Volume 8, Issue14 — January — June — 2022

ECORFAN

Journal-Republic of Guatemala

ISSN-On line: 2410-8849

ECORFAN®

ECORFAN Journal-Republic of Guatemala, Volume 8, Issue 14, June 2022, is a journal edited semestral by ECORFAN. Kilometer 16, American Highway, House Terra Alta, House D7 Mixco Zona 1, Republic of Guatemala. WEB: www.ecorfan.org/republicofguatemala/, journal@ecorfan.org. Editor in Chief: MARTÍNEZ-HERRERA, Erick Obed. MsC. ISSN Online: 2414-8849. Responsible for the latest update of this number ECORFAN Computer Unit. ESCAMILLA-BOUCHÁN, Imelda, LUNA-SOTO, Vladimir, Kilometer 16, American Highway, House Terra Alta, House D7 Mixco Zona 1, Republic of Guatemala, last updated June 30, 2022.

ECORFAN Journal Republic of Guatemala

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Knowledge Area

The works must be unpublished and refer to topics of Biological and Health Sciences, Medical Mycology, Dermatology, Immunology, Human Ecology, Parasitology, Pediatric Infectious Diseasesand other topics related to Medicine and Health Sciences.

Presentation of Content

In the first article we present, *Prevalence of dental calculus in a young adult population in a suburban community of the city of San Francisco de Campeche 2021*, by ROSADO-VILA, Graciella, ZAPATA-MAY, Rafael, OROZCO-RODRIGUEZ, Rubén and VIDAL-PAREDES, Jorge, with adscription in the Universidad Autónoma de Campeche, as the following article we present, *Calculation of optimal environmental indices, to preserve/conserve melipona bees in the State of Puebla*, by SANTOS-OSORIO, Arturo, SOTO-LEYVA, Yasmin and BONES-MARTÍNEZ, Rosalía, with adscription in the Instituto Tecnológico Superior de Huauchinango, as the following article we present, *Differential genes of Arabidopsis thaliana in response to Ustilago maydis infection*, by PLANCARTE-DE LA TORRE, Marco M., CASARRUBIAS-CASTILLO, Kena, ROBLES-MURGUIA Celia and MÉNDEZ-MORÁN, Lucila, with adscription in Universidad de Guadalajara, as the following article we present, *Prevalence of drug-resistant uropathogenic bacteria in canines in the city of Merida, Yucatan*, by BASTO-MIJANGOS, Harold Noe, DUARTE-MENDOZA, Grisell Anahí, PÉREZ-BRETÓN, Susana A. and KIM-MOO, Manuel J., with adscription in the Yi Health Grupo Diagnóstico S.A.S. de C.V.

Content

Article **ECORFAN Journal-Republic of Guatemala**
June 2022, Vol.8 No.14 1-12

Prevalence of dental calculus in a young adult population in a suburban community of the city of San Francisco de Campeche 2021

Prevalencia de cálculo dental en población adulta joven de una comunidad suburbana de la ciudad de San Francisco de Campeche 2021

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DOI: 10.35429/EJRG.2022.14.8.1.12 Received: January 30, 2022; Accepted June 30, 2022

Abstract

Good oral health goes hand in hand with good dental hygiene, this guarantees well-being in people. Unfortunately, people do not have good dental hygiene due to various factors, one of them is personal economy. According to the study carried out by the Key-Stone Consultant under the title, "Patients in the dental sector", 21% of patients admit not going to a dental clinic, despite having some oral problem present, due to economic difficulties, lack of time, lack of importance given. This generates an accumulation of microorganisms in the oral cavity generating a bacterial plaque giving rise to dental calculus or dental tartar and this causes endless oral problems such as lack of dental aesthetics. This research work studies dental calculus as well as the relationship of associated factors for this condition to be generated. This is achieved from the exhaustive review of scientific articles concerning the oral health problem, analyzing the prevalence of dental calculus in young - adults in the suburban neighborhood of the city of San Francisco de Campeche during the year 2021 is the main objective. In interviews conducted with young patients, the analysis showed that of 20 people who were interviewed, 14 brush only twice a day and therefore there is a visualization of the presence of dental calculus presenting other diseases. 50% of people have economic problems which limit their ability to go to the dentist. The lack of time to do an adequate dental cleaning is also a factor that was presented in the suburban area since 70% admitted to having a lack of time. Concerning visits to the dentist, the analysis resulted in 8 people never going to the dentist and only one person going 3 times for braces. In this project, dental calculus is a factor that causes multiple problems in the oral cavity that are associated with the lack of good responsible habits with the mouth. The same dental calculus causes the loss of teeth. It is extremely relevant to promote dental education in young people as in adults with the purpose of raising awareness and changing the attitude of the most vulnerable population of these suburban areas of the city of Campeche. The implementation of oral health programs is necessary in order to improve them.

Dental prosthesis, Oral health, Aesthetics

Resumen

Una buena salud bucal va de la mano con una buena higiene dental, esto garantiza bienestar en las personas. Desgraciadamente, las personas no tienen una buena higiene dental debido a diversos factores, uno de ellos es la economía personal. Según el estudio realizado por la Consultora Key-Stone bajo el título, "Pacientes en el sector dental", el 21% de los pacientes reconoce no acudir a una clínica dental, a pesar de tener algún problema bucodental presente, debido a dificultades económicas, falta de tiempo, falta de importancia que se le da. Esto genera una acumulación de microorganismos en la cavidad bucal generando una placa bacteriana dando lugar al cálculo dental o sarro dental y esto provoca un sin fin de problemas bucales como la falta de estética dental. En este trabajo de investigación se estudia el cálculo dental así como la relación de factores asociados para que se genere esta afección. Esto se logra a partir de la revisión exhaustiva de artículos científicos referentes al problema de salud bucal, analizando la prevalencia de cálculo dental en jóvenes - adultos de la colonia suburbana de la ciudad de San Francisco de Campeche durante el año 2021 es el objetivo principal. En entrevistas realizadas a pacientes jóvenes, el análisis arrojó que de 20 personas que fueron entrevistadas, 14 se cepillan sólo dos veces al día y por lo tanto se visualiza la presencia de cálculo dental presentando otras enfermedades. El 50% de las personas tienen problemas económicos que limitan su capacidad para ir al dentista. La falta de tiempo para hacer una limpieza dental adecuada también es un factor que se presentó en el área suburbana ya que el 70% admitió tener falta de tiempo. En cuanto a las visitas al dentista, el análisis dio como resultado que 8 personas nunca van al dentista y sólo una persona va 3 veces por aparatos dentales. En este proyecto, el cálculo dental es un factor que causa múltiples problemas en la cavidad bucal que están asociados a la falta de buenos hábitos responsables con la boca. El mismo cálculo dental causa la pérdida de dientes. Es de suma relevancia promover la educación dental tanto en jóvenes como en adultos con el propósito de concientizar y cambiar la actitud de la población más vulnerable de estas zonas suburbanas de la ciudad de Campeche. Es necesaria la implementación de programas de salud bucal para mejorarlos.

Prótesis dental, Salud oral, Estética

Citation: ROSADO-VILA, Graciella, ZAPATA-MAY, Rafael, OROZCO-RODRIGUEZ, Rubén and VIDAL-PAREDES, Jorge. Prevalence of dental calculus in a young adult population in a suburban community of the city of San Francisco de Campeche 2021. ECORFAN Journal-Republic of Guatemala. 2022. 8-14:1-12.

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† Researcher contributing first author.

Introduction

The oral cavity is exposed to thousands of bacteria due to the constant microorganisms that enter the oral cavity derived from the food we consume, drinks and the simple fact of being an open cavity from the outside, also due to various factors such as the pH of saliva, mouth temperature, anatomical characteristics, immunological factors and dental hygiene.

A good oral health goes hand in hand with a good dental hygiene which guarantees well being in people, unfortunately people do not have a good dental hygiene due to several factors one of them is the economy according to the study carried out by Key-Stone Consulting under the title, patients in the dental sector, 21% of the patients recognize that they do not go to a dental clinic, This generates an accumulation of microorganisms in the oral cavity generating a bacterial plaque giving rise to dental calculus or dental tartar and this causes a myriad of oral problems.

In this community program we talk in detail about dental calculus and everything related to its generation, this was achieved from research in articles, in interviews with the people we focus on who are young adults, we talk from the age of 15 years to 40 years and the importance we should give to our oral cavity as it is of utmost importance to live a dignified life. It is necessary that the community gives relative interest at the time of brushing your teeth, since having a proper brushing with the use of dental floss that can be accompanied by a mouthwash achieves the goal of mouth care, visiting the dentist could detect oral pathologies in time to prevent, but unfortunately many people have the lack of habit by bad habits.

According to their analysis, people think that by not visiting the dentist they are saving money and avoiding discomfort, it is a risk factor that these bad attitudes continue in the long term and the consequences can be seen, such as dental loss and even problems such as oral instability, studies have shown that having dental problems, such as bad breath.

To be able to analyze the causes and current motives of the population of young people and adults in the suburban area of Campeche, the lack of hygiene gives rise to dental calculus, thus examining the factors that prevent not going to the dentist, the poorly performed techniques at the time of tooth brushing, factors such as bad habits in the living areas as well as the lack of education and the reasons why there should be more implementation in the area of education for dental health from schools, to prevent oral diseases.

Approach to the problem

With the crisis, oral health has become one of the most neglected issues for the majority of public health care patients. In addition to the fear or laziness of having to go to the dentist, there is now a third factor in discord. The economic factor is driving more and more families away from our offices, says Luis Rasal, president of the Official College of Dentists of Aragon, who warns of the health risks involved in neglecting the teeth. Not going to the dentist continues to be a relevant aspect of general health conditions in the Americas. Its importance lies in the fact that it accounts for a large part of the global burden of oral morbidity, because of the costs related to its treatment and the possibility of applying effective preventive measures.

A school of dentists warns that solving a simple problem means savings in the long term, as there is no need to resort to more complicated operations to solve the condition, which is why the ideal is prevention to correct any signs, figures that are more than relevant and show that despite the fact that oral health is gaining social awareness among the population, which is increasingly going to the dentist on a regular basis as a preventive measure to avoid diseases and ailments, there are certain social brakes that prevent oral health care from being completely correct. According to data from a study on the reasons why people do not go to the dentist: According to the study carried out by Key-Stone Consulting under the title, "Patients in the dental sector", 21% of patients admit that they do not go to a dental clinic, in spite of having an oral problem, because of economic difficulties.

The data from the study also shows that 11% of Spaniards admitted that they do not go to the dentist due to lack of time in their daily lives. It is important to point out that the bacteria found in the mouth are very numerous. According to scientific studies, there are around 100 million bacteria per milliliter of saliva. In addition, there is not only one species of bacteria, since up to 600 different species of bacteria have been found in the mouth, and when dental plaque is not removed during brushing and flossing, it hardens over time and forms dental calculus, which together with bacterial plaque are the main etiological agents of periodontal disease.

Since tartar is rough and porous, it facilitates the retention of more plaque and can be located above the gum margin, covering the spaces between the teeth. It has a yellowish color, although it can acquire darker colorations due to tobacco and other pigments. It has a soft consistency. Among the symptoms that dental calculus can cause are: inflammation and bleeding of the gums, bad breath, recession of the gums, damage to the enamel, caries and even total loss of the tooth. Once the calculus is formed, it can be removed only by our dentist by ultrasound or scaling and root planing, depending on its location. In the case of bacterial plaque, oral hygiene instructions are provided to the patient to control its deposit, as well as to prevent its subsequent calcification into tartar.

- Tartar damages the supporting structure of the teeth.

- A good brushing technique, the use of dental floss and irrigator are key to prevent its formation.

- It cannot be eliminated by tooth brushing, so only professional hygiene can do it Prophylaxis at the dentist.

We should not wait until we have pain to go to the dentist's office, since, probably, if we have pain it means that we already have a problem that requires treatment. If we go for regular check-ups and we apply prophylaxis techniques, we will certainly avoid many of the dental and gingival problems that we could develop throughout our lives.

Prophylaxis is a treatment that does not require anesthesia unless the patient has dental hypersensitivity. Ultrasonic appliances are usually used to remove the calculus; these instruments, being refrigerated, can be uncomfortable in the case of sensitive teeth. After the mechanical removal of bacterial plaque and calculus, we will finish the gingival prophylaxis with the elimination of dental stains using a prophylaxis brush and dental polishing paste. The best way to enjoy a healthy smile and healthy teeth is to continue the good oral habits acquired during early childhood. A healthy smile is more than cosmetics.

Studies indicate that the health of your teeth and gums can indicate the state of your overall health. Poor dental hygiene has been associated with a high risk of some diseases in adults. Similarly, the mouth is a wonderful and privileged part of the human body, because through it the cry that announces the arrival of a human being to life emerges; it is a moment of great emotion and importance the act of feeding and love that constitutes breastfeeding where the oral cavity plays a fundamental role. It should be taken into account that during adolescence the permanent teeth fully erupt and, therefore, oral hygiene should be constant, since they are still immature and are more prone to dental caries; in addition, during this period the frequency of ingestion of sweets increases and tooth brushing during school hours decreases (Duarte Lisimón J. Influencia de las técnicas educativas en el conocimiento sobre salud bucal [work for the title of 1st Degree Specialist in Periodontology]. 2007, Santiago de Cuba). Characteristics of this stage, such as: rebelliousness, neglect of personal hygiene habits, rejection of parental authority, among others, cause concern among family members, teachers and health personnel. For the aforementioned reasons, it is important to raise awareness among adolescents to take care of their teeth and take extreme care of oral hygiene measures, as well as to systematize visits to the stomatologist every 6 months, even if their teeth remain healthy. Likewise, they should be guided to perform oral and facial examinations to prevent cancer and to teach the practice of self-examination of this cavity.

Both the adolescent and the mother will be questioned about habits, customs and knowledge related to oral health, specifically aimed at nutrition, hygiene and inadequate habits. Action should also be taken on the risks associated with negative lifestyles for the health of the members of this population group and their environment; prevent accidents and traumas involving the oral or facial region; control adequate supplies of fluoride supplements in accordance with the risks to which they are exposed; carry out plaque controls to ensure correct and effective toothbrushing, as well as taking into account the sequences of dental outbreaks. Regarding the timing of this analysis... 33% of Spaniards are considering not going to the dentist in the next few months due to economic reasons and the uncertainty caused by the coronavirus pandemic. This research reveals that 21% of the families declare serious economic problems: 10% of the families interviewed state that they have suffered a very negative impact due to the loss of employment of one or more of their members, together with 11% who confirm that they are living a difficult situation due to an ERTE or labor instability that will increase, could last in time or worsen later on. Another 16% have managed to resolve problems related to job uncertainty and temporary lay-offs, while only 63% of families remain unscathed by job security issues. People should always keep in mind that dental hygiene is key to maintaining a healthy and balanced bacterial flora in our mouth. Health education in dentistry would be of great help in preventing dental calculus: It is a continuous learning process that begins in childhood, continues throughout our lives and promotes the development of healthy practices.

Nowadays, it is considered a fundamental tool to achieve the strategies proposed by Health Promotion. Health education implies generating a change in behavior through messages that promote the control of behavioral risk factors through the application of various methodologies, theories and models appropriate to the reality or context in which we wish to apply them. In the Faculty of Dentistry of the Universidad Nacional Mayor de San Marcos: - Gutiérrez et al. conducted a quasi-experimental-comparative study in 2020 to test the effectiveness of two types of didactic methods, one traditional (expository by an expert) and the other innovative (playful: Dentoplay rules game) on the attitudes of young people aged 15-18 years for the preservation of their oral health. The study was carried out in a National High School Educational Institution in the district of San Martin de Porres. The population consisted of 70 young people who were divided into two groups Health Education in Dentistry. 239 (A and B) of 35 children each. Group A was assigned the expository didactic method by an expert using a giant model of the upper and lower jaws and a flip chart, while group B was assigned the Dentoplay didactic method. An evaluation form was applied to the youngsters at the beginning and end of the intervention to monitor learning. In both groups the topics were developed equally and the attitude of the youngsters towards their oral health was measured based on the following elements: cognitive (knowledge and beliefs), affective (feelings and preferences), volitional (manifest actions: participation, attention and concentration) and oral hygiene control.

