



Facultad de Ciencias Naturales y Exactas Universidad del Valle



Prevalence and Antimicrobial Susceptibility of Potentially Pathogenic Bacteria and Fungi Isolated from Public Parks of Bogotá, Colombia

Diego Fernando Gonzalez Lozano

Camila Andrea Camacho Ramos

Universidad Militar Nueva Granada

Universidad Militar Nueva Granada

Iván Alberto Méndez Rodríguez

Universidad Militar Nueva Granada

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Abstract

Regular interactions of humans with their environment, animals and different microorganisms as part of everyday routine can cause several infections or diseases that can become a public health problem if these are not properly controlled. It is necessary to identify potentially pathogenic bacteria (with its antimicrobial resistance) and fungi, and their prevalence in public parks in Bogotá city. Four parks were evaluated, where samples were taken from dog's feces and hair, recreational items and environmental material, to perform the microorganism's isolations. The higher prevalence percentages in each sort of sample were for *Escherichia coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Rahnella aquatilis*, *Penicillium* spp, *Cladosporium* spp. and *Mucor* spp. and there were pathogenic species like *Salmonella enteritidis* and *Klebsiella pneumoniae*. We report a strain of *S. aureus* with intermediate resistance to vancomycin (VISA) found in recreational items. The higher rates of antibiotics resistance like ampicillin and trimethoprim were found in the isolations from dog's feces. Some species or genera reported are considerated as opportunistic pathogens; however, these and the pathogenic species reported represent a threat to human health and this is why it is essential to have a personal hygiene plan after stay at a park.

Keywords: public health, pathogenic microorganisms, prevalence, antibiotic resistance.

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1 Introduction

Man has always been in a constant interaction with other organisms, mainly with domestic animals like dogs, cats, rodents, cattle, some birds, and others. However despite the benefits of having a group of animals like these, there are certain risks usually related to infectious agents or pathogens (such as bacteria, fungi and parasites) that can be found in animals mentioned before ⁽¹⁾. Bacteria and fungi that are potentially pathogenic to humans are just a small portion of the total of bacteria and fungi known, which can cause various diseases with specific or varied symptoms ^(2,3), *domestic animals* can be reservoirs *for* these microorganisms *through* environmental *exposition, commensal opportunistic*

bacteria and fungi or a foreign agent ⁽²⁾. Bacteria can be transmitted mainly through direct contact with animal feces or some type of fluid ⁽¹⁾. In the case of fungi transmission, it occurs mostly through the contact with animal's skin or hair ⁽³⁾. Also green areas or parks, where people attend with their pets (usually dogs) are considered an important part in the dynamics of the city population, as they are constantly visited areas ⁽⁴⁾. These zones are mainly made up of areas with vegetation and recreative elements which act as fomites and implies direct or indirect interaction with, for example, pets and possibly their depositions, which in many cases are not handled in a proper way, being left in the area where people and other dogs are exposed to them.

Some bacteria and fungi related to pets or domestic animals that are capable of being transmitted from these and cause human diseases are *Escherichia coli, Salmonella* spp, *Shigella* spp, *Yersinia* spp, *Microsporum* spp, among others ⁽⁵⁻⁷⁾, which *are often associated with* symptoms like fever, chills, muscle pain, diarrhea, vomiting or nausea, tinea (mainly capitis), *among others* ^(6,7). Treatment of diseases caused by bacterial infections usually required antibiotics, which have different mechanisms of action, considering that each type of antibiotic affects a certain group of bacteria as Gram-positive bacteria or Gramnegative bacteria, or even both ^(2,8,9).

Some groups are more vulnerable than others such as children, elderly people and immune compromised individuals ^(10,11). People usually don't know that they can be exposed to a big number of microorganisms in a park or green areas *where dogs attend and recreational elements and plant* material are in constant contact with lots of people and other animals, which can lead to a public health problem where a focus of infection could be generated, unknowing what produce it and how can *it can* be avoided ^(5,7). In Colombia there are few publications on this subject, most of them are mainly focused on zoonosis related with protozoa, helminths, nematodes and other parasites in dogs and cats ^(12,13,14). That's why it is planned to contribute to the knowledge with the identification of bacteria and fungi found in feces, fur of dogs, recreational elements and vegetation in some public parks in the city of Bogotá D.C, Colombia.

