Bacteriological Indicators on the Environment and in Human Health

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ABSTRACT

Mexico has public health problems due to its inadequate systems for sewage treatment, sanitation means and low income and economic levels, which influence the increase of disease manifestation. Determine seasonal variations, frequency and distribution of enterobacteriaairborne aerosols incoming from "San Juan de Dios" River. It is worth mentioning that, these bacteria possess antimicrobial and heavy metals resistance, such as to Pb, Cr, and Cd, and their hemolytic profile. Therefore, an ecological study was conducted during the seasons of summer and autumn. 822 enterobacteria strains were collected, from which 723 were identified under 18 genres and 40 species, from which 63.90% corresponding to summer and 36.09% to the autumn season. As a critical sampling, point number 2 showed to have 265 colony forming units during summer and 124 during autumn. 48 strains had beta-hemolytic profile; the 68.57% of identified strains showed resistance to more than two antibiotics in reference of Pb, Cd and Cr to which also showed resistance. Enter bacteriological recoverability shows values   above 1x103 Gram negative/m3 of air, as risk factors for human health; which allows (due to their characteristics) their implementation as useful indicators of risk exposure.

Key words: Aerosols, Enterobacteria, Resistance, Antimicrobials, Heavy metals, Risk indicators, Environmental and wastewater.

INTRODUCTION

In Mexico, water pollution (as a public health problem) is derived from the irregular and deficient sewage system that most of the times affect low-income people, and low social and economic classes; this influences the development of health problems such as diarrhea diseases[1], specialty among children who are under five years old[2-6] that is one factor that determines death in this country[1,2,3, 4]. In Mexico, one etiological agent corresponds to rota virus (80%), and bacterial agents such as Escherichia *Coli*, *Campylobacter Jejuni*, *Salmonella* sp. y Shigela sp[5, 6,7]. However, *Escherichia Coli* is the most important etiological agent that is associated to wastewater contamination. This bacteria has been categorized into six serotypes ECET, EPEC, EIEC, EHEC, EAGGEC AND DAEC[2,8, 9, 10]. They have been related to break out enteric diseases, such as cholera or typhoid[1]. Wastewater has been considered as a reservoir to a great variety of microorganisms that are resistant to antibiotics and heavy metals, and b-hemolytic microorganisms are associated to virulence, between another factors such as more adherence, toxin production and invasion[11], Studies

on sewage have shown that strain gram negative were resistant to cooper (76 %), mercury (50%) , chrome (13%), lead (22%)[12]. Therefore, resistant strain to antibiotic such as *Salmonella tiphymurimun and Escherichia coli had* been detected from environmental and clinical samples of waste water[11, 13,] we know that bacterial resistance to antibiotic can be exchanged by the conjugation of more bacteria coming from the environment[11]. Therefore, Louis Pasteur found the microbial contamination of air[11]. Today, we know that both microorganisms are accidental pollutants of air, and that air is not a habitat for microorganisms, however, this dispersed pathogenic bacteria. In the air, virulent bacteria is able to synthesize compatible solutes that support the resistance to osmotic stress from environmental increasing virulence, thus both the atmosphere inside operating rooms and pharmaceutical laboratories should to be cleaning avoiding post-surgical infections. We know that $1x10^3$bacillus/m^3 from air of restricted are as depict human health damage because they can be more virulent by synthesizing many proteins that allow surviving at hostile conditions with the modifications of metabolism and shape until better environmental conditions occur[14-16]. These conditions can be associated with both hospital and environmental clanship. However in the hospital some strains, which show antibiotic resistance, have been detected even after the cleaning, being those *E. coli*, *Pseudomona Aeruginosa* and Enterococcus, even had been isolated β lactamase *K. pneumonia*[22]

The air also transports many microorganisms such as saprophytes and products of aerosolization, flagella fragments, genetic material, metabolites, volatile organic compounds, endotoxins and micotoxins. The air also contain edbio aerosols with particles between 0.5-30 micrometers of diameter. The concentration of microorganisms in bio aerosols depended on their dispensation and the deposition of particles. These conditions are associated to size, density, moisture and temperature. In this case, high environmental moisture or extreme conditions occasioned the growth of many micro organims such as fungi, bacteria, virus, and amoeba cysts provoking damage on human health due in part to inhalation, ingestion and the contact to the skin. Therefore, the human inhaled about 10 m ^{3} of air by day, and they are can be allowed particles of 1 a 2 micrometers inside of breath provoking severe infections, asthma, pneumonia, and the others diseases that are associated to aerosols exposition[17, 18].