With regard to oral hygiene, it was observed that the percentage of young people with poor hygiene (at the beginning of the study) in both group A and group B decreased significantly after the application of the methods indicated, with the difference being greater in the group of young people who used the play method. Furthermore, only in group B, at the end of the study was there a percentage of young people with good oral hygiene (8.82%). On the other hand, both in group A and group B the cognitive variables comparing before and after were from fair to good and the affective variables in group A were from not favorable to not very favorable, while in group B they were from moderately favorable and favorable to very favorable. Finally, both methods allowed increasing the degree of knowledge in the young people, but their feelings and preferences towards oral health (affective component) were inclined towards the playful method.

Justification

This research project aims to improve dental health in young people and adults in order to implement a better quality of life. The mouth represents an important part of multiple oral functions. The population segment to which this study is directed is with the purpose of raising awareness and preserving oral health.

The presence of dentobacterial plaque is part of an accumulation of bacteria present in the mouth, currently the suburban community of young adults do not know the proper dental hygiene measures since this population has not been studied, lacks oral health programs that assess the presence of dental calculus, the purpose is to raise awareness and implement previous health measures to this suburban population of young adults with oral hygiene campaigns.

Theoretical framework

Oral health has become with the crisis one of the most neglected in most of the patients of the public health system. In addition to the fear or laziness of having to go to the dentist, there is now a third discordant factor. "The economic factor is driving more and more families away from our offices," says Luis Rasal, president of the Illustrious Official College of Dentists of Aragon, who warns of the health risks involved in neglecting the teeth. Not going to the dentist continues to be a relevant aspect of general health conditions in the Americas. Its importance lies in the fact that it accounts for a large part of the global burden of oral morbidity, because of the costs related to its treatment and the possibility of applying effective preventive measures.

The College of Dentists warns that solving a simple problem means savings in the long term, since it is not necessary to resort to more complicated "operations" to solve the condition, so the ideal is prevention to correct any signs. These figures are more than relevant and show that, despite the fact that oral health is gaining social awareness among the population, which is increasingly going to the dentist on a regular basis as a preventive measure to avoid diseases and ailments, there are certain social brakes that prevent oral health care from being completely correct. According to data from a study on the reasons why people do not go to the dentist: According to the study carried out by Key-Stone Consulting under the title, "Patients in the dental sector", 21% of patients admit that they do not go to a dental clinic, in spite of having an oral problem, because of economic difficulties.

The data from the study also shows that 11% of Spaniards admitted that they do not go to the dentist due to lack of time in their daily lives. It is important to point out that the bacteria found in the mouth are very numerous. According to scientific studies, there are around 100 million bacteria per milliliter of saliva. In addition, there is not only one species of bacteria, since up to 600 different species of bacteria have been found in the mouth, and when dental plaque is not removed during brushing and flossing, it hardens over time and forms dental calculus, which together with bacterial plaque are the main etiological agents of periodontal disease. Tartar, being rough and porous, facilitates the retention of more plaque and can be located above the gum margin, covering the spaces between the teeth, it has a yellowish color, although it can acquire darker colorations due to tobacco and other pigments. It has a soft consistency. Among the symptoms that dental calculus can cause are: inflammation and bleeding of the gums, bad breath, recession of the gums, damage to the enamel, caries and even total loss of the tooth.

Once the calculus is formed, it can only be removed by our dentist by means of ultrasound or scaling and root planing, depending on its location. In the case of bacterial plaque, oral hygiene instructions are provided to the patient to control its deposit, as well as to prevent its subsequent calcification into tartar. - Tartar damages the supporting structure of the teeth. - Good brushing technique, flossing and irrigation are key to prevent its formation. - It cannot be eliminated by tooth brushing, so only professional hygiene can do it Prophylaxis at the dentist... We should not wait until we have pain to go to the dentist's office, since, probably, if we have pain it means that we already have a problem that requires treatment. If we go for regular check-ups and we apply prophylaxis techniques, we will certainly avoid many of the dental and gingival problems that we could develop throughout our lives. Prophylaxis is a treatment that does not require anesthesia unless the patient has dental hypersensitivity. Ultrasonic appliances are usually used to remove the calculus; these instruments, being refrigerated, can be uncomfortable in the case of sensitive teeth. After the mechanical removal of bacterial plaque and calculus, we will finish the gingival prophylaxis with the elimination of dental stains using a prophylaxis brush and dental polishing paste.

The best way to enjoy a healthy smile and healthy teeth is to continue the good oral habits acquired during early childhood. A healthy smile is more than cosmetics. Studies indicate that the health of your teeth and gums can indicate the state of your overall health. Poor dental hygiene has been associated with a higher risk of some diseases in adults. Similarly, the mouth is a wonderful and privileged part of the human body, because through it springs the cry that announces the arrival of a human being to life; it is a moment of great emotion and importance the act of feeding and love that constitutes breastfeeding where the oral cavity plays a fundamental role.

It should be taken into account that during adolescence the permanent teeth erupt completely and, therefore, oral hygiene should be constant, since they are still immature and are more prone to dental caries; in addition, during this period the frequency of ingestion of sweets increases and toothbrushing during school hours decreases. Duarte Lisimón J. Influencia de las técnicas educativas en el conocimiento sobre salud bucal [work to opt for the title of 1st Degree Specialist in Periodontology. 2007, Santiago de Cuba. Characteristics of this stage, such as: rebelliousness, neglect of personal hygiene habits, rejection of parental authority, among others, cause concern among family members, teachers and health personnel. For the aforementioned reasons, it is important to raise awareness among adolescents to take care of their teeth and take extreme care of oral hygiene measures, as well as to systematize visits to the stomatologist every 6 months, even if their teeth remain healthy. Likewise, they should be oriented to perform oral and facial examinations to prevent cancer, and teach the practice of self-examination of this cavity. Both the adolescent and the mother should be questioned about habits, customs and knowledge related to oral health, specifically directed towards nutrition, hygiene and inadequate habits. Action should also be taken on the risks associated with negative lifestyles for the health of the members of this population group and their environment; prevent accidents and traumas involving the oral or facial region; control adequate supplies of fluoride supplements in accordance with the risks to which they are exposed; perform plaque controls to ensure correct and effective toothbrushing, as well as taking into account the sequences of dental outbreaks. Regarding the timing of this analysis...

33% of Spaniards are considering not going to the dentist in the coming months due to economic reasons and the uncertainty caused by the coronavirus pandemic. This research reveals that 21% of the families declare serious economic problems: 10% of the families interviewed state that they have suffered a very negative impact due to the loss of employment of one or more of their members, together with 11% who confirm that they are living a difficult situation due to an increase in unemployment or job instability, which could last for a long time or worsen at a later date. Another 16% have managed to resolve problems related to job uncertainty and temporary lay-offs, while only 63% of families remain unscathed by job security issues. People should always keep in mind that dental hygiene is key to maintaining a healthy and balanced bacterial flora in our mouth. Health education in dentistry would be of great help in preventing dental calculus: It is a continuous learning process that begins in childhood, continues throughout our lives and promotes the development of healthy practices. It is currently considered a fundamental tool for achieving the strategies proposed by Health Promotion. Health education implies generating a change in behavior through messages that promote the control of behavioral risk factors through the application of various methodologies, theories and models appropriate to the reality or context in which we wish to apply them. In the Faculty of Dentistry of the Universidad Nacional Mayor de San Marcos: -Gutiérrez et al. conducted a quasi-experimental-comparative study in 2020 to test the effectiveness of two types of didactic methods, a traditional expository method by an expert and an innovative playful one: Dentoplay rules game on the attitudes of young people aged 15-18 years for the preservation of their oral health.

The study was carried out in a National High School Educational Institution in the district of San Martin de Porres. The population was 70 young people who were divided into two groups Health Education in Dentistry. 239 A and B of 35 children each. Group A was assigned the expository didactic method by an expert using a giant model of the upper and lower jaws and a flip chart, while group B was assigned the Dentoplay didactic method. An evaluation form was applied to the youngsters at the beginning and end of the intervention to monitor learning.

In both groups the topics were developed equally and the attitude of the young people towards their oral health was measured based on the following elements: cognitive knowledge and beliefs, affective feelings and preferences, volitional manifest actions: participation, attention and concentration, and control of oral hygiene. With respect to oral hygiene, it was observed that the percentage of young people with poor hygiene at the beginning of the study in both group A and group B decreased significantly after the application of the methods indicated, with the difference being greater in the group of young people who used the play method. In addition, only in group B, at the end of the study, the percentage of young people with good oral hygiene was 8.82%. On the other hand, both in group A and group B the cognitive variables comparing before and after were from fair to good and the affective variables in group A were from not favorable to not very favorable, while in group B they were from moderately favorable and favorable to very favorable. Finally, both methods allowed increasing the degree of knowledge in the young people, but their feelings and preferences towards oral health affective component were inclined towards the ludic method. through a reduction in the total intake and frequency of consumption of sugars. Protection of the tooth surface can be achieved by ensuring adequate exposure to fluorides.

Likewise, actions can be taken to reduce the effect of biofilm through good oral hygiene. Studies reported worldwide on its prevalence, such as the World Health Organization in 2004, showed 60 to 90% in school children and almost 100% in adults, and the Dental Federation International in 2010, found 44%, affecting almost half of the population. Dental caries is a disease of high prevalence and severity in the populations of various countries. In industrialized countries it affects more than half of the population, and because it is a cumulative process, the severity of the damage increases with increasing age. From the perspective of causality, caries is a complex disease since it is caused by the interaction of different mechanisms. For its analysis, the action of several genes, environmental, cultural, social and local factors should be considered. Several authors mention that dental caries is a public health problem due to its high prevalence. Dental caries is a process that can evolve and cause tooth loss if it is not treated promptly.

In Mexico it has been documented that the prevalence of dental caries is between 70 and 85% in secondary dentition at the age of years. The onset of the carious process cannot be attributed to a single cause, since its development requires a confluence of factors that determine the carious lesion, that is, that the aggression of the dental enamel is of great magnitude, that the resistance of the enamel to acid dissolution is insufficient, and that the mechanisms of remineralization of the enamel do not take place.

When dental caries reaches the deep dental tissues it becomes an emergency in stomatological services, but only if we know how this condition starts and spreads can we cure and prevent it. It is in this direction that the greatest efforts should be directed towards the control of this most frequent disease. It is for these reasons that we were motivated to carry out this work. Dentists fill teeth by removing the decayed dental material with the use of a dental drill and replacing it with a material such as silver alloys, gold, porcelain or composite resin. The latter two materials most closely resemble the natural appearance of the tooth and may be preferred for front teeth. Many dentists consider silver and gold alloy amalgams to be stronger and tend to use them for the back teeth, although there is a tendency to use the highly resistant composite resin in the back teeth as well. Periodontal disease: Undoubtedly in the last decade there has been increasing evidence that periodontal disease is a worldwide public health problem that health systems should take care of and periodontal disease represents the main cause among adults. According to the type of tooth, the twenty-one molars are extracted mainly due to caries and the anterior teeth due to periodontal disease. Dental caries and periodontal disease in advanced stages cause pain and the individual, when he/she does not have sufficient resources, prefers to eliminate it by means of dental extraction, resisting to face a long and expensive conservative treatment, which many times is not available in public health services. Specific bacteria are the essential cause of periodontal diseases. Other important risk factors are smoking, unhealthy diet, genetic factors, stress or excessive alcohol consumption.

Periodontal diseases are also associated with certain systemic diseases such as diabetes, cardiovascular diseases, respiratory diseases and some complications during pregnancy. Periodontal disease is a bacterial disease that causes low intensity infection, with important metabolic consequences and local inflammatory reactions that destroy supporting and protective tissues of the tooth. Gingivitis Periodontal disease begins with gingivitis, a chronic inflammation of the gums, which is very common and reversible for most patients. It can progress to periodontitis, a more serious situation in which there is destruction of the supporting bone. In 15% of the population the disease can progress to severe periodontitis which can lead to tooth loss. The most difficult part is to identify the sites that are already evolving from gingivitis to periodontitis. We can find sites with depths of 4 mm but which still do not show radiographic evidence of bone loss and this is mainly due to the low sensitivity of radiography and probing error. It has been calculated that the probing error is approximately 1 mm and added to the degree of inflammation, we can easily go from 3 mm to 4 mm. This should be carefully analyzed by interpreting all periodontal clinical parameters. The extent of gingivitis can be classified as localized ≤30% of affected sites and generalized >30% of affected sites. Likewise it can be marginal, papillary and diffuse. Periodontitis is the most common chronic inflammatory disease seen in humans. It is a major public health problem, causing tooth loss, disability, masticatory dysfunction and nutritional status. Deficient.

The manifestations of periodontitis, such as bleeding, halitosis, gingival recession and tooth loss, can have an impact beyond the individual sufferer. Periodontitis also compromises speech, reduces quality of life and is an increasing burden on the economy. Chronic Periodontitis Unlike gingivitis, periodontitis is inflammation of the gingiva and supporting periodontium, significantly affecting the gingival connective tissue, periodontal ligament, cementum, bone. As a pathognomonic result we observe inflammation, bleeding on probing, periodontal pocket formation, attachment loss and radiographic bone loss. Aggressive periodontitis Aggressive periodontitis usually occurs in subjects younger than 35 years, but it is suggested that it can occur at any age.

The rate of periodontal destruction is rapid and since it begins early in life, the destruction is seen in young subjects. But the analysis should not be based on age alone but on clinical findings, radiographic findings, family and personal history, laboratory aids. In the early stages of periodontal disease, most of the treatment consists of scaling and root planing, which means removing plaque and calculus in the pockets around the teeth and smoothing the root surfaces. In most cases of early periodontal disease, the above treatment and daily home care is all that is required for a satisfactory result. More advanced cases may require surgical treatment.

Early diagnosis of periodontal diseases is one of the main motivations for today's dental practice. With prevalence data being so contumacious, the prevention of gingivitis and periodontitis is one of the basic commitments of a dentist to the society in which he or she develops as a health professional. Dentobacterial plaque forms as a deposit on the surface of the tooth, and is made up of bacteria, their extracellular products and glycoproteins. ROSS, et al, 1984. Bacterial plaque corresponded to J. Leon Williams between 1852 and 1832, an American dentist, who worked in London, England, and who, in 1897, described a gelatinous accumulation of bacteria attached to the enamel surface in relation to dental caries. Carranza 1997. The researcher G.V. Black, a dentist and microbiologist between the years 1836-1915, discovered in some investigations that there were deposits of bacterial food on the surface of the teeth, proposing the term plaque, suggesting that the bacteria that inhabited it were mediators of forming a gelatinous substance that adhered to the surface of the teeth. The term plaque was first used in 1898 by G. V. Black to describe the microbial mass that coated carious lesions. Carranza1997; Liebana, 1997. At the end of the 21st century, Slots and Taubman published an article in 1992, in which they pointed out that this accumulation of bacteria associated with the dental surface could be easily removed by mouth rinses in its initial stages of development).

Currently, at the beginning of the XXI century, the World Health Organization has introduced a new concept known as Biofilm, this research has concluded that this is the biofilm that bathes the tooth surfaces that corresponds to a proliferating bacterial entity with enzymatic activity that adheres firmly with biochemical and metabolic activity that it possesses, has been proposed today as the main etiological agent in the development of dental caries. In 1987, a bibliographic study on bacterial plaque was carried out at the Evangelical University of El Salvador, whose primary objective was to present in a global manner the morphology of dental plaque and its evolution, as well as aspects of bacterial specificity and methods used for plaque control Dental calculus The period required is very variable, from days to weeks, usually beginning between the first and 14th day of plaque formation. However, there are reports of calcification in as little as 4 to 8 hours, because plaque in the process of calcification can mineralize 50% in 2 days and 60 to 90% in 12 days Carrranza, 1991 It usually adheres strongly to the teeth and a new acquired film can form on its surface and so on. Their main problem is to be an obstacle to the effectiveness of oral hygiene since they are areas of mechanical retention for microorganisms and exit points for bacterial toxic products irritating to the oral soft tissues. Carranza 1997; Calcification involves the fixation of calcium ions with carbohydrate-protein complexes of the organic matrix and the precipitation of crystalline salts of calcium phosphates. Carranza, 1997.