2 Materials and methods

2.1 Parks selection

We selected at northern El Virrey Park, at southern Timiza Park, at eastern Nacional Park and at western Simon Bolívar Park. In each one, four items were sampled: fecal samples, canine fur, environment samples that were near populated zones and elements in recreational areas. A total of 50 samples were taken distributed in ten samples for each park except on Simon Bolivar Park where twenty samples were collected because its bigger extension.

2.2 Cultures procedure and biochemical testing

2.2.1 Fecal samples were cultivated on McConkey or Eosine Methylene Blue agar during 24 hours at 37° C then the macroscopic description was done followed by the culture of the following biochemical test: for Gram-negative bacteria were used Triple

Sugar Iron agar, Citrate, Indole (SIM Medium), Urease, Voges-Proskauer and Methyl Red tests. After the identification of bacteria, we performed the Kirby-Bauer test, ampicillin, ceftriaxone, ciprofloxacin trimethoprim, gentamicin for Gram-negatives and oxacillin, clindamycin, ciprofloxacin, trimethoprim and gentamicin for Gram-positives cocci. This procedure was applied to bacteria isolated from other types of samples.

- 2.2.2 Recreational areas samples were taken to Blood Agar and were incubated for 48 hours at 37°C, followed by macroscopic description and Gram stain. For Gram-positives cocci were performed catalase test and for Gram-positive rods were no further tests performed. Catalase positive bacteria were cultured in Mannitol Salt Agar and coagulase test; in some cases where test were undetermined we performed agglutination test for Staphylococcus aureus.
- 2.2.3 Environmental samples were processed using a hyssop to cultivate in Blood Agar to determinate bacterial microorganism, and in to Saboraud Agar at environment temperature over the course of four weeks to identify fungus microorganisms.
- 2.2.4 Canine fur samples were obtained with previous consent of their owners, a small haircut was done from the dog's back or next to a skin lesion if it was present, and the fur sample was taken to the lab and put on Saboraud Agar at environment temperature over four weeks.

2.3 Identification

For Gram-negative bacteria, identification was done using macroscopic description and biochemical test, Gram-positive stains followed the same procedure with one exception: the agglutination test for *Staphylococcus aureus* on cases not determined. For fungus samples, the first step was doing the macroscopic description followed by the microscopic identification of sexual or asexual structures and its genre.

3 Results

Two hundred samples were taken from the four parks (Table 1), of which 403 isolated found, 197 were bacteria and 206 were fungi.

Park	Bacteria	Fungi	Isolations per park
Nacional	36	43	79
Timiza	57	53	110
El Virrey	41	39	80
Simón Bolívar	63	71	134
Total Isolations	197	206	403

 Table 1. Bacterial and fungal isolations per park and total isolations.

3.1 Bacterial isolations from fecal samples

From 50 fecal samples taken, 50 isolations were performed, of which 11 genus and 5 species of bacteria were identified (Table 2, 3).

Genus/specie	Escherichia coli	Citrobacter spp	Enterobacter spp	Aeromonas spp	Edwardsiella spp	Pantoea spp	Klebsiella spp	Plesiomonas spp	Proteus spp	Yersinia spp	Aeromonas hydrophila	Edwardsiella hoshinae	Klebsiella pneumoniae	Salmonella enteritidis
Prevalence	36	14	12	4	10	8	2	2	2	2	2	2	2	2

Table 2. *Identification and prevalence of bacteria isolated from all fecal samples.*

Table 3. Antibiotic susceptibility from fecal samples isolations. S=Susceptible, I=Intermediate, R=Resistant, n=number of isolations.

	S(n)	%S	I(n)	%I	R(n)	%R
Ampicillin	6	12	8	16	36	72
Ceftriaxone	39	78	4	8	7	14
Ciprofloxacin	24	54.5	12	27.3	8	18.2
Trimethoprim	16	42	1	3	21	55
Gentamicin	34	89	1	3	3	8

3.2 Bacterial isolations from recreational items

From 50 samples taken from recreational items, 76 bacterial isolations were performed, of which 11 genera and 9 species were identified. Isolations of Gram-positive bacilli were reported with a prevalence of 17 % (Table 4, 5).

 Table 4. Identification and prevalence of bacteria isolated from recreational items samples.

