



## RESEARCH ARTICLE

# Bacteria related to health public in the air dust and precipitate outdoors of Morelia, Michoacán, México

Margarita Rodríguez-Cáceres<sup>1</sup>, Lucía Consuelo Jiménez-Juárez<sup>1</sup>, Juan Luis Ignacio de la Cruz<sup>1</sup>, Elda María Beltrán-Peña<sup>2</sup> and Juan Manuel Sánchez-Yáñez<sup>3\*</sup>

<sup>1</sup>Environmental Microbiology Laboratory  
Research Institute in Biology and Chemistry

Universidad Michoacana de San Nicolás  
de Hidalgo, Morelia, Michoacán, Mexico

<sup>2</sup>Research-professor -Signal Transduction  
Laboratory Research Institute in Biology  
and Chemistry-Universidad Michoacana  
de San Nicolás de Hidalgo, Morelia,  
Michoacán, Mexico

<sup>3</sup>Professor and Researcher Laboratory of  
Environmental Microbiology, Institute of  
Chemical Biological Research, Michoacana  
University of San Nicolás de Hidalgo

\*[syanz@umich.mx](mailto:syanz@umich.mx)



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## ABSTRACT

Outdoor air contains suspended dust particles as well as precipitated dust containing microorganisms attached to their surfaces, that are responsible for respiratory health problems and enteric pathogens for humans. In suspended and/or precipitated dust these microorganisms survive depending on environmental conditions: solar radiation, temperature and rainfall. The objectives of this research were to analyze Gram-positive and enteric bacteria in suspended and precipitated dust from outdoor air in places of high and low affluence of people in the city of Morelia, Michoacán, México. For which vacuum pump and surface dust collection techniques were used in the months of October to December 2017 at 9 am and 2 pm in the Independence market, Plaza Valladolid, City Hall, Ocolusen Garden and Gardens of the Universidad Michoacana de San Nicolás de Hidalgo. The results indicated the existence of several genera and species of Gram-positive bacteria: *Staphylococcus epidermis*, *Streptococcus faecalis*, *Bacillus cereus*, as well as, genera and species of Gram-negative bacteria: *Escherichia coli*, *Citrobacter freundii* mainly in the precipitated dust from outdoor air, regardless of temperature, rainfall, wind direction and speed, intensity of solar radiation and time of day. The common presence of *S. epidermis*, *S. faecalis* and *E. coli*, is due to the fact that the wind is one of the ways in which these microorganisms are mobilized in all areas of the city of Morelia, especially those of high population density and economic, educational or housing activity. The existence of microorganisms that are indicators of the microbiological quality of the air for the prevention of skin, respiratory or gastrointestinal diseases, supports the need for basic sanitary health measures to prevent skin, respiratory or food diseases related to contamination of food and beverages of humans living or circulating in these areas. In that sense, health authorities should reinforce public health care measures associated with suspended and/or precipitated dust in the city of Morelia, Mich, México.

**Keywords:** dust, microorganisms, public health, open environments, food, skin, human respiratory diseases.

## Introduction

The suspended and precipitated dust in the outdoor air in cities in the Mexican Republic and around the world depends on human activities related to tourism, education and commerce. The capital city of the state of Michoacán, Mexico, is Morelia, which has approximately 428,486 inhabitants, located 300 km southwest of the country's capital, Mexico City. In general, the air quality of the city of Morelia is generally acceptable for most people, however, groups with respiratory health problems may experience mild to moderate symptoms associated with allergies. In relation to the microbiological quality of the city, there is limited information on the genera and species of fungi and yeasts. The results showed a high and low incidence of microscopic fungi: *Aspergillus fumigatus*, *Penicillium sp.*, *A. niger*, *Monilia sp.*, *Alternaria sp.*, *Mucor sp.*, *Acremonium sp.* *Cladosporium sp.* and *Chrysosporium sp.* including the presence of *Candida tropicalis* most of them associated to allergies and skin diseases. Isolates during the months of October to December 2017, that have been reported in suspended and precipitated dust from the outside air, in main areas of the city Morelia, with intense human activity such as the City Hall, the Independencia Market, one of the most important and largest in the city, as well as the gardens of the Michoacana University of San Nicolás de Hidalgo (UMSNH), during an academic semester with a population of approximately 30,000 students and a residential area with a low population density, regardless of the weather conditions of the season<sup>(1-3)</sup>. However,

there is no information related to the bacterial group of importance in public health, in the same selected areas of the city. Therefore, the objective of this work was: to analyze the presence of Gram-positive and negative bacterial genera and species associated with respiratory or gastrointestinal diseases attached to suspended and precipitated dust from these areas of the city of Morelia, Mich, México.