Background and terminology

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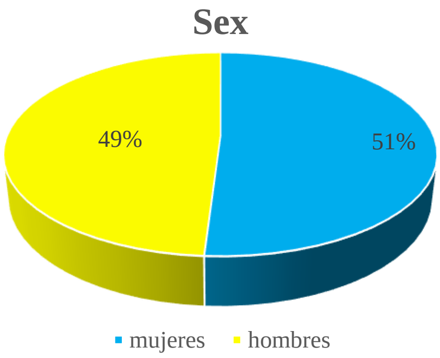
Methodology

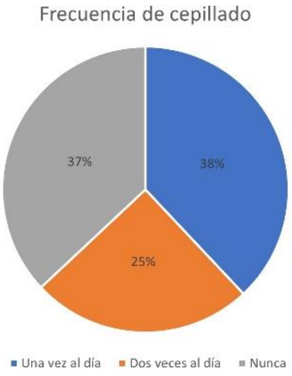
The study design is observational, descriptive and cross-sectional, carried out in a suburban population of the city of San Francisco de Campeche. The sample was obtained by means of the formula for finite sample size with a total of 100 subjects.

Results

Of the study population of subjects n=100, 51% corresponded to male and 49% to female. The mean age was 30 years with a standard deviation of 11 and a range of 53. With respect to the variable times brushing, the majority 37% brushed once a day, 25% twice a day, never 38%. The distribution of the prevalence of dental calculus with respect to sex, reported high dental calculus in males with 28%, followed by moderate dental calculus in males with 30%, low dental calculus in males with 42%, high dental calculus but in females with 29%, moderate dental calculus also prevalent in females with 35%, and finally low dental calculus with 36% in females.



Graphic 1

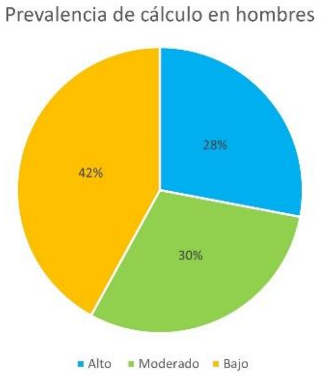


Graphic 2

Conclusion

Dental calculus is a primary factor for the development of multiple problems in the oral cavity, the lack of attitude in good hygiene habits in the oral cavity propitiates the presence of dental calculus which represents a dental loss of great relevance in the young-adult population, the promotion of dental education in young people as well as adults is the purpose of prevention through the implementation of oral health programs.

The study carried out within the community demonstrates the high rate of lack of frequency of brushing that present dental calculus, in the information based on scientific articles where oral health has become with the crisis one of the great forgotten in most of the patients of the public health. In addition to the fear or laziness of having to go to the dentist, there is now a third discordant factor. The economic factor is driving more and more families away from our offices, says Luis Rasal, president of the Official College of Dentists of Aragon, who warns of the health risks involved in neglecting the teeth. Not going to the dentist continues to be a relevant aspect of general health conditions.



Graphic 3

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Article **ECORFAN Journal Republic of Guatemala**
June 2022, Vol.8 No.14 13-22

Calculation of optimal environmental indices, to preserve/conserve melipona bees in the State of Puebla

Cálculo de índices ambientales óptimos, para preservar/conservar abejas meliponas en el Estado de Puebla

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DOI: 10.35429/EJRG.2022.14.8.13.22 Received January 15, 2022; Accepted June 30, 2022

Abstract

The objective of this investigation is to verify that the environmental factors of temperature (°C) and wind (m/s) in the meliponaria of the State of Puebla are optimal for the reproduction of the Melipona Bee. The applied methodology contemplates 3 phases: 1) Analysis of environmental conditions of the region, 2) Selection of measurement devices: Use of two types of thermometers, the Steren Thermo-Hygrometer thermometer assigned for temperature measurements (°C), and the UA-965 Thermo-Anemometer thermometer to quantify temperature and wind speed, 3) Statistical studies of environmental factors of temperature and wind (random sampling) . The contribution is exposed socially, economically and culturally, with respect to the social environment, the optimal levels of temperature and wind are indicated, this action will benefit 70% of the meliponiculturists with the increase in the levels of bee reproduction, this increase will cause greater quantities of products (honey, propolis, wax) which, when sold, will attract greater economic uptake for meliponiculturists. Finally, the cultural contribution focuses on the conservation and preservation of the region's native bees, fostering a reproduction system with adequate environmental conditions.

Environmental conditions, Wind, Temperature, Meliponaria

Resumen

El objetivo de esta investigación es verificar que los factores ambientales de temperatura (°C) y viento (m/s) en los meliponarios del Estado de Puebla sean óptimos para la reproducción de la Abeja Melipona. La metodología aplicada contempla 3 fases: 1) Análisis de las condiciones ambientales de la región, 2) Selección de los dispositivos de medición: Uso de dos tipos de termómetros, el termómetro Steren Thermo-Hygrometer asignado para mediciones de temperatura (°C), y el termómetro UA-965 Thermo-Anemometer para cuantificar temperatura y velocidad del viento, 3) Estudios estadísticos de factores ambientales de temperatura y viento (muestreo aleatorio) . La contribución se expone social, económica y culturalmente, con respecto al ámbito social, se indican los niveles óptimos de temperatura y viento, esta acción beneficiará al 70% de los meliponicultores con el incremento en los niveles de reproducción de las abejas, este incremento provocará mayores cantidades de productos (miel, propóleos, cera) que al ser comercializados atraerán mayor captación económica para los meliponicultores. Finalmente, la contribución cultural se centra en la conservación y preservación de las abejas nativas de la región, fomentando un sistema de reproducción con condiciones ambientales adecuadas.

Condiciones ambientales, Viento, Temperatura, Meliponaria

Citation: SANTOS-OSORIO, Arturo, SOTO-LEYVA, Yasmin and BONES-MARTÍNEZ, Rosalía. Calculation of optimal environmental indices, to preserve/conserve melipona bees in the State of Puebla. ECORFAN Journal-Republic of Guatemala. 2022. 8-14:13-22.

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Introduction

In natural situations, each species occupies a reasonably defined ecological niche where individuals tolerate or adapt to most variations in the physical environment. Therefore, an adapted living being is one that is in perfect harmony with its environment.

However, from the diversity of interrelated factors that make up the environment, the climate turns out to be relevant due to the effects caused to the vegetation and fauna. From this perspective, the fauna turns out to be an element that requires greater preservation. In the case of the State of Puebla, the conservation of native fauna turns out to be one of the primary objectives, with the preservation of the native bee being a priority, which is distinguished by procreating within the meliponaria of the region and area of influence.

In the experience of the meliponiculturists of the region, the adaptability of the melipona bee turns out to depend directly on the temperature conditions and the affectation that the wind causes to the hives established in the meliponaria, although it is true that the optimal reproduction conditions focus In a hot climate, the native bee of the region seeks means that provide regular temperature and wind conditions, for this reason it is important to create the appropriate environment for the proliferation and conservation of these, what has been described above turns out to be an emerging problem in the Municipality of Huauchinango belonging to the State of Puebla where the exposed research was developed, in which the optimal environmental parameters (wind temperature) are established to increase productivity and preserve existing hives.

Specifically, the research carried out analyzes the affectation of environmental conditions on the reproduction of the native species, considering a random sampling in a period of 12 months (January-December 2021), in which measurements were taken with devices (2 different thermometers) to know the real conditions, these random samplings were carried out at different times and days that make up the period, in the same way a follow-up of the existing hives was shown to know the productivity and generation of main products, according to the environmental conditions that were observed. presented.

Once the sampling was carried out, a comparison is made using statistical studies, this comparison links the variables of temperature and wind with the levels of productivity of the hives, seeking to determine if there is a correlation between the aforementioned factors, Once the correlation is detected, we proceed to establish the optimal parameters of temperature and wind that must be fostered for the significant improvement of the productivity of the bees., to finalize the investigation, a quantitative validation of the effectiveness achieved is carried out, through the measurement of the amount of honeyed product (honey, propolis, wax) obtained from the establishment of optimal conditions, it is important to mention that the benefit achieved is notorious in the social, economic and cultural sphere, supporting SDG 15: Life of terrestrial ecosystems "Agenda 2030", which establishes the generation of sustainable methodologies that promote the p reservation of terrestrial ecosystems within the regions and areas of influence.

Objectives

General objectives

Analysis of environmental factors (air and temperature) to verify the optimal indices that maintain and increase the reproduction of the Melipona bee in the meliponaria established in the Huauchinango region, Puebla, through the use of random sampling and correlational analysis with software technological.

Specific objectives

A. Analyze the characteristics of the environment for the reproduction of the Melipona bee in the region.

B. Identify the suitable instruments to quantify the variables of air and temperature.

C. Use the instruments to measure environmental factors through random sampling and make an assertive record of the data.

D. Determine the optimal environmental factors for the conservation, preservation and increased productivity of the native melipona bee.

Rationale

The investigation is proposed to know if the air and the current temperature in the region of Huauchinango, Puebla, is favorable to preserve and conserve the reproduction of the Melipona bee, considering that within the region the contribution to preservation is of vital importance. of the native terrestrial ecosystems. In the same way, the economic benefit for the meliponiculturists of the State of Puebla is sought, taking into account that the contribution of the sale of the products sustains the family economy, having as antecedent that the affectation of the variables (air/temperature), brings with it the decrease in productivity levels and the physical affectation of the hives that are located inside the meliponaria.

By providing adequate environmental conditions, it seeks to reduce the main problems and create the correct environment for the natural air conditioning of the meliponaria in the State of Puebla.

Theoretical framework

For a better understanding of the aspects that make up this research, the theoretical foundations that support the formulation of the Analysis of environmental conditions for the reproduction of melipona bees in the State of Puebla are described below.

Climate

In the experience of Torres (2018), the climate is conceptualized as the set of physical conditions coming from the atmosphere for an indeterminate period which directly influence human activities, exerting an influence on natural elements such as vegetation, soils, fauna.

Climatic classification

It is known as the topographic action that results from environmental influence, which generates various climatic changes according to the homogeneous characteristics of each area, these characteristics combine to establish two temperature zones, exposed in two sections: 1) Effective zoning (Climate changes are linked to the existence of native vegetation or fauna), 2) Genetic zoning (Formed from the dynamic events that occur in the circulation of the atmosphere) (CEPAL, 2020).

Types of weather

According to the zone analyzed in the present investigation, two types of climate are evident during the year: Warm, temperate.

Hot weather

Characterized by presenting high temperatures because the areas expose low latitudes, causing the sun's rays to have a greater impact due to the inclination (López, Sánchez and Valverde, 2019).

Temperate climate

In the experience of Flores (2019), this type of climate maintains moderate temperatures, leaning towards cold temperatures at certain times of the year.

Elements of weather

Rivas (2018) indicates that the elements of the climate are represented by the following factors: a) Temperature, b) Precipitation, c) Wind, d) Humidity, e) Atmospheric pressure and cloudiness, according to the theme exposed in this research will define below the elements that infer the most:

Temperature

It is considered as a physical indicator of the degree of heating of the air, establishing the following correlation: the greater the heating of the air, the greater the molecular agitation. The unit with which it is measured internationally is the °C.

Wind

It is contextualized from the movement of the air by means of gusts that move through the physical unit of m/s, this displacement is carried out with a route that goes from the high areas to the low areas, regularly showing three dimensions spatial (X, Y, Z).

Measuring instruments

According to the measurement variables, it is decided to use the following means: 1) UA-965 thermometer, 2) Thermo-Anemometer to quantify temperature and wind speed.

Meliponiculture in the State of Puebla

Currently meliponiculture in the State of Puebla, represents the livelihood of various municipalities in which Cuetzalan and Huauchinango stand out, these municipalities contemplate the preservation and reproduction of native bees, with 5% of the producing families depending directly and indirectly on this economic activity, it is important to mention that in recent years various problems have arisen that bring with them the decrease in the production levels of the main honeyed products, among which honey, propolis and wax stand out, these problems in the Most of the cases are derived from the lack of control of the environmental conditions of the region and area of influence, being these a factor that requires prompt control and the taking of actions that contribute to the conservation of native bees (Ávila, 2021).

Environmental conditions for the reproduction of bees

Environmental conditions turn out to be determining variables for the preservation and conservation of native bees, according to the main abiotic factors that affect or benefit the proliferation of bees are the following (González, Flores and Chiappa, 2019):

a) Environmental temperature.

b) Luminous intensity

c) Relative air humidity.

d) Wind speed

As we can see, temperature is one of the factors that generate the greatest negative effects when they are extreme or positive when they are moderate, this in response to the fact that honey bugs are characterized by being apiform organisms. Wang, Liu, Li, & Song , (2009). deduce that the correlation between the different species of native bees and environmental factors is relevant, indicating that the increase in temperature and the decrease in relative humidity notably increases the feeding of these insects, causing honeyed products of considerable quality, the existing correlation (R ^{2}=R2) for sunlight exposes the following statistical relationship (1):

$$> \text{Sunlight} = \text{R2}(0.7426) \tag{1}$$

While for temperature the relationship is (2):

$$> \text{Temperature} = \text{R2}\ (0.7901) \tag{2}$$

On the other hand, the statistical relationship of the wind with honey bugs is contemplated within the following parameters (3):

$$< \text{Wind} < \text{Humidity} = \text{R2} = \ 0.674 \tag{3}$$

Methodology

Below is the methodological description by which the calculation of optimal environmental indices was carried out, to preserve/conserve the melipona bees in the State of Puebla (Figure 1. Phases of the methodological process for the development of the research).

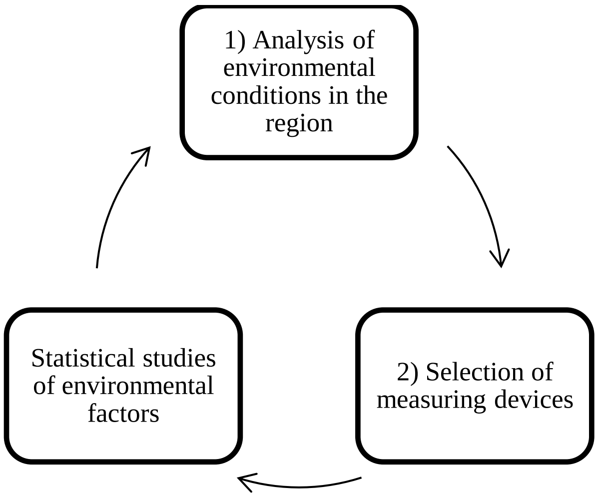


Figure 1 Phases of the methodological process for the development of the research
Source: Own elaboration

Phase 1) Analysis of environmental conditions in the region

In this phase, we proceed to theoretically investigate the environmental factors that have occurred in the last annual period, determining the following variables:

Climate Huauchinango Puebla

Specifically, in the research area (Huauchinango Puebla), a temperature ranging from 9 °C to 28 °C is shown and rarely drops below 5 °C or rises above 32 °C, from from March to June (Figure 2. Summary of the Huauchinango Puebla climate).

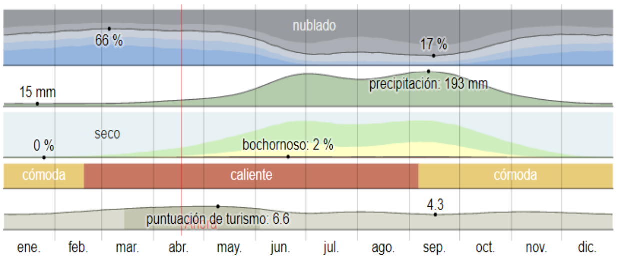


Figure 2 Summary of the Huauchinango Puebla climate
Source: https://es.weatherspark.com/y/6888/Clima-medio-en-Huauchinango-M%C3%A9xico-durante-todo-el-a%C3%B1o#Sections-Humidity

Temperature Huauchinango Puebla

The temperate season lasts for 2.3 months, from March 27 to June 6, with an average daily high temperature above 80°F. The hottest day of the year is May 10, with an average maximum temperature of 28 °C and an average minimum temperature of 15 °C (Figure 3. Average hourly temperature).