Genus/specie	Staphylococcus epidermidis	Staphylococcus aureus	Staphylococcus spp.	Enterobacter spp	Pantoea spp	Streptococcus spp	Yersinia spp	Aeromonas hydrophila	Aeromonas salmonicida	Chryseobacterium idologenes	Klebsiella terrigena	Ochrobactrum antrophi	Salmonella gallinarum	Yersinia enterocolitic
Prevalence (%)	19.7	13.1	14.5	7.9	2.6	3.9	1.3	1.3	3.9	1.3	2.6	2.6	2.6	1.3

Gram-negative	S(n)	%S	I(n)	%I	R(n)	%R	Gram-positive	S(n)	%S	I(n)	%I	R(n)	%R
Ampicillin	16	72,7	2	9,1	4	18,2	Oxacillin	26	89,6	0	0	3	10,4
Ceftriaxone	18	75	3	12,5	3	12,5	Clindamycin	26	89,6	1	3,4	2	7
Ciprofloxacin	24	100	0	0	0	0	Ciprofloxacin	30	93,8	1	3,1	1	3,1
Trimethoprim	20	100	0	0	0	0	Trimethoprim	31	96,9	0	0	1	3,1
Gentamicin	20	100	0	0	0	0	Gentamicin	31	96,9	1	3,1	0	0

Table 5. Antibiotic susceptibility of Gram-negative and Gram-positive bacteria from recreational items isolations. S=Susceptible, I=Intermediate, R=Resistant, n=number of isolations.

Two *Staphylococcus aureus* (label as A and B, obtained from El Virrey park) and one isolation of *Staphylococcus* spp. (obtained from Simón Bolívar park) showed oxacillin resistance. E-test for vancomycin was applied in both *S. aureus* and one of them showed susceptibility to vancomycin $(3\mu g/ml)$ and the other one showed an intermediate pattern $(8\mu g/ml)$ (Table 6).

 Table 6. Vancomycin E-test values for two strains of Staphylococcus aureus resistant to oxacillin.

Strain	E-test	Park
Staphylococcus aureus (A)	3μg/ml	El Virrey
Staphylococcus aureus (B)	8µg/ml	El Virrey

3.3 Isolations from environmental material

3.3.1. Bacterial identification and prevalence

From 50 samples taken from environmental material, 71 bacterial isolates were performed, of which 14 genera and 11 species were identified. Isolations of Gram-positive bacilli were reported with a prevalence of 28% (Table 7, 8).

 $\textbf{Table 7.} \ \textit{Identification and prevalence of bacteria isolated from environmental material samples}.$

Genus/specie	Staphylococcus spp.	Staphylococcus epidermidis	Staphylococcus aureus	Rahnella aquatilis	Aeromonas spp.	Burkholderia spp.	Edwardsiella spp.	Enterobacter spp.	Pantoea spp.	Pasteurella spp.	Streptococcus spp.	Aeromonas salmonicida	Chryseobacterium idologenes	Enterobacter amnigenus	Enterobacter gergoviae	Enterobacter intermedius	Ewingella americana	Pseudomonas aeruginosa	Salmonella gallinarum	Yersinia enterocolitica
Prevalence (%)	9.8	8.4	4.2	9.8	1.4	1.4	1.4	5.6	2.8	1.4	2.8	1.4	2.8	1.4	1.4	2.8	4.2	1.4	2.8	4.2

Gram-negative	S(n)	%S	I(n)	%I	R(n)	%R	Gram-positive	S(n)	%S	I(n)	%I	R(n)	%R
Ampicillin	20	74	3	11	4	15	Oxacillin	13	81,2	0	0	3	18,8
Ceftriaxone	24	89	1	3,7	2	7	Clindamycin	13	81,2	1	6,3	2	12,5
Ciprofloxacin	23	85,2	1	3,7	3	11,1	Ciprofloxacin	11	92	0	0	1	8
Trimethoprim	13	72	1	5,5	4	22	Trimethoprim	15	100	0	0	0	0
Gentamicin	18	100	0	0	0	0	Gentamicin	16	100	0	0	0	0

Table 8. Antibiotic susceptibility of Gram-negative and Gram-positive bacteria from environmental material isolations. S=Susceptible, I=Intermediate, R=Resistant, n=number of isolations.

Likewise, from the same 50 samples taken from environmental material, 112 fungal isolates were performed of which 14 genera were identified and also fungal colonies with sterile mycelium were reported with a prevalence of 7.1%.