## Material and methods

### SELECTION OF SAMPLING ZONES FOR COLLECTING DUST SUSPENDED AND PRECIPITATE OF MORELIA

In this investigation we applied accurate air bubbling and surface swabbing method better than the Microbial Contamination Index (MCI) was used to determine the health risk associated with microbial flux associated with surface contamination by microbes<sup>4-7</sup>. For the detection of *Escherichia coli* and another enteric bacterium, *Staphylococcus aureus*, *Streptococcus pyogenes* and in some cases *Bacillus cereus* in the five sites above mentioned of Morelia, the air was collected at different sites according to the intensity of human activity and vehicular traffic. The sampling of each site was carried out at two different times of the day: 9:00 a.m. and 14:00 pm and in Autumn, as shown it in table 1. The dust collection in open environments used in this study was carried out in five specific sites in the city at 9:00 a.m. and 14:00 p.m. The techniques used in this research derive from the selection of methods carried out by several work research<sup>(8,9,10)</sup>

**Table 1.** Sites for the analysis of *E. coli* and another enteric bacterium: *S. aureus* and *S. pyogenes* on dust suspended and deposited on the surfaces outdoors of Morelia, city, Mich, México.

Place / population density	Collection sites
Downtown/high*	Independence market (IM)
Downtown/high	Valladolid Square (VS)
Downtown/high	City Hall (CH)
Residential/ low**	Ocolusen Garden (OG)
Open Garden and school zone/high	UMSNH Gardens (UG)

\*High density= 800 inhabitants per hectare, \*\*Low density= 50 inhabitants per hectare

### Method 1

Evar® brand vacuum pump described in Figure 1 was used to bubble 180 L of air/3 minutes in a 1 L Kitasato flask with 1 ml of sterile saline solution, 0.1 ml of 0.1% Tween 20, at pH of 7.2 according to technique reported<sup>(1,11)</sup>. This solution was shaken for 20 min, centrifuged at 3000 rpm to concentrate the sample, the sediment was resuspended in 10 ml of sterile saline solution, vortexed for 2 min, filtered on a 0.2-micron Millipore membrane. This membrane was washed with 50 ml of sterile saline solution and the washing solution was used for the analysis of enteric bacteria and *Streptococcus pyogenes* from the dust suspended of the air outdoor of the Morelia, City<sup>(12,14)</sup>.

### Method 1 modified

Evar® brand vacuum pump described in Figure 1<sup>(1,7,13)</sup> was used to bubble 180 L of air/3 minutes in

a 1 L Kitazato flask with 100 ml of nutrient broth supplemented with 1% methylene blue, 2g/L sodium chloride and 2g/L sodium thiosulfate for the isolation possible genera and enteric species: *E. coli* and *S. pyogenes* from the dust suspended in the air outdoor of the Morelia City. Sodium azide 0.3g/L was added to the nutrient broth. Nystatin 3ml/L was included in the nutrient broth supplemented with methylene blue and sodium azide to inhibit fungal and yeast growth<sup>(14-16)</sup>. Each nutrient broth was placed in a 500 ml Erlenmeyer flask. The isolation and counting technique were not modified. No positive results were obtained for any enteric, so the surface contact technique was used to collect the precipitated dust from the air outside<sup>(1,17)</sup>.



Figure 1. Method of bubbling dust from the air of Morelia with the vacuum pump<sup>(1,18,19)</sup>. (a) Bubbling in nutrient broth with sodium azide for the recovery of *S. aureus*, *S. pyogenes*, *E. coli* from the dust suspended and precipitate of the air outdoors of the UMSNH Garden. (b) Nutrient broth with methylene blue for enteric bacteria in dust suspended of air outdoor of the UMSNH Garden. (c) Nutrient broth with sodium azide dust from dust of the air outdoor in Valladolid Square and City Hall (d).