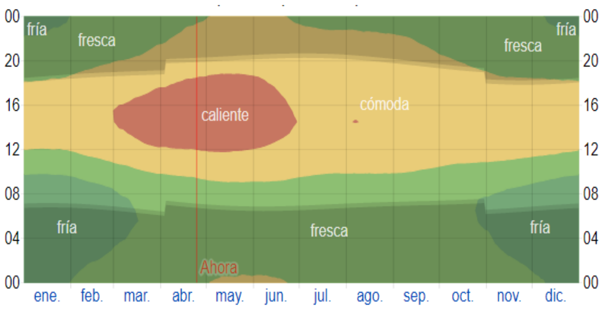


Figure 3 Average hourly temperature
Source:https://es.weatherspark.com/y/6888/Average-weather-in-Huauchinango-M%C3%A9xico-during-all-a%C3%B1o#Sections-Humidity

Wind Huauchinango Puebla

For the case analyzed, the gusts of wind indicate that the wind is a climatic condition exposed during 7.8 months, from February 19 to October 13, with average wind speeds of more than 8.2 kilometers per hour. Specifically, the windiest day of the year on April 29, with an average wind speed of 9.5 kilometers per hour (Figure 4. Average wind speed).

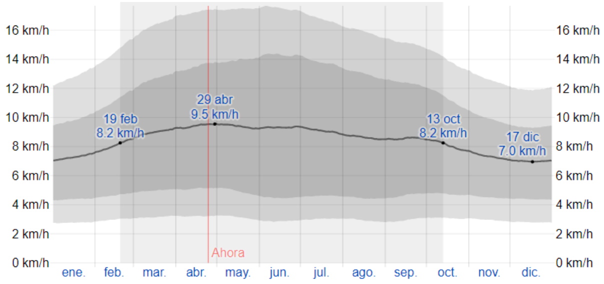


Figure 4 Average wind speed
Source: https://es.weatherspark.com/y/6888/Clima-medio-en-Huauchinango-M%C3%A9xico-durante-todo-el-a%C3%B1o#Sections-Humidity

Phase 2) Selection of measurement devices:

According to the stated need, two models of thermometers are selected, which are:

1. Steren Thermo-Hygrometer assigned for temperature measurements (°C) (Figure 5. Steren Thermo-Hygrometer).

Figure 5 Steren Thermo-Hygrometer
Source: Own elaboration

2. UA-965 Thermo-Anemometer to quantify temperature and wind speed (Figure 6. UA-965 Thermometer).

Figure 6 UA-965 thermometer
Source: Own elaboration

Phase 3) Statistical studies of environmental factors

Once the variables have been determined, a sequence of random samplings of the temperature and wind variables is carried out (Table 1. Abstract of taking measurements).

Day	Steren Thermo-Hygrometer	UA-965 Thermometer
01/03/2021		
06/22/2021		
10/10/2021		

Table 1 Abstract of taking measurements
Source: Own elaboration

These samplings are carried out over a period of 12 months and the results are recorded by means of a control log (Table 2. Abstract of measurement records).

Sampling	Date	Time	Teren Thermo -Hygrometer °C				UA-965 °C				m/s			
			-	X1 _	X2 _	+	-	X1 _	X2 _	+	-	X1 _	X2 _	+
1	05/03/2021	10:00	24.2	27.6	28.8	27.3	26.6	28.6	29.7	29	0	0	0.5	1.5
2		12:00	30.3			30.5	30.5			31.1	0			0
3		2:00 p.m.	28.4			28.7	28.8			28.9	0			0
4	05/05/2021	9 o'cloc k	27.7	30.7	30.9	27.9	25.1	29.7	31.1	28.9	0	0	3	1.8
5		11:00	32.3			32.6	32.2			32.2	0			3.2
6		13:00	32.1			32.2	31.9			32.1	0			4
7	05/07/2021	10:00	27.3	27.8	28.7	28.3	27.8	28.2	28.9	29.1	0	0	2	0.9
8		12:00	27.9			29.3	28 .6			29 .3	0			23
9		2:00 p.m.	28.2			28.6	28.1			28.4	0			2.8

Table 2 Abstract of measurement records)
Source: Own elaboration

Results

The first beneficial result for the present investigation is exposed with the taking of measurements directly from the meliponarios located in the Sierra Norte of the State of Puebla, these measurements allowed to observe the climatic changes and the affectations to the meliponarios. According to the results obtained from the applied measurements, two statistical analyzes are carried out, the first statistical analysis contemplates the decrease or increase of honeyed products (honey, propolis, wax) on a monthly basis due to the affectation of the temperature and the second implies the affectation by the wind (Table 3. Comparison of behavior of environmental factors with production levels).

Average monthly temperature	Wind speed	Productivity levels (Average in grams) per hive		
		Honey	Propolis	Wax
26°C	4m/s	600	13	5.4
33°C	9 m/s	550	12.2	8.8
30°C	3m/s	700	15.6	8.2
21°C	4m/s	450	13.9	3.4
28°C	2m/s	600	14	7.8
21°C	1m/s	350	16	5.2
29°C	4m/s	670	25	15.2
21°C	8m/s	375	24	5.8
26°C	7 m/s	800	28	7.5
31°C	3m/s	870	fifteen	eleven
22°C	6m/s	500	19	5
30°C	6m/s	470	22	8.9
$\bar{X}$ =26.5°C	$\bar{X}$ =4.75 m/s			

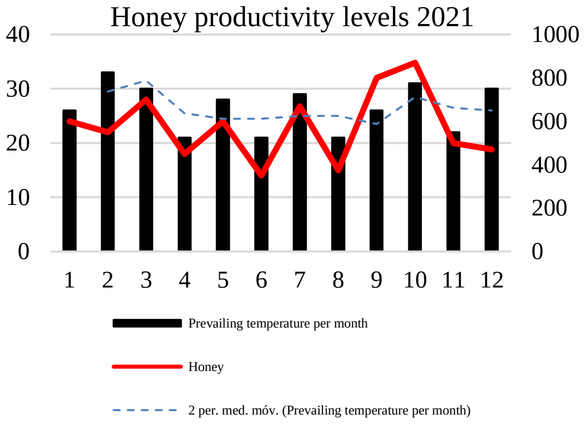
Table 3 Comparison of behavior of environmental factors with production levels
Source: Own elaboration

Temperature inference

The first comparison seeks to determine the influence of the temperature factor to obtain higher levels of productivity. This statistical analysis is carried out for each product.

A. *Statistical analysis of honey/temperature*

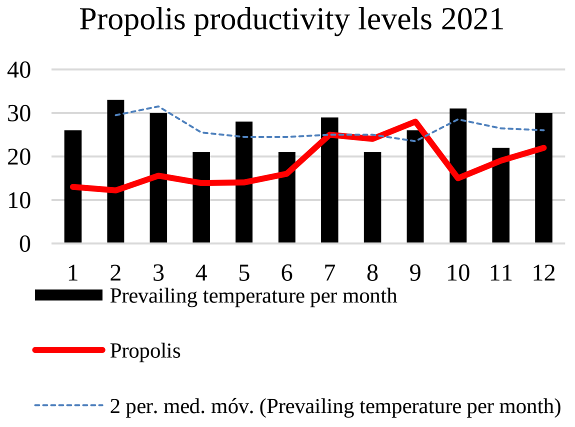
As we can see, the correlation between temperature and honey production follows the following relationship, the higher the temperature, the greater the amount of honey production (Graph 1. Honey productivity levels 2021).



Graphic 1 Honey productivity levels 2021
Source: Own elaboration

B. *Statistical analysis of propolis/temperature*

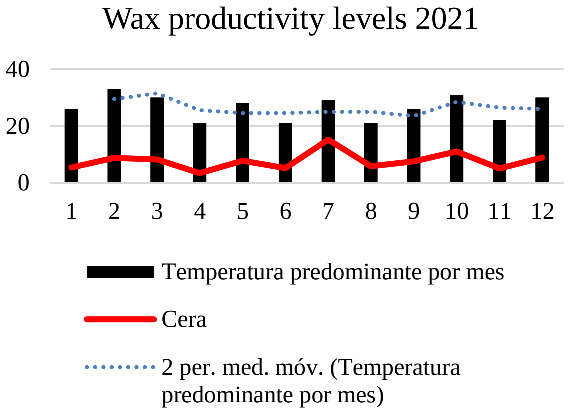
According to the exposed analysis, propolis production does not show an affectation or benefit according to temperature (Graphic 2. Propolis productivity levels 2021).



Graphic 2 Propolis productivity levels 2021
Source: Own elaboration

Wax/temperature statistical analysis

For this specific product, it is notorious that there is an interrelationship with respect to the amounts produced per hive, as we can see the higher the temperature, the greater the amount of wax produced (Graphic 3. Wax productivity levels 2021).

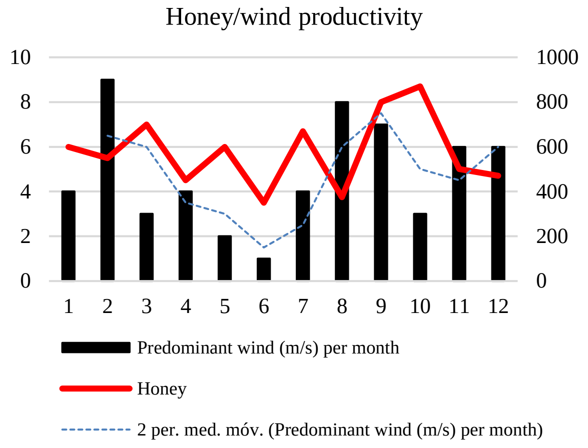


Graphic 3 Wax productivity levels 2021
Source: Own elaboration

Wind inference

A. *Honey/wind statistical analysis*

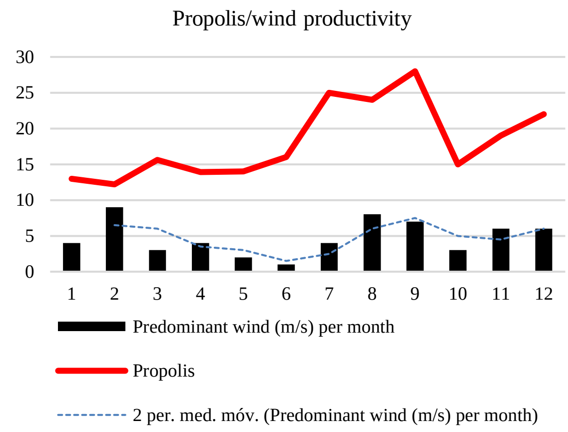
For this statistical analysis, it can be deduced that there is a positive correlation between both measured variables, concluding that the less wind, the higher the honey production index (Graphic 4. Honey/wind productivity levels 2021).



Graphic 4 Honey/wind productivity levels 2021
Source: Own elaboration

B. *Statistical analysis of propolis/wind*

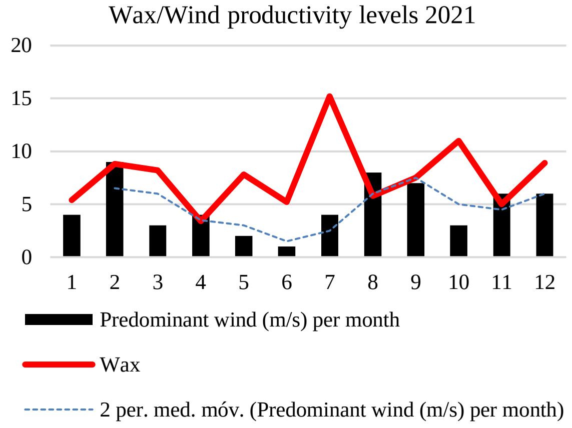
According to the information analyzed, it is concluded in this variable that the lower the amount of wind, the higher the propolis productivity levels will be (Graph 5. Propolis/wind productivity levels 2021).



Graphic 5 Propolis/wind productivity levels 2021
Source: Own elaboration

C. Statistical wax/wind analysis

In this last product, it is notorious that the increase or decrease in wind gusts does not affect or benefit (Graph 6. Wax/wind productivity levels 2021). Now, concluding with the statistical analysis, it is considered that the wind and the existing relationship with honey, propolis and wax derive from a particular source of origin, which is: If the wind gusts are high, they tend to destroy the hives or disorient the insect, causing loss of hives and a decrease in the population of meliponas.



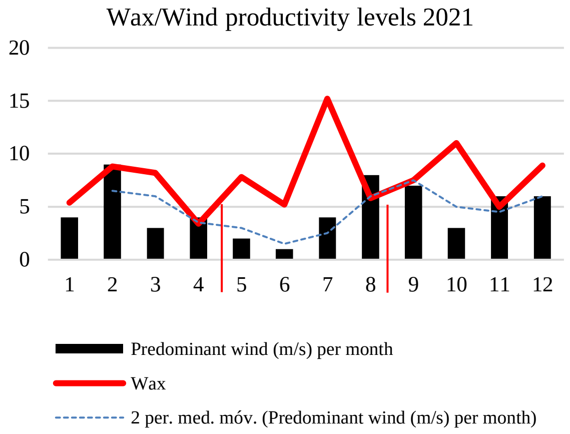
Graphic 6 Wax/wind productivity levels 2021
Source: Own elaboration

In accordance with the above, it is notorious that there is a correlation between the environmental factors of temperature and wind with the reproduction and conservation of the melipona bee, quantitatively a level of affectation is observed in two of the main products in the case of inferred temperature. in honey and wax, while the environmental factor of wind is correlated with honey and propolis, for which these environmental factors greatly influence the meliponaria.

This brings with it that the optimum values of temperature and wind are determined. that must be maintained, for the calculation of these indices the sampled values (Phase 3) are taken as a reference, and they are subjected to two normal distribution tests, contemplating for the temperature an average ($\bar{X}$)= 26.5° C and for the wind a mean ($\bar{X}$)= 4.75 m/s with a sigma σ level of +-3 σ and an interval of 0.5.

Test 1: Normal distribution (Temperature)

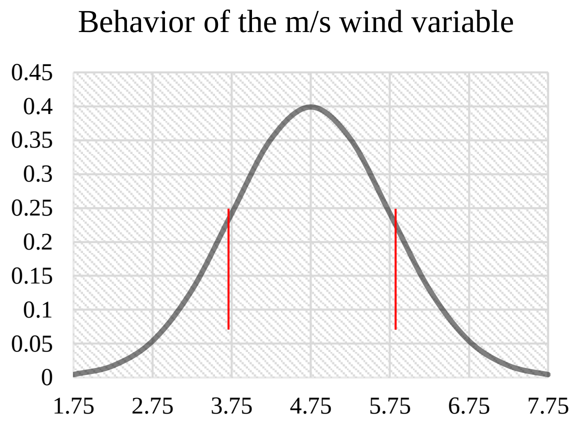
As we can see, the minimum acceptable temperature is 23.5° C, while the maximum is 29.5° C, bringing with it that the ideal parameters to meet the objective of the research carried out are established from 25.5° C-27.5° C (Graphic 7. Behavior of the temperature variable °C).



Graphic 7 Behavior of the temperature variable °C
Source: Own elaboration

Test 2: Normal distribution (Wind)

As we can see, the minimum displacement of the gusts of wind is 1.75 m/s, while the maximum displacement is 7.75° m/s, bringing with it that the ideal parameters to meet the objective of the research carried out are established at 3.75 ° C-5.75° C (Graph 8. Behavior of the wind variable m/s).