3.3.2. Fungal identification and prevalence

Table 9. *Identification and prevalence of fungi isolated from environmental material samples.*

Genus/specie	Penicillium spp.	Cladosporium spp.	Mucor spp.	Absidia spp.	Alternaria spp.	Bipolaris spp.	Chrysonilia spp.	Epicoccum spp.	Fusarium spp.	Humicola spp.	Nigrospora spp.	Rhizopus spp.	Sordaria spp.	Trichoderma spp.
Prevalence (%)	21.4	17.8	12.5	0.9	7.1	0.9	5.3	8.9	8.9	1.8	1.8	2.7	0.9	1.8

3.4 Fungal isolations from canine hair

From 50 samples taken from canine hair, 94 isolates were performed, of which 13 genera were identified. Yeast colonies and fungal colonies with sterile mycelium were reported with a prevalence of 8.5% and 4.2% respectively (Table 10).

Table 10. *Identification and prevalence of fungi isolated from canine hair samples.*

Genus/specie	Penicillium spp.	Cladosporium spp.	Epicoccum spp.	Absidia spp.	Alternaria spp.	Chrysonilia spp.	Cunninghamella spp.	Fusarium spp.	Mucor spp.	Nigrospora spp.	Phoma spp.
Prevalence (%)	38.2	22.3	9.5	2.1	2.1	3.1	1.1	1.1	5.3	1.1	1.1

4 Discussion

4.1 Bacterial isolation from fecal samples

The high prevalence of *Escherichia coli* is because this specie is part of the normal intestinal microbiota of mammals like dogs, however most strains of *E. coli* are non-pathogenic, while those that are pathogenic can cause infections such as travelers diarrhea, abdominal cramps, vomit, urinary tract infections among others ⁽¹⁵⁾. Like *E. coli*, genus *Citrobacter* spp, *Enterobacter* spp, *Edwardsiella* spp, *Pantoea* spp, *Klebsiella*, *Plesiomonas* spp, *Proteus* spp, *Salmonella* spp, and *Yersinia* spp. belong to the Enterobacteriaceae family, within which it has been reported species that normally are part of the intestinal microbiota of some animals or which are isolated from the feces of them ⁽²⁾.

Additionally, a portion of the species belonging to the mentioned genera, such as *Klebsiella pneumoniae* and *Salmonella enteritidis* reported here, have medical importance because they generate pathologies in humans related to bacteremia, urinary and lung or respiratory infections, gastroenteritis, meningitis, septicemia and other infections, mainly in people with immunodeficiency (16,17). In the case of *S. enteritidis*, human infections occur mostly by eating contaminated foods. It is possible to happen the same with a dog, which becomes in the vector of the pathogen and as in this particular case where *S. enteritidis* was isolated from a feces sample (18).

A few species of *Aeromonas* spp, can be isolated from some animals and occasionally from human feces, or may also be isolated from freshwater ⁽²⁾. It has been reported that some species of this genus, such as *A. hydrophila* can cause chronic diarrhea or gastroenteritis, mainly in infants, from enterotoxin production ⁽¹⁹⁾.

4.2 Bacterial isolations from recreational and environmental material

Recreational items are constantly manipulated by people who are in contact with other elements of the area or with organic materials such as fallen leaves, plants, water, including soil, in which microorganisms form biofilms of one or more species, like *Staphylococcus epidermidis* and *S. aureus*, which can explain the prevalence presented ⁽¹⁵⁾. In this situation, both species showed the highest prevalence in both type of samples, recreational items and environmental material. *S. aureus* (see Figure 1) and *S. epidermidis* can be found normally in the human skin, however *S. aureus* is found in a lesser proportion, and both *S. aureus* and *S. epidermidis* are species of high clinical importance, because these can cause nosocomial infections, skin infections with a variable severity and in some cases infections by ingestion of contaminated food, being more vulnerable people who have immunodeficiency ^(2,20). In addition, the strain of *S. aureus* (B) reported, isolated from El Virrey Park and which showed an intermediate pattern to vancomycin (VISA), have a high clinical importance because these strains represent a considerable threat as potential pathogens that can cause difficulties during treatment ⁽²¹⁾.

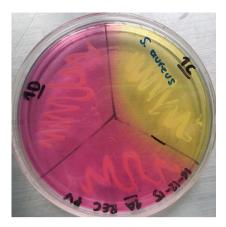


Figure 1. Mannitol salt agar test for Staphylococcus spp. obtained from a sample from El Virrey Park.

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Moreover the species *Rahnella aquatilis* showed the same prevalence percentage as the genus *Staphylococcus* spp. in the environmental samples, possibly because *R. aquatilis* is isolated from soil, freshwater and in some cases from small invertebrates $(^{22,23})$. However, it has been reported unusual cases of bacteremia, sepsis and other infections produced by *R. aquatilis* in wounded patients, elderly or immunocompromised $(^{23})$.