Many investigations have shown that bacteria concentration of 1 x 10 Gram negative bac/ M^3 from environmental induced mucosal inflammatory response, such concentration is equivalent to the exposition of the 0.1mg/M3 of enterotoxin (26, 27, 28, 29,34). In the experimental conditions one bacteria colony can grow on tryticase-soy agar in a period of 15 minutes after being exposed, which means exposure to 38 bacteria colony/m^3 as estimated for enterotoxin/m from air. This study had the fundamental objective of diagnosing the biodiversity of enterobacteria found on air and sewage water by using hemolytic, antibiotic resistance and heavy metals resistance tests to predict both biological and chemical contamination and environmental and human health risk bio indicators. 36 samples from air and wastewater were employed for this research.

The aim was taking samples of biology particles from air. These conditions can depend on the biological and physical characteristics while the sample equipment on quantitative monitoring of bio aerosols such as their impact, broth media and filtration, gravity on opening agar dish, Andersen sampler, and filtration on the broth; and other tests such as microscoy, biochemistry, immunoassays and PCR[16].

In Guadalajara City from Jalisco State, Mexico 14 wastewater tracks have a dual design, which means that they are closed at some point but are kept opened in most of the cases.

The most important waste water track is known as San *Juan de Dios*. This waste water track has suffered changes and transformation show ever its waste water hasn't received the proper treatment yet. In this point, aerosols samples were taken in order to determinate enterobacteria. What species of enterobacteria are found in the aerosols of *San Juan de Dios* River from Guadalajara, Jalisco State? Why this problem is still unsolved? and what is the hemolytic profile of microorganisms that show resistance to antibiotics and heavy metals such as

Pb, Cd, Cr that cause damage on the human health of people who of downtown near of wastewater track

MATERIALS AND METHODS

Study design

An ecological study was conducted in order to investigate air quality in terms of entero-bactereological biodiversity and in reference to the hemolytic profile of such microorganisms as well as their antimicrobial resistance to heavy metals such as Pb, Cd and Cr.

Sample side

This study aims to diagnose the quality of air in terms of its entero-bacterial biodiversity, antimicrobial and heavy metals resistance as well as their hemolytic level. It was also important to know the people's point of view about contamination of *San Juan de Dios* wastewater track located at the North of *Guadalajara* City, *Mexico.* The samples were taken near the downtown of *Villas de San Juan at* the peripheral road and a total six samples at the month by duplicating three points along the topography of the place. The point 1. Located near San Juan neighborhood. This place is opened up to Rancho Nuevo neighborhood and Santa Elena de la Cruz, El Paso without turbulence of forming water reservoirs. The point 2. Located near both Rancho Nuevo and San Juan. This place was characterized by the secondary joint cannel "Canal de San Juan" with the inclination of a waterfall that provokes both turbulence and aerosols. The point 3. Located near San Juan Neighborhood to the borderline with less turbulence than in the second point. The samples were taken during summer and autumn every Monday at the same time and at three different points on the Trypti case Soy agar by 15 minutes. The samples and controls were moved to The Microbial Ecology Laboratory and they were incubated at 37°C during 24 hours. Then, the bacterial growth was quantified using to Colony forming plaques. This colony was isolated on a plaque as follow.

1.   The colony delimited was isolated to avoid contamination.
2.   The colony with Gram negative bacilli, and the carbohydrates fermentative was selected.
3.   The strain of enterobacterial resistance to

both antibiotic and Pb, Cm, Cr heavy metals with the hemolytic profile was selected by high impact at the human health.

Identification of Enterobacterial Strain

The enterobacterial strain was cataloged using biochemistry smples on the Lisine and Iron agar, Simons Citrate, Ornitine Indolmovility, Mannitol, Urea agar, Sucrose, and MR-Vp following Bergey´s Manual Determinative Bacteriology-9 a, and the Gamelaya software developing to the Epidemiology and Cybernetic Laboratory of Gamelaya Institute from Russian Academic of Medical Sciences of Moscu.

Hemolysis tests

The bacteria hemolysis was evaluated by using blood on agar following standard methods, and being incubated on the template for 14 strains by sterile conditions during 24 hours at the 37°C. The clear zone around colony was considered as positive test for hemolysis[11].