Graphic 8 Behavior of the m/s wind variable
Source: Own elaboration

Gratitude

To the Instituto Tecnológico Superior de Huauchinango and the Industrial Engineering Division for the facilities provided for the preparation of this chapter.

Financing

Not applicable, carried out with own resources.

Conclusions

During the time in which the investigation was carried out, we worked on the quantitative and qualitative analysis of the abiotic factor (wind) and the physical factor (temperature), through the use of measuring instruments (Thermo-Hygrometer and Thermo -Anemometer) whose unit of measurement is m/s and in degrees Celsius (°C), undoubtedly these factors directly affect the levels of productivity and the preservation of the melipona bees established in the meliponaria of the State of Puebla. It should be noted that currently environmental conditions are decisive for the increase or decrease of the main products (honey, propolis and wax) that are produced within the hives or colonies.

However, according to the experience of the meliponiculturists of the region, the null control of these factors brings about the destruction or weakening of the hives and the decrease in the populations of insects that are born and reproduce in the different colonies, in response to this problem, a statistical study was generated to determine the exact parameters to which the meliponaria should be maintained, the study was classified according to the normal distribution, with $\bar{X}$ according to the samplings carried out in a period of 12 months and a sigma level of +- 3 σ, it is important to mention that when establishing the optimal indices, it was recommended to the meliponiculturists that due to the climate that the area presents in periods where humidity or wind is higher, they should condition or air-condition the meliponaries to maintain or reach the levels indicated., to finish the investigation carried out in Huauchinango Puebla turns out to be of great benefit for the population because contributes to the conservation and preservation of the melipona bee, which is the native insect of the region.

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Article **ECORFAN Journal Republic of Guatemala**
June 2022, Vol.8 No.14 23-29

Differential genes of *Arabidopsis thaliana* in response to *Ustilago maydis* infection

Genes diferenciales de *Arabidopsis thaliana* en respuesta a la infección por *Ustilago maydis*

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DOI: 10.35429/EJRG.2022.14.8.23.29 Received January 30, 2022; Accepted June 30, 2022

Abstract

Arabidopsis thaliana - Ustilago maydis integrate the pathosystem used to study the plant-pathogen interaction, in order to know the molecular mechanisms involved in the response of the plant to the pathogenesis of *U. maydis*, a differential expression bank was constructed 72 hours after inoculation, using the subtractive hybridization technique. The fragments obtained were sequenced and subjected to bioinformatic analysis which allowed us to locate 36 different sequences with homology to *Arabidopsis thaliana* in response to *U. maydis* infection, several of them with roles in photosynthesis, reactive oxygen species, defense, and signaling among others, involved either directly or indirectly in the early response to infection. The results of this work are focused on understanding the plant-pathogen interaction and can be extrapolated to other model plants of agronomic importance.

Pathosystem, Defense, *Arabidopsis*

Resumen

Arabidopsis thaliana - Ustilago maydis integran el patosistema utilizado para el estudio de la interacción planta-patógeno, con la finalidad de conocer los mecanismos moleculares implicados en la respuesta de la planta a la patogénesis de *U. maydis* se construyó un banco de expresión diferencial a las 72 horas posteriores a la inoculación, mediante la técnica de hibridación sustractiva. Los fragmentos obtenidos fueron secuenciados y sometidos a un análisis bioinformático el cual permitió localizar 36 secuencias distintas con homología a *Arabidopsis thaliana* en respuesta a la infección por *U. maydis*, varios de ellos con roles de fotosíntesis, especies reactivas de oxígeno, defensa, y señalización entre otros, involucrados ya sea de manera directa o indirecta en la respuesta temprana a la infección. Los resultados de este trabajo están centrados en entender la interacción planta patógeno y pueden ser extrapolados a otras plantas modelo de importancia agronómica.

Patosistema, Defensa, *Arabidopsis*

Citation: PLANCARTE-DE LA TORRE, Marco M., CASARRUBIAS-CASTILLO, Kena, ROBLES-MURGUIA Celia and MÉNDEZ-MORÁN, Lucila. Differential genes of *Arabidopsis thaliana* in response to *Ustilago maydis* infection. ECORFAN Journal-Republic of Guatemala. 2022. 8-14:23-29.

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Introduction

In recent years *Ustilago maydis* has been a fungal model widely used in biological and genetic studies (Bolker, 2001), it requires a host to complete its sexual cycle, and is pathogenic in maize (*Zea mays* L.), its natural host causing the disease known as common bunt or "huitlacoche" (Bolker, 2001; Kahmann and Kämper, 2004).

In studies conducted by León-Ramírez et al. in 2004, the possibility of using alternative hosts to study *U. maydis* infection was raised. The *Ustilago maydis-Arabidopsis thaliana* non-natural pathosystem proved to be a useful model for the study of plant-pathogen interaction and in the analysis of virulence factors of Ustilaginales (Méndez-Morán et al., 2005), where results were achieved with a haploid strain that facilitates the analysis of the fungus in this system.

Currently, most of the work on the arabidopsis-ustilago pathosystem has focused on characterising the response genes of the fungus (Aleman-Duarte, 2009; Martínez-Soto et al, 2013) and not on the plant side, with the aim of understanding the molecular mechanisms involved in the interaction between *U. maydis* and *A. thaliana*, in the present work, a differential cDNA expression bank was constructed to analyse *A. thaliana* genes involved in the early infection of *U. maydis*, considering a time of 72 h after inoculation (hpi) of the fungus in the plant. Gene fragments obtained from an arabidopsis subtractive library were subjected to bioinformatic analysis to determine the identity of the differentially obtained sequences, with the perspective that the results obtained in this work can be extrapolated to other plant-fungus models of agronomic importance.

Materials and methods

Plant growth, fungi and growing conditions

The wild variety of *Arabidopsis thaliana Landsberg erecta* (Ler.) was used as host for *Ustilago maydis*. Seeds of *A. thaliana* were disinfected with 70 % ethyl alcohol for 5 minutes, then transferred to sodium hypochlorite solution (20 %) for 10 minutes, and washed 3 times with sterile distilled water. Seeds were sown on solid Murashigne and Skoog (MS) culture medium (0.8% v/v) and incubated at controlled temperature conditions (25°C), with a photoperiod of 16 h light and 8 h dark.

The wild-type *U. maydis* strain (*a1b1*) was grown in vitro in liquid MC medium (Hollyday, 1974) at 28°C under constant agitation for 18-24 h to obtain a concentration of $1x10^8$ sporidia/µL, cells were recovered by centrifugation and resuspended in sterile distilled water.

Inoculation

Seedlings 4 days post germination (dpg) were inoculated by placing 0.5 µL ($5X10^4$ sporidia/µL) of the $1x10^8$ sporidia/µL suspension of *U. maydis* (grown in vitro) on the epidermis of the first two true leaves. Then they were placed in growth chambers at 25°C with a photoperiod of 16 h light and 8 h dark, after 72 hpi these infected plants were collected and considered within the experiment as the problem material, and as controls 7-day-old uninoculated *A. thaliana* seedlings and the haploid culture of *U. maydis* grown in vitro were used. The material was collected in liquid nitrogen and macerated in cold mortar.

Molecular assays

RNA isolation

RNA was extracted according to the method of Sambrook et al., 2012 and in addition the PureLink Micro-to-midi Total RNA extraction protocol PureLink Micro-to-midi Total RNA Purification System (Invitrogen, Carlsbad, CA, USA) was used. All samples were analysed by gel electrophoresis for integrity.

RT

From the total RNA, 1 µL of Oligo dT, 1 µL of the dNTPs mixture was added and made up to a final volume of 12 µL with sterile distilled water. It was left at 65°C for 5 min. Then 4 µL of 5x buffer, 2 µL of 0.1 M DTT and 1 µL of Superscript II reverse transcriptase enzyme (Invitrogen, Carlsbad, CA, USA) were added. Incubation was left for 50 min at 42°C and then 15 min at 70°C.

Polymerase chain reaction (PCR)

Approximately 2 ng cDNA or 2 ng plasmid DNA were used. The amount of primers was 10 µM each, the mixture of DNTP's was 10 mM and 3 mM $MgCl_2$ in a volume of 50 µL, 5 U of Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) and its 10x reaction buffer were added to the mixture, The amplification programme was as follows: initial denaturation at 94°C, for 3 min, followed by 30 cycles with denaturation at 94°C for 45 sec, alignment at temperature close to the Tm of the primers for 30 sec and polymerisation at 72°C for a time that complies with the following rule: for every 1000 bp, 1 min is required.

Subtractive hybridization

Differential banks were constructed using the "PCR-Select™ cDNA Subtraction Kit" technique (Clontech Mountain View, CA, USA) according to the supplier's specifications. The cDNA of the test sample (72 hpi seedlings), and the control cDNA (uninfected seedlings and *U. maydis* grown in vitro) were used. The test samples were separated into two populations. To one population the first adaptor (Ad1) was ligated and to the other population the second adaptor (Ad2R) was ligated. No adapters were added to the control cDNA. The Ad1 and Ad2R populations were hybridised separately to the control cDNA. The hybridisation products were then mixed and two PCR amplifications were performed to obtain only the amplification of those double-stranded cDNAs (hybrids) with different adaptors, which amplified exponentially. The products of this PCR reaction were inserted into a T/A cloning vector (pDrive, in *E. coli* cells), with ampicillin and kanamycin resistance.

Cloning and transformation of subtracted PCR products

The TOP10 F strain (Invitrogen, Carlsbad, CA, USA) was used with the conditions described by Sambrook et al. (1989), then for transformation 2 µL of the transforming DNA was added to 100 µL of TOP10 F competent cells, left on ice for 10 min, heat shocked for 2 min at 42 °C and left on ice for 5 min, then 900 µL of LB medium was added and incubated for 1 h at 37 °C under agitation.

Finally, the mixture obtained was spread on LB medium plates spiked with the appropriate antibiotic. The extraction of plasmid DNA from *E. coli* was performed according to the protocols described by Sambrook et al. (1989) using the alkaline lysis of Birboim and Doly (1979). When higher purity plasmid DNA was required for use in DNA sequencing, it was obtained using anion exchange columns (Qiagen, Hilden GER), according to the supplier's instructions.

Sequencing and bioinformatics analysis

Sequencing was carried out at the Centro de Investigaciones y Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Unidad IRAPUATO with an "ABI PRISM 377 DNA sequencer from Perkin Elmer" to obtain the nucleotide sequence and perform bioinformatic analysis of the sequences. The sequences obtained were analysed with the "Basic Local Alignment Search Tool" (BLAST, http://www.ncbi.nlm.nih.gov/BLAST/) algorithm developed by the National Center for Biotechnology Information (NCBI) of the US government (Altschul et. al., 997) using the BLAST'n tools (compare a nucleotide sequence against a database containing also nucleotide sequences). The database for *Arabidopsis thaliana*: https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=3701 was used to search the sequences.

Results and discussion

Selection of positive clones from subtractive hybridization

Of these, only 181 positive clones (white) were recovered and 105 negative clones (blue) were discarded), Once the 181 positive clones were isolated, the presence of the plasmid was verified, the clones that did not present visible bands were discarded, from the 181 positive clones obtained in the subtractive library, 144 clones were selected to be sequenced, 6 of these were discarded because they did not present a sequence that could be analysed, therefore 138 sequences were submitted to bioinformatic analysis.

Once the sequences were obtained, the adapters were searched for (TCGAGCGGCCGCCCGGGGGCAGGT [sense]; ACCTGCCCGGGGGCGGCCGCTCGA [complementary antisense] on strand 3' or AGCGTGGTCGCGGGGCCGAGGT [antisense] ACCTCGGCCGCGACCACGCT [complementary sense] on strand 5)' flanking the cloned fragments, so that the sequence belonging only to the fragment was subjected to bioinformatics analysis. The 138 clones were subjected to alignment analysis with the BLAST tool in the nucleotide mode (BLAST'n) considering the alignment significant when the error (E) was less than 1e-3. Figure 1 shows the distribution of the 106 sequences, with homology to *Arabidopsis thaliana* (76.8 %) representing 36 different genes, 22 sequences with homology to *Ustilago maydis* (15.9 %) coding for 19 different genes, and 10 more with no significant alignment (7.2 %), and 10 sequences with homology to Arabidopsis thaliana (76.8 %) representing 36 different genes.

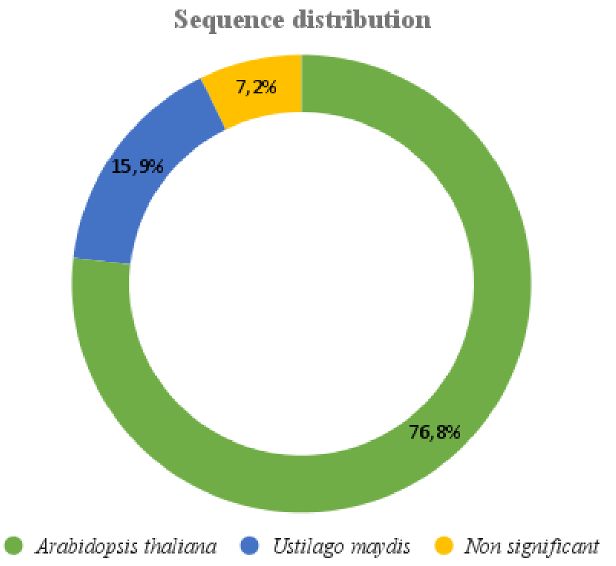

Figure 1 Sequence distribution. The number of sequences with homology to *Arabidopsis thaliana* is shown in green, sequences with identity to *Ustilago maydis* in blue and sequences with no significant alignment in yellow

Sequences with homology to Arabidopsis thaliana

Of the sequences with homology to *Arabidopsis*, 36 different sequences were obtained, which are presented in table 1 with the description at transcript level obtained from the NCBI databases, and in table 2 the annotation according to the Gene Ontology (GO) databases, which provides information on the functionality of the genes.

