalientes (AGS2003-CO2-11326) Rev Latinoam Microbiol 2007; 49 (1-2): 25-30

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