Resistance to Antibiotic test

The bacteria was incubated on the Muller-Hilton agar (pH 7.4) at 4 °C using sterile swabs keep out 3 min and then put on disc and the plaque were incubated at 37°C during 18 hours. They culture with the resistance presented clear zone around colony. The resistance to antibiotic was cataloged as high, intermediate, and sensitive.

Resistance to Heavy metals test

Each metal was diluted by part/millions of Pb, Cd, Cr, and the concentration used to Pb was 207 ppm, 2. 20.7 ppm, 2.7 ppm, 0.207 ppm, 0.027 ppm, 2.7 x10-3 ppm, 2.07x10-4 ppm, 2.07x10-5 ppm, 2.07-6 ppm, 2.07x10-7 on the tryticase soy broth. Each concentration was incubated with the 100 ìm of bacteria culture. Alike, the concentration used to Cd was 12 ppm, 11.2ppm, 1.12ppm, 0.112ppm, 1.12x10-3ppm, 1.12x10-4ppm, 1.12x10-5ppm, 1.12x10-6ppm, 1.12x10-7ppm on the tryticase soy broth. Each concentration was incubated with the 100 ìm of bacteria culture during 24 hours at 37°C. Alike, the concentration used to Cr was 104ppm, 10.4ppm, 1.04ppm, 0.0104ppm, 1.04x10-3ppm, 1.04x10-5 ppm, 1.04x10-7 ppm, 0.104ppm, 1.04x10-4ppm, 1.04x10-6 ppm on the tryticase soy broth. Each concentration was

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incubated with the 100 ìm of bacteria culture during 24 hours at 37°C.

RESULTS

Ours results showed significative different between Colony Forming Unit and the sampling place (p=0.010075), according to Kruskal Wallins Test. We also found significative different between Enterobacteria group and the sampling place (p=0.034885), with the break point in the sampling place no. 2 during summer than autumn without meaningful different (p=0.096408). However, the number of Colony Forming Unit presented increased at the summer (Table 1). The sampling point no. 1 was selected with 89 Colony Forming Unit of Enterobacteria, and a mean of the 15 Colony Forming Unit of Enterobacteria at summer and a mean of the 125 Colony Forming Unit of Enterobacteria. In the sampling point no. 2 presented a break point with the 265 Colony Forming Unit of Enterobacteria and the mean of 44 Colony Forming Unit of Enterobacteria at the summer. At the autumn, the Colony Forming Unit of Enterobacteria was 21 and the point 3 was detected 116 Colony Forming Unit of Enterobacteria with the mean 19 Colony Forming Unit of Enterobacteria at summer and Colony Forming Unit of Enterobacteria was 103 with mean of 17 at the autumn (Figure 1). We identified 723 strain of enterobacteria with a mean 462 Colony Forming Unit of Enterobacteria at the summer and a mean of 261 Colony Forming Unit of Enterobacteria at the autumn belong 18 genres and 40 species (Table 1). They had beta hemolytic proprieties with 48 strains belong 13 genrs and 20 species both

seasonal summer with the 39 and 9 autumn (Table 2). They had resistance to antibiotic with 24 strains resistance to antibiotic (68,57%) according to antibiogram test conducted on 35 strains. Theses enterobacterial had different spectrum of resistance to antibiotic, thus one strain was resistance to two antibiotics (2.85%), and three strains were resistance to one antibiotic (8.57%). Salmonella I strain was resistance to 12 antibiotics. Some strains showed sensitiveness to 12 antibiotics (20.0%), (Table 3).

The strains isolated showed resistance to Nitro furantoine (71.0%), Pefloxacine (60.0%), Amikacine (48.5%), Cloranfenicol (42.8%), Carbeniciline (40.0%), Ampicilin (37.1%), Cefotaxima (25.7%), Netilmicine (25.7%), y Trimetoprim-Sulfametoxazol (8.5%), (Figure 2). The strains resistance to antibiotics were *Enterobacter cloacae, Edwarsiellaictaluri, Samonellaparatyphi and serotype I Salmonella*, and *Rahnellaacuatilis* was resistance to pefloxacine.