ID	# pb	Identity	ID Locus	Access No. NCBI	Annotation (Blast Result)
1	293	99 %	AT4G02520	NM_116486.3	Glutathione S-transferase PHI 2 (GSTF2)
2	520	100%	QBI37805.1	MK353213.1	Isolate 180404IB4 chloroplast, complete genome
3	495	99 %	AT5G60620	NM_125455.4	Glycerol-3-phosphate acyltransferase 9 (GPAT9)
4	210	100 %	36335705	NC_037304.1	Ecotype Col-0 mitochondrion, complete genome
5	490	99%	AT4G14030	NM_117478.6	Selenium-binding protein 1 (SBP1)
6	208	97%	AT3G21610	NM_113056.3	Acid phosphatase/vanadium-dependent haloperoxidase-related protein
7	196	98%	AT5G01530	NM_120231.4	Light harvesting complex photosystem II (LHCB4.1)
8	328	100%	AT2G39730	NM_179989.3	Rubisco activase (RCA)
10	689	99 %	AT1G76080	NM_106257.3	Chloroplastic drought-induced stress protein of 32 kD (CDSP32)
11	450	97 %	AT1G67090	NM_105379.4	Ribulose bisphosphate carboxylase small chain 1A (RBCS1A)
12	628	98 %	AT1G27450	NM_102509.4	Adenine phosphoribosyl transferase 1 (APT1)
13	370	99 %	AT1G79040	NM_106555.4	Photosystem II subunit R (PSBR)
14	792	99 %	AT2G25110	NM_128068.3	Stromal cell-derived factor 2-like protein precursor (SDF2)
15	81	100 %	AT4G28750	NM_119019.4	Photosystem I reaction centre subunit IV / PsaE protein (PSAE-1)
16	272	100 %	AT3G61470	NM_116012.5	Photosystem I light harvesting complex protein (LHCA2)
17	212	99 %	AT1G20620	NM_001332452.1	Catalase 3 (CAT3)
18	347	99 %	AT1G55670	NM_104443.2	Photosystem I subunit G (PSAG)
19	244	100 %	AT5G66570	NM_126055.4	PS II oxygen-evolving complex 1 (PSBO1)
20	180	82 %	9316370	XM_002880257.2	PREDICTED: Arabidopsis lyrata subsp. lyrata signal recognition particle
21	155	95 %	9299655	XM_002863533.2	PREDICTED: Arabidopsis lyrata subsp. lyrata berberine bridge enzyme-like 28
22	304	98 %	AT2G34420	NM_128994.3	Photosystem II light harvesting complex protein B1B2 (LHB1B2)
23	387	98 %	AT2G45470	NM_130109.3	FASCICLIN-like arabinogalactan protein 8 (FLA8)
24	154	100 %	AT2G45470	NM_001342565.1	Histone superfamily protein (AT4G40040)
25	135	99 %	AT5G40450	NM_001344333.1	A-kinase anchor-like protein (AT5G40450)
26	217	99 %	AT4G22690	NM_118395.3	Cytochrome P450, family 706, subfamily A, polypeptide 1 (CYP706A1)
27	145	99 %	AT1G28290	NM_102594.3	Arabinogalactan protein 31 (AGP31)
28	129	96 %	AT2G41840	NM_129748.4	Ribosomal protein S5 family protein (AT2G41840)
29	190	99 %	AT2G34430	NM_128995.3	Light-harvesting chlorophyll-protein complex II subunit B1 (LHB1B1)
30	293	93 %	AT1G78630	NM_106510.3	Ribosomal protein L13 family protein (emb1473)
31	185	98 %	AT4G34150	NM_119578.4	Calcium-dependent lipid-binding (CaLB domain) family protein (AT4G34150)
32	232	97 %	AT2G41110	NM_180013.3	Calmodulin 2 (CAM2)
33	244	100 %	AT3G50820	NM_114942.3	Photosystem II subunit O-2 (PSBO2)
34	102	100 %	At1g61670	AK117715.1	Unknown protein, complete cds
35	195	99 %	AT1G07720	NM_001331724.1	3-ketoacyl-CoA synthase 3 (KCS3)
36	400	99 %	Q5HZ38	AM489730.1	SnRK1-activating protein kinase-1 (SnAK1 gene)

Table 1 *Arabidopsis thaliana* genes obtained from subtractive hybridization

Locus ID TAIR	Cell function annotated in Gene Ontology
AT4G02520	Cadmium ion response, cold response, oomycete response, zinc ion response, toxin catabolic process
AT5G60620	Diacylglycerol biosynthetic process, triglyceride biosynthetic process
AT4G14030	Cellular response to selenium ion, response to cadmium ion, response to hydrogen peroxide, sulphate assimilation, protein binding, selenium binding
AT3G21610	Biological processes
AT5G01530	Photosynthesis, light harvesting in photosystem I, response to light stimulus
AT2G39730	Leaf senescence, response to cold, response to jasmonic acid, response to light stimulus, ADP binding, ATP binding, ATP hydrolysis activity, enzyme regulatory activity, mrna binding, ribulose-1,5-bisphosphate carboxylase/oxygenase activating activity
AT1G76080	Bacterial defence response, heat acclimation, cellular redox homeostasis, response to oxidative stress, response to water deprivation, protein binding
AT1G67090	Photosynthesis, cold response, ribulose bisphosphate carboxylase complex assembly, copper ion binding, mrna binding, protein binding, salicylic acid binding
AT1G27450	Adenine rescue, circadian rhythm, cytokinin metabolic process, AMP rescue, adenine rescue, cytokinin metabolic process
AT1G79040	Assembly of photosystem II oxygen-evolving complex, mrna binding, protein binding
AT2G25110	Bacterial defence response, fungal defence response, pattern recognition receptor signalling pathway
AT4G28750	Process of biosynthesis of aromatic compounds, process of biosynthesis of cellular nitrogen compounds, process of biosynthesis of heterocycles, process of biosynthesis of cyclic organic compounds, response to light stimulus, response to temperature stimulus
AT3G61470	Photosynthesis, light harvesting in photosystem I, response to cold, response to high light intensity, response to light stimulus, response to low light intensity stimulus
AT1G20620	Cellular response to nitrogen starvation, cellular response to phosphate starvation, cellular response to sulphate starvation, catabolic process of hydrogen peroxide, peptidyl-cysteine S-trans-nitrosylation, response to cold, response to light stimulus, catabolic process of hydrogen peroxide, response to hydrogen peroxide, response to oxidative stress
AT1G55670	Photosynthesis, photosynthetic NADP+ reduction, photosynthetic electron transport in photosystem I, stabilisation of photosystem I, stabilisation of proteins
AT5G66570	Photoinhibition, photosynthesis, reaction to light, assembly of photosystem II, stabilisation of photosystem II, regulation of protein dephosphorylation
AT2G34420	Photosynthesis, light harvesting in photosystem II
AT2G45470.1	Meiotic cell cycle process, oxoacid metabolic process, lipid response, tissue development
AT5G40450	Organisation of lytic vacuoles molecular_function
AT4G22690	Defence response to bacteria, defence response to fungi, hormone-mediated signalling pathway, oxo acid metabolic process, regulation of defence response, response to alcohol, response to lipids, response to oxidative stress, response to water deprivation, secondary metabolic process
AT1G28290	Jasmonic acid response, rhamnogalacturonan biosynthetic process II
AT2G41840	Translation
AT2G34430	Photosynthesis, photosynthesis, light uptake in photosystem II
AT1G78630	Negative regulation of translation, translation
AT4G34150	Cellular response to hypoxia, response to cold
AT2G41110	Pollen germination, protein catabolic processing, calcium ion binding, enzyme regulatory activity, protein binding
AT3G50820	Photoinhibition, photosynthesis, reaction to light, assembly of photosystem II, stabilisation of photosystem II, regulation of protein dephosphorylation
AT1G07720	Response to cold, response to light stimulus, fatty acid biosynthetic process

Table 2 Functionality of *Arabidopsis thaliana* genes

Subtractive hybridisation was performed at 72 hpi, which is considered an early response to infection by *U. maydis* infection, we can observe that there is an induction of photosynthesis genes (photosystems I and II); some genes related to response to pathogens such as bacteria and fungi; other genes related to oxidative stress; some genes involved in signal transduction; Calvin cycle genes and several other genes related to both anabolic and catabolic metabolism, transport, biological processes, protein binding, histones, and protein of unknown function.

The arabidopsis pathosystem has been described by Mendez-Morán and collaborators in 2005, additionally, in a previous work this same pathosystem was analysed under a microarray scheme finding a set of differential genes (unpublished data), from these results it was possible to characterise the *PLA2A* gene in response to ustilago infection (Casarrubias-Castillo et al., 2021).

In plants, defensive responses are costly and therefore require internal adjustment, which may be related to reductions in growth, reproduction or storage, as well as increases in carbon assimilation to cover metabolic demands (Schwachtje and Baldwin, 2008), which can be reflected in the induction of genes related to the photosynthetic process in both photosystem I and photosystem II, and clearly the chloroplast is highly active and the induction of these genes is represented in the results (table 1). The chloroplast also provides biosynthetic functions to generate defence hormones such as salicylic acid (SA) and jasmonic acid (JA), and the reactive oxygen species (ROS) response, and this organelle is an important plant defence mediator in maize plants in response to ustilage infection (Kretschmer et al., 2017 and literature cited therein).

Increases in photosynthesis can lead to changes in the source-consumer relationship as a result of the increased demand for energy and carbon sources required for the production of defensive compounds (Schwachtje and Baldwing, 2008).

As a result, the induction of genes related to signal transduction such as calmodulin (CAM2), protein kinases (SnAK1 and A-kinase anchor-like protein), a regulator of defence responses (CYP706A1) and a PRR receptor (SDF2), indicating that there is an initial response to the attack, and that there are genes induced both in response to JA and SA according to the functionality of several genes (table 2) and that both, in many cases, are antagonistic to each other, while ethylene signalling (ET) can be synergistic with JA signalling, both associated with resistance to necrotrophic pathogens (Pieterse et al., 2012). *U. maydis* possesses a Jsi1 effector, which triggers the response of genes related to ethylene response, probably attenuating the SA pathway that is effective against biotrophic pathogens (Darino et al., 2021).

Some JA response genes were also obtained such as the gene encoding for Arabinogalactone protein 31 (AGP31) and FASCICLIN-like arabinogalactan protein 8 (FLA8), both with a possible role in the response to pathogenic infection (Wu et al., 2020), and genes involved in the detoxification of reactive oxygen species such as Glutathione-S-transferase (GSTF2) and catalase 3 (CAT3) were also observed.

Conclusions

The subtractive hybridisation technique besides helping us to identify low abundance genes, allowed us to identify genes involved in several processes such as: photosynthesis, reactive oxygen species, defence, and signalling among others, and may be involved either directly or indirectly in the early response to infection, coupled with these results and other data confirmed by microarrays (unpublished data) what happens in this *Arabidopsis thaliana-Ustilago maydis* pathosystem occurs the same as in maize plants, the pathogen in its haploid stage keeps the plant alive and the fungus lives at the expense of its energy, reducing the size, maintaining the tumours and increasing the anthocyanin content (Méndez-Morán et al. , 2005). The results obtained in this work can be extrapolated to other plant-fungus models of agronomic importance and to understand the plant-pathogen interaction.

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Article ECORFAN Journal Republic of Guatemala
June 2022, Vol.8 No.14 30-42

Prevalence of drug-resistant uropathogenic bacteria in canines in the city of Merida, Yucatan

Prevalencia de bacterias uropatógenas drogorresistentes en caninos de la ciudad de Mérida, Yucatán

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DOI: 10.35429/EJRG.2022.14.8.30.42 Received January 30, 2022; Accepted June 30, 2022

Abstract

Antibiotic treatment is key to the improvement of canine patients with urinary tract infections; however, the irrational use of antimicrobials has led to the emergence of resistance mechanisms in uropathogenic bacteria. The objective of the study was to identify the bacteria present in urine cultures from canines in the state of Yucatán, determine their sensitivity to antibiotics, the prevalence of resistance to methicillin (MR) and production of extended-spectrum beta-lactamase (ESBL). Identification and sensitivity to antibiotics were performed using the MicroScan commercial kit and the Kirby-Bauer technique. MR was determined by sensitivity to oxacillin/cefoxitin and ESBL production through synergy techniques with beta-lactamase inhibitors. The most frequently isolated bacteria was *Proteus mirabilis* and the antibiotic with the highest percentage of resistant strains was norfloxacin; 30.2% of the Enterobacterales strains manifested ESBL production and 39.4% of the *Staphylococcus* spp. showed MR. The development of drug resistance is an important problem, only the knowledge about the prevalence of these uropathogenic bacteria and their drug-resistant strains in our state will allow us to propose effective treatment protocols.

ESBL, UTI, Canine

Resumen

El tratamiento antibiótico es clave para la mejoría de los pacientes caninos con infecciones del tracto urinario, sin embargo, el uso irracional de los antimicrobianos ha provocado el surgimiento de mecanismos de resistencia en las bacterias uropatógenas. El objetivo del estudio consistió en identificar las bacterias presentes en urocultivos provenientes de caninos del estado de Yucatán, determinar su sensibilidad a antibióticos, la prevalencia de resistencia a meticilina (MR) y producción de betalactamasa de espectro extendido (BLEE). Se realizó la identificación y sensibilidad a antibióticos mediante el kit comercial MicroScan y la técnica de Kirby-Bauer. Se determinó la MR mediante la sensibilidad a oxacilina/cefoxitina y la producción de BLEE a través de técnicas de sinergia con inhibidores de betalactamasas. La bacteria aislada con mayor frecuencia fue *Proteus mirabilis* y el antibiótico con mayor porcentaje de cepas resistentes fue norfloxacina; 30.2% de las cepas de Enterobacterales manifestaron producción de BLEE y 39.4% de las cepas de *Staphylococcus* spp. mostraron MR. El desarrollo de farmacorresistencia es un problema importante, sólo el conocimiento sobre la prevalencia de estas bacterias uropatógenas y sus cepas farmacorresistentes en nuestro estado nos permitirá proponer protocolos de tratamiento eficaces

BLEE, ITU, Canino

Citation: BASTO-MIJANGOS, Harold Noe, DUARTE-MENDOZA, Grisell Anahí, PÉREZ-BRETÓN, Susana A. and KIM-MOO, Manuel J. Prevalence of drug-resistant uropathogenic bacteria in canines in the city of Merida, Yucatan. ECORFAN Journal-Republic of Guatemala. 2022. 8-14:30-42.

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Introduction

In the small animal clinic, one of the most frequent causes of consultation is urinary tract infection (UTI), caused by the adhesion, multiplication and persistence of an infectious agent in the urogenital tract (Wong *et al.*, 2015; Yu *et al.*, 2020).

In México, lower urinary tract infections occur regularly in clinical practice; however, scientific reports are limited. Mendoza, *et al.* (2017), conducted a retrospective study in which bacterial lower urinary tract infection was identified as the most frequent cause of UTI (34.02%). The main bacterial agent causing acute UTI in canines is *Escherichia coli*, and in recurrent or persistent infections, *Enterococcus spp.* and *Pseudomonas aeruginosa.* (Thompson *et al.*, 2011). In another research conducted by Brložnik *et al.*, (2016) reported that 39% of isolates in canines with UTI correspond to *Escherichia coli*; 27.3% of cases to *Staphylococcus spp*., 13.5% to *Proteus spp.* and only 8.5% to *Enterococcus spp*. Many of the frequently isolated bacteria are considered potentially zoonotic; an example is *Escherichia coli*, which can produce infections in the human urogenital tract.

On the other hand, the high frequency of resistance to antibiotics used empirically for their treatment has made UTIs relevant to public health (Johnson *et al.*, 2003). Initial empiric antimicrobial therapy is indicated to alleviate patient discomfort in most cases while awaiting the results of urine culture and antibiotic sensitivity testing. However, antimicrobial treatments have an impact on the resistance patterns of the dog's resident microbiota, as well as opportunistic pathogens (especially after the use of broad-spectrum antibiotics) (Galarce *et al.*, 2019; Garza *et al.*, 2018; J. Weese *et al.*, 2011).

The irrational use of antimicrobials in infections where they are not required (viral diseases), inadequate therapeutics and lack of commitment on the part of the owners, contribute to the generation of selective pressure on bacteria and with it the selection of resistance genes that allow the survival of microorganisms. This is the case of the emergence of methicillin resistance (MR) in *Staphylococcus*, a mechanism that confers resistance to all β-lactam antibiotics. Another resistance mechanism of special importance is the production of extended-spectrum beta-lactamases (ESBL) in Enterobacteriaceae, which confers resistance to penicillins, cephalosporins and monobactams (Acosta & Vargas, 2018; Angles, 2018; Garza *et al.*, 2018; J. Gómez & Sánchez, 2018; Villegas *et al.*, 2016).

There are classifications that catalog antibiotic resistance in bacteria: multidrug resistance (MDR), resistance to 3 or more groups of antibiotics; extended drug resistance (XDR), resistance to all categories except 1 or 2 groups of antibiotics; and pandrug-resistance (PDR), when there is resistance to all antibiotics available for treatment (Angles, 2018; Jiménez *et al.*, 2019).

Multidrug-resistant pathogens in animals and humans produce diseases with greater lethal potential due to their virulence and frequent treatment failures (Galarce, *et al.*, 2019). In this context, taking measures to improve the use of antibiotics to reduce bacterial resistance becomes more important. The World Health Organization (WHO), during 2016, raised the need to contain and delay the emergence of bacterial resistance, for this purpose it promoted epidemiological surveillance as the first step of multiple strategies against this serious public health problem (Angles, 2018; J. Gómez & Sánchez, 2018; Madero & Justo, 2021; OMS, 2016). Despite the evidence of increased percentages of antibiotic resistance in bacteria from UTI in canines, there are no studies in the state of Yucatán that evaluate the presence of drug resistance, so the use of empirical therapies may not be in accordance with our local epidemiology.

The objective of this study was to identify the bacteria isolated from urine cultures from canine patients, by means of biochemical tests and to determine their sensitivity profile to frequently used antibiotics, to determine the prevalence of bacterial species, drug-resistant strains, and antibiotics with a higher percentage of resistance. Likewise, to identify the prevalence of MR strains or extended spectrum beta-lactamase (ESBL) producers to determine if there is a relationship between these and the presence of resistance to other important antibiotics.