The bacterial genera and species and its prevalence reported here, can be related with the contact not only people have but also other organisms such as birds, rodents, dogs, etc. with the recreational items and organic sources or material nearby which are full or covered with microorganisms as the biofilms mentioned before (24). For example, *Salmonella gallinarum* is a species that is mainly spread through birds and can cause gastrointestinal infections (25). Also, it is important to point out the genus *Streptococcus* spp. which has saprophytic species that are normally isolated from skin, mouth and respiratory tract of humans. Yet, some species are of clinical importance as they can produce diseases like pneumonia, meningitis, tonsillitis and other infections (2,26). Other species reported are potential pathogens because they can produce many infections by its ingestion (in the case of *Yersinia enterocolitica*) or can also be considered as opportunistic microorganisms, such as *Ochrobactrum anthrophi*, although its pathogenicity is uncommon and is mainly related to nosocomial infections and immunocompromised patients (27,28).

The antimicrobial susceptibility test results of all isolates showed a considerable high percentage of resistance to ampicillin (44.4%) and, although not as high, trimethoprim (21.1%), being ampicillin only used in the Gram-negative bacilli isolations and trimethoprim in both Gram-negative bacilli and Gram-positive cocci. The high percentage of resistance to both antibiotics can be seen specifically in the feces samples results, where isolations reach the 72% and 55% ampicillin and trimethoprim resistance, which can be associated to the uncontrolled supply of antibiotics to dogs and possible exposure to resistant bacteria in places such as veterinary clinics, where there have been reported a high percentage of multi-resistant strains of potentially pathogenic bacteria that can infect dogs (2,29).

4.3 Fungal isolations from environmental material and canine hair

In both type of samples the genus *Penicillium* spp. showed the highest prevalence, probably because is a cosmopolitan genus and it is isolated from the environment, mainly from the soil, thus generating the prevalence result in both environmental material samples and canine hair samples (24). Few cases have been reported where some species of *Penicillium* spp. cause infection or disease in humans, where the reported ones have generally been in immunocompromised patients (24,30)

The genus *Cladosporium* spp. can be found widely distributed in the environment (like *Penicillium* spp.) and some species can produce diseases like black tinea, onychomycosis, phaeohyphomycosis, among other, in healthy people ⁽³¹⁾. Other genera that can produce "feohifomicosis" in healthy and immunocompromised patients are *Alternaria* spp, (see Figure 2) *Bipolaris* spp, *Phoma* spp. and *Nigrospora* spp. ^(31,32). Also, some of the species of the genus *Fusarium* spp. (see Figure 3) and *Trichoderma* spp, can cause hyalohyphomycosis ⁽³²⁾.



Figure 2. Conidia from Alternaria spp. obtained from an environmental sample, observed through a light microscope with 100x. Author: Diego Gonzalez Lozano



Figure 3. Characteristic septate conidia from Fusarium spp. observed through a light microscope with 100x.

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The genus *Mucor* spp, had a high prevalence compared to the other fungi genera in samples of environmental material, because it is a genus that is distributed in soil and decaying organic material ⁽³¹⁾. *Mucor* spp, as *Rhizopus* spp. (see Figure 4) and *Absidia* spp, can cause infections or mucormycosis, affecting eyes and respiratory structures ^(31,33). Additionally, it has been reported the genus *Chrysonilia* spp, with a relevant prevalence, where it is important to point out a case of occupational asthma reported in Paris caused by a constant exposure to *C. sitophila* in a healthy grown man without relevant medical history related to respiratory diseases ⁽³⁴⁾.

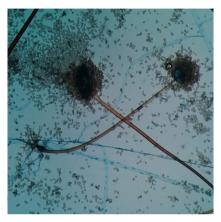


Figure 4. Asexual structure of Rhizopus spp. and coenocyte hypha with rhizoids observed through a light microscope with 100x. Author: Diego Gonzalez Lozano

A large proportion of fungal isolates from environmental material are normally found distributed in the environment, carrying out the decomposition of organic matter, and in some cases they don't represent a threat to human health, except in people with underlying disorders that impair their immune response which makes them vulnerable to infections by opportunistic microorganisms ⁽³¹⁾. In the canine hair samples we reported mainly environmental genera without relevant dermatophyte genus being identified, probably because the samples were taken from a long part of hair or from the outermost part of the dog's hair, in which fungi found in the environment can adhere as the dog have contact with the soil, plant material, water, etc. bearing in mind that the results of fungal identification in both types of samples are similar.