The resistance to heavy metals as Pb, Cd and Cr was identified on 32 strains. A 9.3 % of strains were resistance to Pb, they were growth to 207 ppm concentration of heavy metal, and another bacteria growth to 20.7 ppm of metal (90.6%). *15.6%* of strains were resistance to Cd and they were growth to 112ppm concentration of metal, and 21 strains growth to 11.2 ppm concentration of metal (65.6%). The resistance to heavy metals were also minor to some strains, thus they were growth to 0.0112 ppm of heavy metals concentration (3.12 ppm), and another bacteria growth minor concentration of heavy metals (6.25%), and three

Table 1: Frequency and average CFU of microorganisms detected per sampling point

Stations of theyear	Sampling point 1	Sampling Point 2	Sampling point 3
Summer	675 CFU μ=113 CFU	3892 CFU μ=648 CFU	679 CFU μ=113 CFU
Fall	448 CFU μ=75 CFU	1574 CFU μ=262 CFU	623 CFU μ=262 CFU
Hypothesis test Kruskal Wallis	p=0.010075	p=>0.05 Point 2 . resulted the most critical	By station no meaningful difference was detected p=0.096408

Source: Direct Aerosol Samplingsewage canal Rio San Juan de Dios, Guadalajara Jalisco, Mexico

strains (9.3%) could not grow in the concentration of heavy metals, and they were cataloged as sensitive to them 28 strains were resistance to Cr and they were growth to 10.4 ppm (87.5%) of concentration of heavy metal, or they were growth to 1.04 ppm (3.12%) , and some bacteria were not resistance to heavy metal (Table 4).

DISCUSSIÓN

Seasonal variation, regarding the microbial concentration in the environment is determined by the own factors of each of the seasons. In this way, we could make reference to global climate changes expressed though the modification of the average temperature as well as the force and orientation of winds, as well as the growth and development of typical endemic plants[14].

Bacterial findings collected through air sampling and study were directly associated to the aerosols which are generated from sewage water, whose sanitarian design also influences in a meaningful way, due to the particularities that the cannel represents, there are sectors that are irregular in their topography. These differences in their construction are more prominent particularly in sampling point 2, which has a cascading decline and the binding of a secondary channel for the same; this is where they had the highest number of microorganisms which significantly exceeds 1x103 bacilli Gram negative/M3 that determines the start of the presentation of adverse effects to human health. Moreover, at points 2 during the summer and autumn, this limit was exceeded but to a lesser extent in a seasonal way.

Table 2: Frequency and percentege of Enterobacteriaceae detected and identified by biochemical tests

Station of summer	462	64
Station of fall	261	36
total	723	100

Source: Direct Aerosol Samplingsewage of the "San Juan de Dios" River, Guadalajara Jalisco, Mexico

Table 3: Antimicrobial resistance of 35 strains to which they applied the antibiogram

Strains	Frequency	Percentage
Resistant to more than two antimicrobial	24	68.57
Withtwoantimicrobialresistance	1	2.85
Withresistance to antibiotics	3	8.57
Note: these Salmonella strains resistant to 12 antibiotics	1	2.85
Withsensitivity to 12 antibiotics	7	20

Source: Direct Aerosol Samplingsewage of the "San Juan de Dios" River, Guadalajara Jalisco, Mexico

Source: Direct Aerosol Samplingsewage of the "San Juan de Dios" River, Guadalajara Jalisco, Mexico

**Fig. 1: Enterobacterial and average frequency detected per sampling point**

It is worth mentioning that, during summer much more enterobacteria were identified, whose biodiversity is an important advantage for practical use, and that can be used as biological indicators of environmental pollution because, by the nature of their habitat in the human gut, or other animals, as well as the respiratory tract, represent in person the continuing cyclical interaction in the health and environment management of their own waste products that are generated from the corresponding physiological actions, but which, however, impact the stability as the current sewage waters which have become the old "San Juan de Dios " River, as a natural reservoir whose cause, partially covered, passes through densely populated areas of the city of Guadalajara Jalisco Mexico (Table 1).

Moreover, one of the most highlighted of the present research is the findings about bacteria such as Rahnella Acuatillis whose particular niche is water. However, those bacteria were isolated from aerosols generated by wastewater and which as part of their phenotypic profile, their synthetic capacity of enzymes beta-hemolytic is highlighted, as well as its edema of high resistance to heavy metals like Pb to 207ppm, Cd to 112 ppm, Cr to 104 ppm and their resistance to PEF, besides the presence of *Escherichia Blattae whose* normal niche constitutes the intestinal track of cockroaches and in whose strain beta hemolytic capacity was found, besides the presence of *Salmonella biotype 1* with an hemolytic profile and a multiple resistance to 12 antimicrobial.