This study is a milestone for future research focused on the identification of circulating resistance genes in bacteria isolated in canine urinary tract infections in the state. In addition, it will allow knowing the best treatment options based on the frequency of resistance in the local environment.

Methodology

Sampling

Dog urine samples for aerobic urine culture were received from different veterinary centers in Yucatán, between June 2020 - August 2022; they were also reviewed and catalogued according to the collection procedure: spontaneous urination, catheter collection and cystocentesis. The sample submitter was responsible for the choice of the appropriate collection method for each case, handling, transport, and preservation of the sample.

Microbiological culture

Inoculation of the sample was performed using a previously sterilized round calibrated bacteriological loop, on CHROMagar™ Orientation agar (Becton Dickinson, Mexico) for the identification and differentiation of pathogens in the urinary tract, and incubated for 24-48 hours in a bacteriological oven at 37°C. The amounts of CFU/mL isolated for each case were interpreted as proposed by Feijoó & Gómez, (2012).

Bacterial identification

After the incubation period, presumptive identification was performed by interpretation of CHROMagar™ Orientation chromogens (guide provided by the manufacturer), complementary tests (oxidase, catalase, bound coagulase, growth on Mac Conkey agar) and Gram staining; Based on the results of this staining, the commercial panel "MicroScan Neg/Urine Combo Panel Type 84" or "MicroScan Combo Panel Type 33" (Beckman Coulter, México) was used for biochemical identification and antibiogram. The analysis of the results was performed based on the biochemical reaction tables proposed by Procop *et al*. (2017), Cowman *et al*. (2016) and the ABIS online platform. (Online Advanced Bacterial Identification Software, 2022).

Based on the identified bacteria and the results of the antibiotics contained in the MicroScan panel (broth microdilution technique), an antibiogram was performed by the Kirby-Bauer method with antibiotic discs: ciprofloxacin 5 μg, enrofloxacin 5 μg, levofloxacin 5 μg, cefotaxime 30 μg, ceftazidime 30μg, cefoxitin 30 μg, penicillin 10 U, ceftazidime 30μg, amoxicillin/clavulanic acid 20/10 μg and fosfomycin 200 μg (Biorad, EU).

For phenotypic identification of ESBL production, the disc approach technique described by Calvo *et al*. (2011), combined double-disc synergy described in document M100 (CLSI, 2022) and microdilution in broth contained in the MicroScan panel were used. On the other hand, for the phenotypic detection of MR strains, the cefoxitin disk induction test and broth microdilution with oxacillin described in M100 (MicroScan panel) were used.

Statistical analysis

The numbers of isolates were compared using Kruskal-Wallis, ANOVA and Pearson's correlation statistics. The existence of association between ESBL/MR production and the presence of antibiotic resistance was performed through the χ^2 test and Fisher's exact F test; an $\alpha = 0.05$ was used for all tests. Statistical analyses were performed using Past 4.05 and IBM SPSS statistics 25 statistical software.

Results

Urine culture was performed on 260 urine samples from canines from the city of Mérida, Yucatán, collected in the period June 2020 - August 2022, of which 108 were from males, 141 were from females and in 11 cases the sex was not specified. According to the way the sample was collected; 180 were obtained by cystocentesis, 29 by catheter, 9 by spontaneous urination and in 42 cases it was not stated.

Once identification was performed, 143 positive urine cultures were obtained (55%, 143/260), of which 139 had only one isolation (97.2%) and 4 had two (2.8%). *Proteus mirabilis*, *Escherichia coli* and *Staphylococcus aureus* were isolated in greater proportion (Table 1).

The data were analyzed to determine whether there was any correlation between the total number of cases with respect to time (months), using the r test (Pearson) with a significance of α=0.05. However, no significant correlation was found between the variables (r = 0.0859, p=0.6699). The same statistic was used to determine whether there is a correlation between positive cases and time (months), and no significant correlation was found (r = -0.1367, p=0.4962).

Microorganism	Percentage
Proteus mirabilis	21.8 %
Escherichia coli	15.0 %
Staphylococcus aureus	14.3 %
Klebsiella pneumoniae	12.2 %
Enterococcus faecalis	10.9 %
Citrobacter freundii	6.1 %
Pseudomonas aeruginosa	5.4 %
Staphylococcus saprophyticus	4.1 %
Enterococcus spp.	2.0 %
Staphylococcus epidermidis	2.0 %
Acinetobacter baumannii/calcoaceticus Complex	1.4 %
Proteus vulgaris	1.4 %
Staphylococcus pseudintermedius	1.4 %
Edwardsiella tarda	0.7 %
Kluyvera ascorbata	0.7 %
Staphylococcus spp.	0.7 %

Table 1 Bacterial isolates in canine urine cultures

The mean, median and variance of monthly cases were calculated for each year: 2020, $\bar{X}$=10.29, $\tilde{X}$=11, S=11.9; 2021: $\bar{X}$=8.66, $\tilde{X}$=6.5, S=15.51; 2022, $\bar{X}$=10.5, $\tilde{X}$=10, S=17.14. The Kruskal-Wallis's test with a significance of α=0.05 was used to determine whether there are differences between the medians of monthly cases according to the year. No significant differences were found (p=0.4845).

On the other hand, to determine if there are any variations in the number of monthly positive cases according to the year (2020: $\bar{X}$= 6.43, $\tilde{X}$=7, S= 3.1; 2021: $\bar{X}$= 4.75, $\tilde{X}$=3.5, S= 10.35; 2022: $\bar{X}$= 5.13, $\tilde{X}$=4, S= 5.61), variances were compared by ANOVA test with a significance of α=0.05. It was found that there is no significant difference (p= 0.4591) between the groups (year variable).

The sensitivity profiles of each isolate to different antibiotics were evaluated (Table 2). The average percentage of resistance per antibiotic was 36.4%.

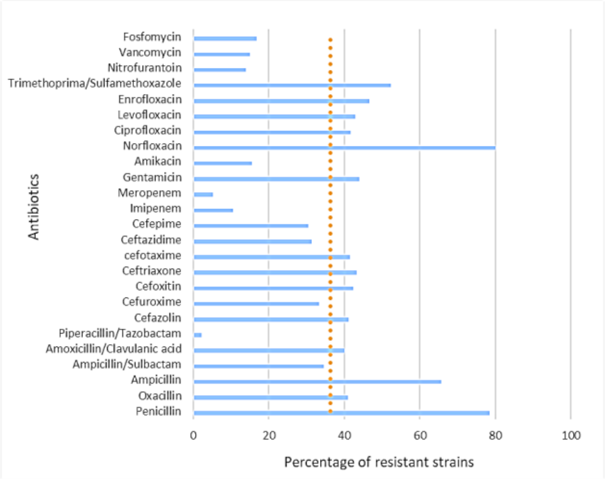
Antibiotic	Sensitive	Medium	Resistant
Penicillin	21.6% (11/51)	-	78.4% (40/51)
Oxacillin	59.1% (13/22)	-	40.9% (9/22)
Ampicillin	34.3% (23/67)	-	65.7% (44/67)
Ampicillin/Sulbactam	51.3% (45/78)	14.1% (11/78)	34.6% (27/78)
Amoxicillin/Clavulanic Acid	52% (39/75)	8% (6/75)	40% (30/75)
Piperacillin/Tazobactam	97.7% (86/88)	-	2.3% (2/88)
Cefazolin	58.8% (30/51)	-	41.2% (21/51)
Cefuroxime	66.7% (2/3)	-	33.3% (1/3)
Cefoxitin	57.6% (57/99)	-	42.4% (42/99)
Ceftriaxone	51.1% (45/86)	5.7% (5/88)	43.2% (38/88)
Cefotaxime	52.9% (46/87)	5.7% (5/87)	41.4% (36/87)
Ceftazidime	64.6% (62/96)	4.2% (4/96)	31.3% (30/96)
Cefepime	68.3% (56/82)	1.2% (1/82)	30.5% (25/82)
Imipenem	74.5% (70/94)	14.9% (14/94)	10.6% (10/94)
Meropenem	93.8% (90/96)	5.2% (5/96)	1% (1/96)
Gentamicin	54.4% (68/125)	1.6% (2/125)	44% (55/125)
Amikacin	78.9% (71/90)	5.6% (5/90)	15.6% (14/90)
Norfloxacin	20% (1/5)	-	80% (4/5)
Ciprofloxacin	49.3% (71/144)	9% (13/144)	41.7% (60/144)
Levofloxacin	51.8% (29/56)	5.4% (3/56)	42.9% (24/56)
Enrofloxacin	41.4% (48/116)	12.1% (14/116)	46.6% (54/116)
Trimethoprim/Sulfamethoxazole	47.7% (53/111)	-	52.3% (58/111)
Nitrofurantoin	69.8% (60/86)	16.3% (14/86)	14% (12/86)
Vancomycin	75% (15/20)	10% (2/20)	15% (3/20)
Fosfomycin	83.3% (5/6)	-	16.7% (1/6)

Table 2 Overall results of susceptibility profiles by antibiotic

To determine the antibiotics with the highest and lowest percentages of resistant strains, the data were grouped for analysis with respect to the overall mean, 36.4% (graph 1).

It was found that the 3 antibiotics with the highest percentage of resistant strains were: norfloxacin, penicillin and ampicillin; while the drugs with the lowest percentage were: piperacillin/tazobactam, meropenem and imipenem. It was also observed that of the group of third generation cephalosporins, only ceftazidime had a lower-than-average percentage of resistance. In addition, it was found that the 4 quinolones studied (norfloxacin, ciprofloxacin, levofloxacin and enrofloxacin) showed above-average resistance.

On the other hand, the results of the sensitivity tests for strains of each species identified were classified by level of resistance: multidrug resistance (MDR), extended drug resistance (XDR) and pan-drugresistance (PDR). The above was performed based on the classification proposed by the Pan American Health Organization (PAHO) and other works (Jiménez *et al*., 2019; Magiorakos *et al*., 2012; Ríos, 2016); however, no strains classified as XDR or PDR were observed. (Table 3).



Graphic 1 Comparison of percentages of resistant strains among antibiotics. The dotted orange line represents the overall mean (36.4%)

Microorganism	MDR	XDR	PDR	Non MDR
Proteus mirabilis	59.4% (19/32)	-	-	40.6% (13/32)
Escherichia coli	59.0% (13/22)	-	-	41.0% (9/22)
Klebsiella pneumoniae	66.7% (12/18)	-	-	33.3% (6/18)
Citrobacter freundii	66.7% (6/9)	-	-	33.3% (3/9)
Proteus vulgaris	50% (1/2)	-	-	50% (1/2)
Edwardsiella tarda	100% (1/1)	-	-	-
Kluyvera ascorbata	100% (1/1)	-	-	-
Pseudomonas aeruginosa	50% (4/8)	-	-	50% (4/8)
COMPLEJO *Acinetobacter baumannii/calcoaceticus*	50% (1/2)	-	-	50% (1/2)
Staphylococcus aureus	42.9% (9/21)	-	-	57.1% (12/21)
Staphylococcus saprophyticus	16.7% (1/6}	-	-	83.3% (5/6)
Staphylococcus epidermidis	33.3% (1/3)	-	-	66.7% (2/3)
Staphylococcus pseudintermedius	50% (1/2)	-	-	50% (1/2)
Staphylococcus spp.	-	-	-	100% (1/1)
Enterococcus faecalis	31.2% (5/16}	-	-	68.8% (11/16)
Enterococcus spp.	33.3% (1/3)	-	-	66.7% (2/3)

Table 3 Comparison of degree of resistance between species. MDR: resistant ≥ 1 antibiotic in ≥ 3 antimicrobial categories; XDR: resistant ≥ 1 antibiotic in all antimicrobial categories except to 1 or 2 groups; PDR: resistant to all antibiotics; non-MDR: not resistant or resistant ≥ 1 antibiotic in < 3 antimicrobial categories

On the other hand, phenotypic detection of ESBL production in isolates of Enterobacteriaceae with low- or high-level resistance (intermediate or resistant strains) to third generation cephalosporins was performed.

Of a total of 85 Enterobacteriaceae isolates, 26 were identified as ESBL producers (30.2%), of these, 8 were identified in *Klebsiella pneumoniae* strains (8/18, 44.4%) ¸ 7 in *Escherichia coli* (7/22, 31.8%), 6 *in Proteus mirabilis* (6/32, 18.7%), 4 in *Citrobacter freundii* (4/9,44.4%) and 1 in *Proteus vulgaris* (1/2, 50%).

The production of ESBL in Enterobacteriaceae leads to the development of resistance to cephalosporins, particularly to third and fourth generation cephalosporins. In this study, resistance to ceftriaxone and cefotaxime was observed in all 26 ESBL -producing strains.

We analyzed whether there is an association between the presence of ESBL and the level of resistance to ceftazidime and cefepime, using the $\chi 2$ and Fisher's exact tests. Likewise, because ESBL-producing strains have been related to the development of corresistances to quinolones and aminoglycosides, the existence of association between the presence of ESBL and the level of resistance to ciprofloxacin, enrofloxacin, gentamicin and amikacin was determined through χ^2 and Fisher's exact tests (Table 4).

Dependent variable	Test	P value	Other Coefficients
Ceftazidime resistance level	Exact F	$0.00\bar{0}$*	Cramer´s V = 0.590 λ= 0.419
Presence of resistance to Cefepime	χ^2=35.55	$0.00\bar{0}$*	Cramer´s V = 0.708 λ= 0.609
Resistance level to Ciprofloxacin	Exact F	$0.00\bar{0}$*	Cramer´s V = 0.564 λ= 0.455
Resistance level to Enrofloxacin	χ^2=19.82	$0.00\bar{0}$*	Cramer´s V = 0.480 λ= 0.273
Resistance level to Gentamicin	Exact F	0.027*	Cramer´s V = 0.268 λ= 0.034
Resistance level to Amikacin	Exact F	0.25	-

Table 4 Analysis of variables associated with the existence of ESBL (independent variable) (*) Statistically significant P-value

Phenotypic detection of MR in Staphylococcus isolates was also performed. Of a total of 33 *Staphylococcus* isolates, 13 were identified as MR (39.4%), of these, 7 were in *Staphylococcus aureus* (7/21, 33.3%) ¸ 2 in *Staphylococcus pseudintermedius* (2/2, 100%), 2 in *Staphylococcus saprophyticus* (2/6, 33.3%), 1 in *Staphylococcus epidermidis* (1/3, 33.3) and 1 in *Staphylococcus spp.* (1/1, 100).

MR strains have been related to the development of quinolone and aminoglycoside resistance, so we determined the existence of an association between the presence of MR and the level of resistance to ciprofloxacin, levofloxacin, enrofloxacin and gentamicin by χ^2 and Fisher's exact tests (Table 5).

Dependent variable	Test	P Value
Ciprofloxacin Resistance	χ^2=0.075	0.784
Levofloxacin Resistance	Exact F	0.411
Enrofloxacin resistance	Exact F	1
Gentamicin resistance	Exact F	0.119

Table 5 Analysis of variables associated with the existence of RM (independent variable) (*) Statistically significant P-value

Discussions

Urinary tract infections (UTI) are considered an important reason for consultation with the veterinarian. Studies have estimated that about 14% of dogs present some UTI event during their lifetime, so it is also considered as the second cause of antibiotic use in canines. On the other hand, reports indicate that UTIs usually involve the presence of uropathogenic bacteria frequently from gastrointestinal origin (Hernando *et al.*, 2021; Yamanaka *et al.*, 2019).

With the intention of observing whether there is any trend in the results of total cases or positive cases, an analysis of the results was performed using Pearson's r statistic; however, no correlation was found. Likewise, no differences were detected between the monthly mean of total or positive cases according to the year. This made it clear that there has been no significant increase or decrease in total or positive cases.