### Method 2. Contact technique for surface

In this case, a sterile swab was used, that was spread over a certain area of approximately 25 cm<sup>2</sup>, to collect the precipitated air dust outdoor of the Morelia, City that was suspended in 4 ml of nutrient broth and incubated for 24–48h/37°C (Fig. 2). Then the tubes were shaken in Vortex for 60

seconds to homogenize the sample. The isolation of the microorganisms enteric from precipitated dust of the air outdoors of Morelia, City was carried out by sowing the swab from the tube on EMB and SSA, sodium azide agar at pH 7 with 3ml/L Nystatin to avoid fungi and yeast growth<sup>(1,20,21)</sup>.





a



b



c

Figure 2. Surface contact technique with sterile swabs and selective broth to collect precipitated dust particles from the air of outdoors of Morelia, City (a) Sampling surface of the precipitated dust of air outdoors in UMSNH Garden. (b) Nutrient broth with methylene blue to isolate enteric bacteria from the precipitated dust of air outdoor, after passing the swab over the surface in UMSNH. (c) Collection of dust particles from the surface from air of outdoor in Valladolid Square at Morelia, City.

#### BACTERIAL ISOLATION AND COUNTING

For bacterial isolation and counting, the following viable plate count technique was used (VPC); 1.0 ml of the washing solution was taken and diluted 1:10, 1:100 and 1:1000 in sterile saline solution. Then 0.1 ml of 1:1000 dilutions was transferred to four Petri dishes in EMB agar: for *Escherichia coli* and other enteric bacteria like *Salmonella*, was used the medium *Shigella* agar (SSA); for *Streptococcus pyogenes*, sodium azide agar (SZA). Was used for *Staphylococcus aureus* and the spore forming *B. cereus*, S-100<sup>(7,11)</sup>. Then each sample was seeded with a sterile Driglasky loop and the Petri dishes were incubated at 37°C/24h-48h. By this method there were no positive results except for *S. aureus*, so this method was modified<sup>(1,19)</sup>.

#### ISOLATION AND IDENTIFICATION OF GRAM POSITIVE AND ENTEROBACTERIA GRAM NEGATIVE FROM OF THE DUST SUSPENDED OR PRECIPITATE OUTDOORS OF MORELIA

After applying the bubbling and surface contact methods for the analysis of suspended and precipitated dust from the air of outdoors of Morelia City, air dust samples were culture<sup>(20)</sup> to isolate Gram positive bacteria as *Staphylococcus* on potassium tellurite agar, Baird Parker's and S-110 agar. *S. epidermidis* was distinguished by the formation of black colonies irregular size and an opaque area around the colony<sup>(21,22)</sup>. *Streptococcus faecalis* and *Bacillus cereus* were culture on Blood Agar. The Gram-negative enteric bacteria: *Escherichia coli*, *Salmonella* and other related were

culture on Eosin Methylene Blue (EMB) and *Salmonella* and *Shigella* on agar (SSA). The identification of *Staphylococcus spp* and *B. cereus* were made with the following biochemical tests: catalase, cytochrome oxidase, lipase, coagulase, use of sugars: sucrose, mannitol and xylose, potassium tellurite reduction and novobiocin sensitivity. Identification of *S. pyogenes* was based on the  $\beta$ -hemolysis produced in blood agar, the catalase activity was performed on slides with hydrogen peroxide (3%), on colonies from sodium azide agar to avoid false positives. Enteric bacteria identification was by the following biochemical tests: Lysine Iron Agar (LIA), Triple Sugar Iron agar (TSI), Motility-Indole-Ornithine (MIO), Simmons

Citrate (SC), Methyl Red (MR), Voges- Proskauer (VP) and Malonate (MAL)<sup>(16,20)</sup>.

## Results and discussion

In Table 2 show that in all outdoor air dust samples from all sites of Morelia, were found brown colonies with an entire edge, Gram-positive rods of *B. cereus* on par with *S. epidermis*<sup>(8,9)</sup>. *Staphylococcus spp* is a Gram-positive bacterium, that growth in Baird Parker's medium, through the appearance of black, convex, shiny colonies surrounded by a transparent halo as shown in figure 3.

**Table 2.** Distribution and frequency of *S. aureus*, *S. epidermidis* and *Bacillus cereus* adhered to the dust of air outdoors of Morelia, City, Michoacán, México.