Table 4: FCU Heavy metal resistant ppm Pb, Cd and Cr

Frequency of strains	Tubo	Pb/ppm	%	Cd/ppm	%	Cr/ppm	%
23	2	20.7	90.6				
3	1	207	9.3				
21	2			11.2	65.6		
5	1			112	15.6		
3	0			sensitive	9.3		
2	4			0.112	6.25		
1	5			0.0112	3.12		
28	2					10.4	87.5
1	3					1.04	3.12
3	0					sensitive	9.3

Source: Direct Aerosol Samplingsewage of the "San Juan de Dios" River, Guadalajara Jalisco, Mexico
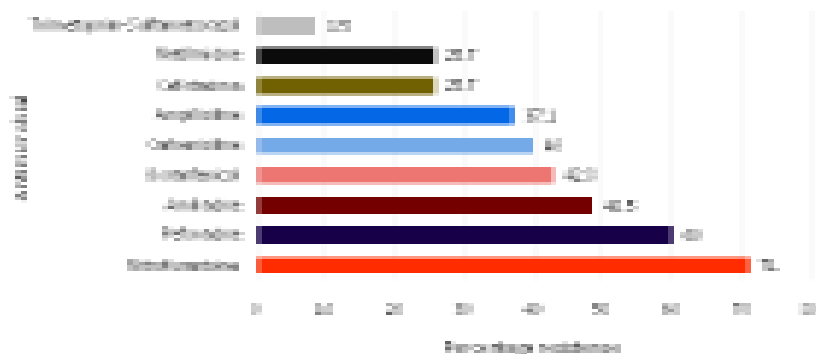
Source: Direct Aerosol Sampling sewage of the "San Juan de Dios" River, Guadalajara Jalisco, MexicoNote: Among the multiresistant strains were found *Entrobacter cloacae*, *Edwarsiellaictaluri, Salmonella paratyphi and Salmonella biotype I,* resulted *Rahnellaacuatilis*pefloxacin resistant.

Fig. 2: Antimicrobials the studied strains showed higher resistance antibiogram.

Finally the ecological-environmental meaningful findings of multiple airborne bacterial species that presents high resistance to heavy metals is also meaningful because apart from their presence in the air is considered as biological indicators of environmental pollution.

At the same time, they also acquire additional usage value in order to function as bio-indicators of chemical pollution. This, under the dynamic interaction that is maintained in its environment, under extreme conditions, as are the industrial wastes that contain metallic residues which stimulate the environmental typical micro flora that acquires high resistance levels towards the same as part of a natural response to their adaptation and ulterior survival in the aerosols generated from the waste water and which allow to regulate their own virulence factors (21,22,23), having an impact on the manifestation of health risks of inhabitants who live nearby these type of environments.

The speech employed by the interviewees reflects for instance, the meaning of pollution associated with environmental conditions present in their community, which indicates that one must pay attention to this type of problems that show the causes of the problems to a public health level in which it is necessary to work focusing on sanitization problems from an environmental methodology, trying to find the control or elimination of risks associated to environmental conditions, which if we remember have an impact on the virulence of bacteria as well as on human health[22]. The Evolution of Yersinia pestis of speciation from an environmental, non-pathogenic ancestor is a good example of the evolution steps that are involved in the emergence of bacterial pathogens. This process began with the acquisition of the plasmid pCD1 by environmental Yersinia.This process is fully explained Wren (2003), Keim and Wagner (2009)[23] hence the importance of living in a healthy environment, not laden bioaerosols contaminated with Enterobacteriaceae.

The air transport of virulent microbiological agents, as well as the density of antibiotics and resistant bacteria may enhance the adverse effects on public health and environmental surroundings, hence the importance of considering the implementation of inter hospital epidemiology And at the community level by exposure of virulent organisms, as they impact on human health in the family economy and health care.

CONCLUSIONS

1. It is feasible to recover enteric microorganisms in aerosols generated from sewage, through the methodology employed in this study.
2. During the summer season, a high quantity and biodiversity of enteric strains was identified, particularly at the second sampling point.
3. The estimated concentration was higher than $1\times10^3/M^3$, besides classical strains and the virulence factors identified, which suggests there is a social-ecological relationship with the inhabitants of neighboring communities to sewage waters.
4. There is a relationship between the "topographic" design sewage and the amount of airborne Enterobacteria.
5. The presence and characteristics of resistance to antibiotics and heavy metals in some enterobacteria strains allows us to propose them as bio-indicators of environmental, microbiological, chemical pollution and as indicators of risk to human health.

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