In this study, the most frequently isolated bacteria was *Proteus mirabilis* (21.8%); this microorganism is strongly related to the generation of UTI, the precipitation of phosphate-ammonium-magnesium crystals (struvite) in urine and the formation of uroliths. *Proteus mirabilis* utilizes urea and generates ammonium and carbon dioxide, this raises the urine pH and increases the availability of phosphates and ammonium to finally, by increasing their concentration together with magnesium, lead to the formation of crystals (Mendóza *et al.*, 2017; Rinkardt & Houston, 2004).

The prevalence of *Proteus mirabilis*, as the most frequently isolated bacteria in this study, contrasts with reports from other investigations. Marques *et al.*, (2018), reported that 45.1% of canine UTI cases in Portugal were caused by *Escherichia coli*. With similar results, a study conducted in Spain by Hernando *et al.*, (2021), found the presence of *Escherichia coli* in 45.3% of UTI cases in dogs. In our study, *Escherichia coli* was the second most frequent isolate, with 15% of the cases. The search for virulence factors in circulating clones of *Proteus mirabilis* isolated from UTI in canines from our state may provide information on the high prevalence of this microorganism.

On the other hand, the choice of the appropriate antibiotic is crucial for the improvement of patient's health. The International Society for Companion Animal Infectious Diseases (ISCAID) proposes the use of antibiotics empirically for the treatment of cystitis; specifically, it proposes the use of amoxicillin/clavulanic acid and trimethoprim/sulfamethoxazole (J. Weese *et al.*, 2011; J. S. Weese *et al.*, 2019). In our study, the percentages of resistance for the two antibiotics were 40% and 52.3%, respectively. These results contrast with those described by other authors. McGovern *et al.*, (2019); in a retrospective study conducted in Louisiana, United States, it was reported the presence of resistance to amoxicillin/clavulanic acid in 24.6% of isolates and to trimethoprim/sulfamethoxazole in 14.1%. For their part, Hariharan *et al.* (2016) found that 35.7% and 57.1% of isolates were resistant to amoxicillin/clavulanic acid and trimethoprim/sulfamethoxazole, respectively.

In the human medical field, it has been established that the use of empirical treatments for UTI should contemplate drugs whose resistance percentages do not exceed 20% in the local environment; however, this has not been properly determined in the veterinary field. (Batalla *et al.*, 2007; Gupta *et al.*, 2011).

Veterinary guidelines only suggest that the choice of antibiotic should be based on local epidemiology (J. Weese *et al.*, 2011; J. S. Weese *et al.*, 2019). In our case the observed percentages were higher than the global mean (36.4%), which discourages its use empirically.

The use of appropriate alternatives for their use as empirical treatments is under discussion. ISCAID suggests the use of nitrofurantoin or fosfomycin as an alternative in the treatment of multidrug-resistant infections; in contrast, these drugs are considered excellent options for empirical treatment of cystitis in the human setting due to their high concentration in urine, short treatment regimens, low resistance rates, etc. (Batalla *et al.*, 2007; J. S. Weese *et al.*, 2019).

In our study, we found a percentage of resistant strains of 14% and 16.7% for nitrofurantoin and fosfomycin, respectively. It is worth mentioning that the results reported for fosfomycin are limited only to *Escherichia coli* and *Enterococcus spp*. due to the lack of reference values for other species.

It is necessary to consider that some frequent bacteria, according to the local epidemiology observed, may be intrinsically resistant to certain antibiotics recommended for UTI, for example: *Proteus spp.* against nitrofurantoin (CLSI, 2022). Likewise, the use of broad-spectrum drugs with distribution in multiple tissues (fluoroquinolones, third generation cephalosporins, etc.) may generate the selection of multiresistant strains during or after treatment. (Yudhanto *et al.*, 2022).

In the present study, resistance percentages higher than the overall average (36.4%) were observed for all fluoroquinolones and 2 of the 3 third generation cephalosporins studied. This is a concerning problem, because it is an indication of selection for resistance genes, such as genes coding for ESBL (Bush, 2018).

The results found in studies on resistance to members of fluoroquinolones and third generation cephalosporins are contrasting with each other. Chan *et al.*, (2022), conducted a retrospective study with data obtained from canine urine cultures between 2018 and 2020 in China. The team found resistant strain percentages of 20% for ceftriaxone, ciprofloxacin, and enrofloxacin. In contrast, Amphaiphan *et al.*, (2021), noted percentages above 50% of *Staphylococcus spp*. strains, *Escherichia coli*, *Proteus spp. strains, Klebsiella spp.* resistant to enrofloxacin.

Gómez *et al*. (2020) reported that more than 30% of the strains of the Enterobacteriaceae family were resistant to enrofloxacin. In the case of ciprofloxacin in *Escherichia coli* it was 35% and in other Enterobacteriaceae it was 15.6%. For their part, García *et al*. (2019), described that of the total number of strains isolated from *Proteus spp*. and *Escherichia coli* (period 2012 - 2017) were resistant to ceftriaxone 27.3% and 38.7%; for ciprofloxacin, 27.6% and 35.9% and for enrofloxacin, 51.9% and 75%, respectively.

The percentages of resistant strains vary according to the region in which they are found, although percentages higher than 25% prevail. It is possible that the abuse of these broad-spectrum drugs in unnecessary treatments is an important factor in the development of resistance. (Bush, 2018; WHO, 2016; Valdés, 2017; Yudhanto *et al.*, 2022).

Resistance to fluoroquinolones has been mainly related to the presence of mutations in the *gyrA* and *gyrB* genes (coding for DNA gyrase subunits, as well as mutations in *parC* (coding for topoisomerase IV); These mutations have been described in Enterobacterales and *Pseudomonas aeruginosa* isolated in dogs and are even implicated in the existence of resistance to last generation quinolones such as enrofloxacin, marbofloxacin and pradofloxacin (Liu *et al.*, 2012; Vingopoulou *et al.*, 2018). Likewise, the above-mentioned mutations are related to cross-resistance between multiple quinolones (EUCAST, 2021). In the same context, Vingopoulou *et al.*, (2018) reported the presence of plasmid-mediated quinolone resistance genes in *Escherichia coli* and *Pseudomonas aeruginosa* strains in canine samples; the identified genes (*qnrA1*, *qnrB1*, *qnrS1* and *qnrS2*) encode for proteins that protect DNA gyrase from the action of fluoroquinolones by preventing their binding.

On the other hand, they determined the percentage of MDR, XDR and PDR strains for each of the bacterial species identified. He emphasized that in all cases of Gram-negative bacteria, a percentage of MDR higher than 50% was observed; this correlates with findings reported by Amphaiphan *et al*. (2021).

The group reported MDR percentages higher than 50% for *Escherichia coli* (69.7%), *Proteus spp.* (81.5%), *Klebsiella spp.* (92.9%), *Enterobacter spp.* (100%), *Pseudomonas aeruginosa* (92.3%) and *Acinetobacter spp.* (100%). As for Gram-positive bacteria (*Staphylococcus* and *Enterococcus*), the results were lower than those reported in the previous investigation. The high percentages of MDR in Gram-negative bacteria observed in the present study are concerning in view of the continuous development of resistance; these raise the need for the correct management of antibiotics in the veterinary clinical environment.

The phenotypic manifestation of ESBL in Enterobacterales and *Staphylococcus* MR strains was determined. In the present study it was found that 30.2% of the Enterobacterales strains presented ESBL production; the most frequent strains were *Klebsiella pneumoniae* and *Escherichia coli*. These results contrast with the data observed in the study conducted in China by Li *et al*., (2017), where the group found 3 ESBL-producing *Escherichia coli* strains (3/118, 2.54%); additionally, the team identified ESBL type CTX-M-15 and TEM-1, these enzymes have been found in strains isolated from humans and animals. On the other hand, a study conducted in Europe by Bogaerts *et al*., (2015), noted the identification of genes coding for ESBL type CTX-M-15 and CTX-M-14 from *Escherichia coli* and *Klebsiella pneumoniae* strains isolated from canine samples; these proteins have been described in strains from human and animal samples.

ESBLs are hydrolytic enzymes that degrade certain β-lactams such as penicillins, cephalosporins and monobactams, and are therefore related to resistance to these antibiotics. Treatment alternatives have been proposed (carbapenemics, fluoroquinolones, etc.) which should be confirmed by antibiogram. The use of antibiotics with inhibitors such as amoxicillin/clavulanic acid for the treatment of ESBL-producing bacteria lacks conclusive studies, so they should be used with caution and in particular situations (Esparza *et al.*, 2015; Mederos *et al*., 2018; Villegas *et al*., 2016; J. S. Weese *et al*., 2019).

It is evident that the observed percentages of ESBL-producing strains are a particularly important problem for public health; the genes involved are found in plasmids that allow their transfer between strains, in conjunction with other resistance genes. Likewise, the isolation of these bacteria from companion animals, such as dogs, poses a potential risk of zoonosis (Bogaerts *et al.*, 2015).

The association between ESBL production in Enterobacterales and the level of resistance to ceftazidime (third generation cephalosporin), the presence of resistance to cefepime (fourth generation cephalosporin) and the level of resistance to aminoglycosides and fluoroquinolones (co-resistance) was evaluated. An association was observed between the presence of ESBL (independent variable) and the level of resistance to ceftazidime (dependent variable), as well as the presence of resistance to cefepime (dependent variable). For ceftazidime, the intensity of association is moderate and the ability to reduce the error to predict the dependent variable is moderate. In the second case, the association is strong and the ability to reduce the error to predict the dependent variable is high. This result was expected, because cephalosporins are substrates of these enzymes; more than 300 classes have been described with greater or lesser affinity for some third generation cephalosporins. The emerging problem of ESBL-producing strains has led to the proposal of alternative antibiotics that allow the correct management of infections caused by these bacteria, avoiding the abuse of carbapenemics (Calvo *et al.*, 2011; Dueñas *et al.*, 2021; Mederos *et al.*, 2018; J. S. Weese *et al.*, 2019).

The presence of corresistance to other antibiotics is frequent in ESBL-producing strains, since the plasmids where the genes are transported also have coding sequences for other resistance mechanisms (Torres & Zarazaga, 2007). Bogaerts *et al*., (2015), reported the presence of corresistances to trimethoprim/sulfamethoxazole and gentamicin; in another study, Li *et al*., (2017), described isolates of ESBL-producing Escherichia coli strains with simultaneous resistance to enrofloxacin, marbofloxacin, trimethoprim/sulfamethoxazole and tetracycline.

The present study analyzed whether there was an association between the presence of ESBL in the strains studied and the presence of corresistance (level of resistance) to ciprofloxacin, enrofloxacin, gentamicin and amikacin. In general, associations with moderate intensity were observed for the two fluoroquinolones, as well as a moderate capacity to reduce the error to predict the dependent variable. In the case of aminoglycosides, we only found an association with the level of resistance to gentamicin with a moderate intensity and a low capacity to reduce the error. The above findings could be explained if we consider that the presence of corresistance genes to other families of antibiotics in plasmids is variable; therefore, it is proposed to perform molecular tests to know which genes cause the development of resistance in this group of bacteria, as well as the frequency in which they occur. This information will allow us to know the genes and plasmids of resistance circulating in the veterinary environment of our state.

On the other hand, the phenotypic manifestation of MR in *Staphylococcus* strains was determined; it was observed that about 40% of *Staphylococcus* isolates presented the MR phenotype. The data obtained contrast with reports from several studies: Marques *et al*. (2018) reported that 9.2% of the isolates of *Staphylococcus* species during the study period presented MR; they also pointed out that the main gene identified was the *mecA* gene. Chan *et al*., (2022) conducted another contrasting study; the team reported that 19% of *Staphylococcus spp*. isolates in the period 2018-2020 presented the MR phenotype (oxacillin resistance). On the other hand, McGovern *et al*., (2019); reported that approximately one third of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* cases presented MR. It is evident that the prevalence of MR *Staphylococcus* cases is a function of the geographical area in which the study was conducted, however, the data observed in this study are alarming, because they surpass the percentages reported in other investigations.

MR comes from the acquisition of *mecA* or mecC genes coding for a PBP2a (penicillin binding protein 2a) that has a lower affinity for beta-lactams; therefore, it preserves the integrity of the peptide-glycan layer (cell wall) by maintaining transpeptidase activity (Aguayo *et al.*, 2018).

According to several studies, *Staphylococcus* strains with MR should be considered resistant to all beta-lactams (CLSI, 2022). Moreover, *mecA* or *mecC* genes, regulatory genes (*mecI* and *mecR*) and other resistance genes are located within a mobile chromosomal element called staphylococcal chromosomal cassette (*SCCmec*), so sometimes MR strains can also develop co-resistance to other antibiotic families. (Aguayo *et al.*, 2018).

The search for therapeutic options for the treatment of Staphylococcus MR is under debate; in general, it is proposed that the management of cases should be individualized due to the presence of corresistances; thus, antibiotics such as vancomycin should be reserved exclusively for cases indicated by the specialist in charge due to their importance in human medicine. ISCAID proposes the use of various antibiotics in whose spectrum of action encompasses *Staphylococcus* MR (trimethoprim/sulfamethoxazole, nitrofurantoin, quinolones, etc.) (J. S. Weese *et al*., 2019). In recent years, the possible use of doxycycline has been explored as an "off-label" alternative in the use of urinary tract infections by multidrug-resistant strains such as *Staphylococcus* MR, although this has not been proven. (Jodlowski *et al.*, 2021; Rubi & Gaunt, 2011).

Finally, this study analyzed the association between the presence of MR in the *Staphylococcus strains* studied and the presence of corresistance (presence of resistance) to ciprofloxacin, levofloxacin, enrofloxacin and gentamicin. In none of the cases was an association found, which could be explained if we consider that the presence of resistance genes to other antibiotic families in the plasmids is variable. Therefore, it is proposed to perform molecular tests to know the type of *SCCmec* circulating in the MR strains isolated in the state of Yucatán; likewise, it is proposed to identify resistance genes to other families in *Staphylococcus* (Aguayo *et al.*, 2018).

Conclusions

The development of antibiotic resistance in the veterinary field is a major problem affecting our state; it compromises the health status of patients by reducing effective therapeutic options. The indiscriminate use of broad-spectrum antibiotics for the treatment of UTIs has led to the selection of bacteria with resistance genes such as ESBL-producing Enterobacterales and *Staphylococcus* MR. Only the knowledge about the prevalence of bacterial species causing UTI in our state and antibiotic resistance will allow us to propose better treatment protocols based on the reality of our environment.

Financing

This study was financed with internal resources.

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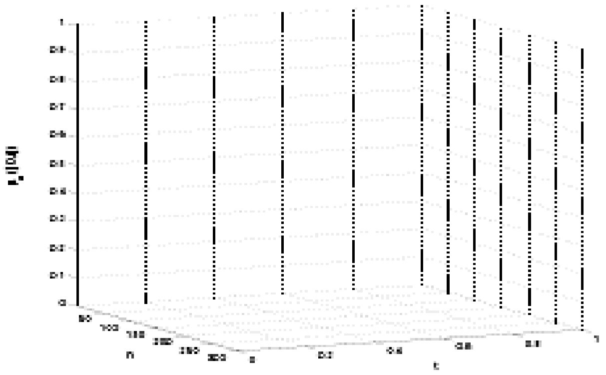

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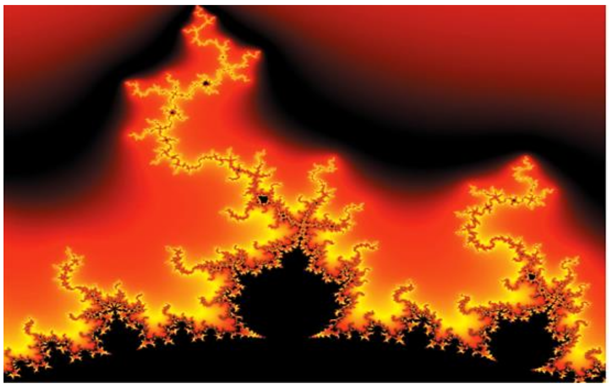

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ISSN 2414 - 8849
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