Dust air outdoors Samples	Sample of the Morelia city				
	City Hall	Valladolid Square	Independence Market	Ocolusen Garden	UMSNH Gardens
<i>Staphylococcus epidermidis</i>	*4/8+	2/8	1/8	4/8	2/8
<i>S. aureus</i>	0/8	0/8	0/8	0/8	0/8
<i>Bacillus cereus</i>	2/8	3/8	3/8	2/8	3/8

\*Positive Detection frequency according to total analysis. +Number of samples where the genus and species were found.

We could not find *S. aureus*, however *S. epidermidis* was found in all places analyzed of Morelia, this genus and specie has been reported in other cities of the world<sup>(1,3,24)</sup>. As is show in Figure 3, *S. epidermis* is regarding an opportunistic human pathogen considered the causal agent of different clinical entities, including: in-hospital urinary tract infections, after eye surgery, infections of medical devices or foreign bodies: intravenous catheters, fistulas for hemodialysis, catheters dialysis, pacemakers, prosthetic joints, vascular grafts, prosthetic heart valves and breast implants, usually inside of close places at hospital's environmental<sup>(5,23,26)</sup>. *S. epidermidis* can survive due, to its pigment which protect it against sun radiation reason why we found in dust precipitate of city Morelia<sup>(1,2,15)</sup>.

Figure 3. Isolation of *S. epidermis* from dust suspended in the air outdoor of Morelia City. (a) Characteristic black colonies isolated on Baird Agar S-110. (b) Colonies white in Blood agar. The identification of *S. epidermis* isolated from air dust was made by biochemical tests: catalase, cytochrome oxidase, fermentation of sucrose, mannitol, xylose and positive, novobiocin sensitivity in *S. epidermidis*, coagulase test was positive and lipase negative<sup>(15,17)</sup>.

*B. cereus* at laboratory level is distinguished by the formation of black colonies, irregular size, opaque area around the colony<sup>(20,24)</sup>. While *B. cereus* growth on Blood Agar like white, convex and shiny colonies, as seen in figure 3b<sup>(23,25)</sup>. *B. cereus* was found in this work, due to formation of dark and irregular colonies, its spores are reported to

contaminate food and produce enterotoxin that causes food poisoning, so it is important to protect food from dust<sup>(16,17,20)</sup>. Spores of *B. cereus* is related to potential risk for consume food at outdoors<sup>(26,27)</sup>. *B. cereus* spores are capable of surviving the environmental conditions of outdoor air: desiccation, solar radiation, even some bactericidal chemical agents<sup>(20-21)</sup>.

In the case of Gram-positive cocci, the presence of *S. aureus* was looked for in Braird Parker S-100 agar, potassium tellurite was an effective agent to

prevent the growth of Gram-negative bacteria, and the egg yolk allowed the appearance of *S. epidermis*. black, convex and shiny colonies with a transparent halo as shown in figure 4. *S. epidermidis* is distinguished by the formation of black colonies, of irregular size, opaque area around the colony. While on Blood Agar it shows white, convex and shiny colonies, In the dust, but also were detected sporulated *Bacillus cereus*<sup>(23,24)</sup>.



a



b

Figure 3. *Staphylococcus epidermidis* isolated from dust precipitated of air of outdoors of the Morelia City: a) in Braird Parker S-100 agar b) on Blood Agar

Figure 4. show *S. faecalis* from dust suspended of the air of outdoors of Morelia City. In blood agar, partial lysis ( $\alpha$ -hemolysis) of the erythrocytes surrounding the colonies was observed, producing a greenish-gray or brownish color change in the culture medium<sup>(23,25,26)</sup>. The primary identification was based presence of Gram-positive cocci

arranged in pairs and chains was evident, the catalase test of colonies from azide of sodium selective culture medium. For this genus and species whose origin is human or animal fecal contamination in open environments<sup>(1,27,28)</sup>.



Figure 4. Growth of *Streptococcus faecalis* on blood agar isolated from suspended dust of the air outdoor of Morelia City.

**Table 3.** Distribution and frequency of *Streptococcus faecalis* and *Escherichia coli* and other enteric bacteria in dust suspended in outdoor air in the city of Morelia, Michoacán, Mexico.