5 Conclusion

According to data reported in this article, public parks are areas where several microorganisms can be found, such as fungi and bacteria, which may be pathogens or potential pathogens spread all over the place and some of those can be deposited by animals or by people themselves. It is necessary to have a correct handling of dog feces in these areas because medical importance microorganisms such as *Escherichia coli* or *Salmonella enteritidis can be found*, which are capable of causing infections specially with the increasing resistance strains, leading to failure of medical treatment.

It is important to mention that the recreational items and the environmental material of the parks are sources of microorganism's infections, often opportunistic ones like *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter* spp, *Rahnella aquatilis*, *Mucor* spp, *Cladosporium* spp, and other listed here. In consequence, it is essential to have a plan of personal hygiene after having contact with any of these elements or after attending to a park, because many of the reported microorganisms can cause public health problems and can affect a broad range of people.

As we can observe in Tables 11, 12, 13, there is an important prevalence of many bacterial and fungal isolates, some of them with real or potential pathogenicity to humans, besides the resistance to antibiotics used in therapy undoubtedly could be revealed a real risk in case of acquisition particularly for immunocompromised people.

Table 11. Antibiotic susceptibility from all isolation	ns. S=Susceptible, I=Intermediate, R=resistant.
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	S(n)	%S	I(n)	%I	R(n)	%R
Ampicillin	42	42.4	13	13.1	44	44.4
Ceftriaxone	81	80.2	8	7.92	12	11.9
Ciprofloxacin	112	77.8	16	11.1	16	11.1
Trimethoprim	95	77.2	2	1.63	26	21.1
Gentamicin	119	96.7	1	0.81	3	2.44
Oxacillin	42	87.5	0	0	6	12.5
Clindamycin	42	87.5	2	4.17	4	8.33

Table 12. Total bacterial identification and prevalence.

Genus/specie	Prevalence %
Aeromonas hydrophila	1.0
Aeromonas salmonicida	2.0
Aeromonas spp.	1.5
Burkholderia spp.	0.5
Chryseobacterium idologenes	1.5
Citrobacter spp.	3.6
Edwardsiella hoshinae	0.5
Edwardsiella spp.	3.0
Enterobacter amnigenus	0.5
Enterobacter gergoviae	0.5
Enterobacter intermedius	1.0
Enterobacter spp.	8.1
Escherichia coli	9.1

Ewingella americana	1.5
Klebsiella pneumoniae	0.5
Klebsiella spp.	0.5
Klebsiella terrigena	1.0
Ochrobactrum antrophi	1.0
Pantoea spp.	4.1
Pasteurella spp.	0.5
Plesiomonas spp.	0.5
Proteus spp.	0.5
Pseudomonas aeruginosa	0.5
Rahnella aquatilis	5.1
Salmonella enteritidis	0.5
Salmonella gallinarum	2.0
Staphylococcus aureus	6.6
Staphylococcus epidermidis	10.7
Staphylococcus spp.	9.1
Streptococcus spp.	2.5
Yersinia enterocolitica	2.0
Yersinia spp.	1.0
Gram-positive bacilli	16.8

 Table 13. Total fungal identification and prevalence.

Genus/specie	Prevalence %
Absidia spp.	1.5
Alternaria spp.	4.9
Bipolaris spp.	0.5
Chrysonilia spp.	4.4
Cladosporium spp.	20.4
Epicoccum spp.	9.2
Fusarium spp.	5.3
Humicola spp.	1.0
Mucor spp.	9.2
Nigrospora spp.	1.5
Penicillium spp.	29.1
Phoma spp.	0.5
Rhizopus spp.	1.5
Sordaria spp.	0.5
Trichoderma spp.	1.0
Yeasts colonies	3.9
Fungal colonies with sterile mycelium	5.8

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Author's address

Diego Fernando Gonzalez Lozano

Facultad de Ciencias Básicas, Universidad Militar Nueva Granada, Bogotá – Colombia u0500801@unimilitar.edu.co

Camila Andrea Camacho Ramos

Facultad de Medicina y Ciencias de la Salud, Universidad Militar Nueva Granada, Bogotá – Colombia u0401564@unimilitar.edu.co

Iván Alberto Méndez Rodríguez

Facultad de Medicina y Ciencias de la Salud, Universidad Militar Nueva Granada, Bogotá – Colombia ivan.mendez@unimilitar.edu.co