Enterobacteria genus and species	Ocolusen garden	City Hall	Valladolid squared	Independence market	UMSNH gardens
<i>Escherichia coli</i>	*3/8	3/8	2/8	2/8	2/8
<i>Citrobacter freundii</i>	0/8	0/8	0/8	1/8	0/8
Enteric bacteria non identified	1/8	1/6	0/6	0/6	0/6
<i>Streptococcus faecalis</i>	1/6	2/6	0/6	0/6	0/6

\*The number of positive signs represents how many of the samples the indicated genus was detected; (0) = no genus and specie of any bacteria were detected.

Table 3 shows the presence of enterobacteria in the dust precipitated from the outside air in five sites of the city of Morelia, regardless of the location, commercial activity or human population density, which was found in these sites, according to the morning and afternoon hours. It was evident that in the dust precipitated from the outside air of the city of Morelia, genera and species of microorganisms that are associated with the human respiratory system were found, as well as those microbes that can contaminate food during the preparation, consumption and conservation of food that cause gastrointestinal problems<sup>(17,23,29)</sup>. The results indicate that in the precipitated dust there was a high frequency and distribution of *E. coli* since it was isolated in the dust from the outside air of all the sampled areas of the city of Morelia. *E. coli* is an important indicator of fecal contamination of human and animal origin<sup>(20,24)</sup>. Therefore, there is a potential risk associated with the ingestion of food or water contaminated with *E. coli* attached to dust in outdoor air<sup>(17,18,21)</sup>. *S. faecalis* was also isolated, although the medical literature had only reported it isolated from patients, hospital environments and dental offices since it also has the human pharynx as its natural habitat<sup>(5-7,27)</sup>. The detection of *S. faecalis* from dust precipitated from outdoor air in different areas of the city of Morelia, supports that it has the capacity to survive in extreme environments such as solar radiation, desiccation and drastic changes in

average minimum and maximum temperature on those days that varied from 12.6 and 25.2; 8.6 and 26.6; 7.8 and 23.2oC, in October, November and December 2017 respectively. In contrast to *Citrobacter freundii* (biochemical profile in Table 4), that was only isolated in the Independencia Market, as well as an enteric bacterium that could not be identified detected in the precipitated dust of the Ocolusen garden, in the Municipal Palace and the gardens of the UMSNH, however, it is reported that *C. freundii* normally exists in sites where pathogens such as Salmonella and Shigella are of high risk to human health, so research in progress will determine how important *C. freundii* could be in public health in this City of Morelia.

Table 4 show the biochemical profile of *E. coli* and *C. freundii*, two main patterns of genus and enteric species that were detected in the airborne dust suspended and precipitated from outdoor of some specific sites in Morelia city. Based on the fact that *E. coli* does not use sodium citrate as a carbon and energy source, a unique differential characteristic with *C. freundii*<sup>(17,18)</sup>.



**Table 4.** Biochemical tests used for the identification of Enterobacteriaceae from airborne dust of Morelia city, Michoacán, México

Enterobacteria genus and species	Biochemical tests						
	Three Sugar Iron (TSI)	Agar lisina (LIA)	Sodium Citrate	Motility Indole Orinithine	Red Methyl.	Vogues-Proskauer	Sodium malonate
<i>Escherichia coli</i>	A/A	alk/alk	-	+/-	+	-	-
<i>Citrobacter freundii</i>	A/A	A/A	+	+	+	-	-

(+) =positive; (-) =negative; (+/-) =both; (A/A) acidic; (alk/alk) alkaline, dust from Morelia city.

## Conclusion

In the dust from the air suspended and precipitated from outside various sites in the city of Morelia, it was shown that there are microorganisms of importance in public health such as the genera and species of bacteria in spherical chains *Staphylococcus epidermidis*, *Streptococcus faecalis*, as well as *Bacillus cereus*, a sporulated recognized as an opportunistic pathogen in food poisoning. While *Escherichia coli* was the main representative of the enteric group found in the dust from the air both suspended and precipitated from all sites in the city of Morelia. The above confirms the need to ensure and maintain basic sanitary measures for the handling of food and beverages, as well as to prevent diseases caused by opportunistic pathogens in the dust from the air suspended and precipitated from outside of open environments of the city of Morelia, Michoacán, México.

## Conflict of Interest:

The authors declare no conflicts of interest.

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