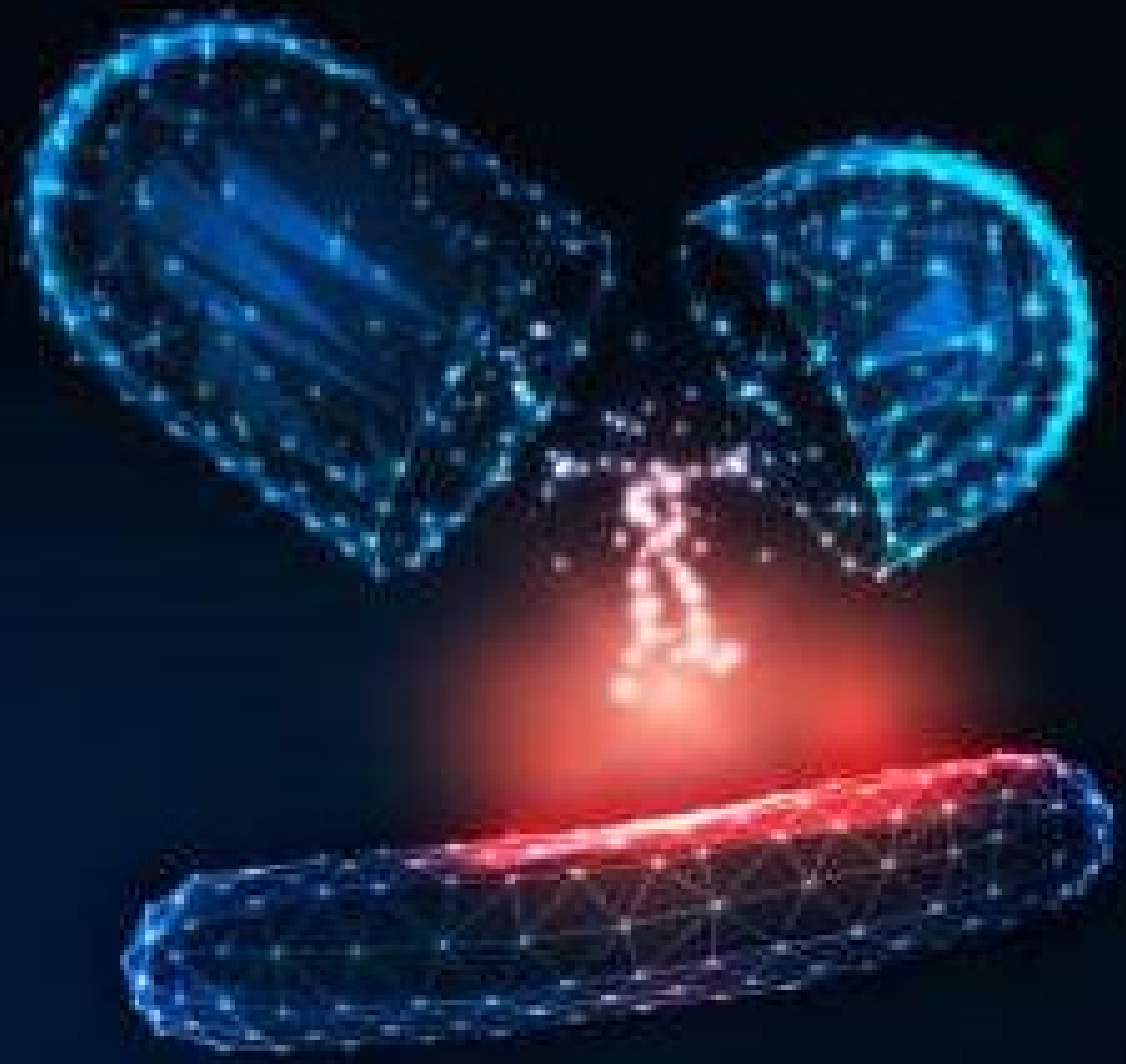
J. MICROBIOL. & HEALTH EDUC.
VOL. 3 (NUM. 1) ***2021***

ISSN: 0013-1091 (print)



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J. Microbiol. & Health Educ.
Vol. 3 (num. 1 – January - December) 2021
Printing date: December 15, 2021
Av. Zoquipan 1022, Zoquipan, C P. 45150, Zapopan, Jalisco, México.
Contacto con el editor: journalmhe@gmail.com

INDEX:

Editorial

The editorial team of *J. Microbiol. & Health Educ.* Vol. 3 (num. 1): 2021, wants to express its gratitude to the CONACYT Scientific and Technological Research Fund for the support granted.

We welcome new members of the Editorial Committee and invited reviewers.

We thank readers for their interest in our magazine. We have observed a significant increase in the number of referrals and potential evaluators. Our index and databases that index our articles have also been increased, such as Google scholar, Google Books, DOAJ-Directory of Open Access Journals and ROAD-Directory of Open Access scholarly Resources.

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JOURNAL OF MICROBIOLOGY & HEALTH EDUCATION, Vol. 3. No. 1, 2021, Pages 62-69.
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ORIGINAL ARTICLE

Determination of fungi and their aflatoxins in embryonated eggs a production batch

Determinación de hongos y sus aflatoxinas en huevos embrionados en un lote de producción

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Article history:
Received 26 Oct 2021
Received in revised from 16 Nov 2021
Accepted 18 Nov 2021
Available online 15 Dec 2021

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A B S T R A C T

The fungal load of origin and the presence of aflatoxins were determined in a batch of embryonated egg production, for this, aliquots of dissolution medium were seeded after a gentle rubbing of the eggshells of a batch, were incubated and then the total load of fungi of origin was quantified. Subsequently, the presence of any of the four main aflatoxins (B_1, B_2, G_1 and G_2) was determined using the ELISA method following the kit recommendations. All of the embryonated eggs had the presence of fungus, the fungal loads varied between 100 and 10 520 MFU/egg. 76 % (38/50) of the samples were positive for any of the four main aflatoxins and the estimated concentration of aflatoxins was 5.4 ± 2.9 µg/egg. It was possible to demonstrate the fail to the quality and safety of the food, both for human or animal consumption, or for animal production, since there is evidence of the high embryonic mortality generated by the mycotoxins that manage to enter the egg, in addition, it is evident the need to develop "organic" strategies for fungal control in the embryonic eggshell.

Keywords: Mycotoxins, Aflatoxin, Embryonated egg

R E S U M E N

Se determinó la carga fúngica de origen y la presencia de aflatoxinas en un lote de producción de huevo embrionado, para ello se sembraron alícuotas de medio de disolución tras un frotado suave de las cascaras de huevo de un lote, se incubaron y se realizó la cuantificación de la carga fúngica total de origen. Posteriormente se determinó la presencia de alguna de las cuatro aflatoxinas principales (B_1, B_2, G_1 y G_2) empleando el método de ELISA siguiendo las recomendaciones del kit. Todos los huevos embrionados tuvieron presencia de hongo, las cargas fúngicas variaron entre 100 y 10 520 UFM/huevo. El 76 % (38/50) de las muestras resultaron positivas a alguna de las cuatro aflatoxinas principales y la estimación de la concentración de aflatoxinas fue de 5.4 ± 2.9 µg/huevo. Se logró evidenciar el compromiso de la calidad e inocuidad del alimento, tanto para consumo humano o animal, o para la producción animal, ya que existen evidencias de la alta mortalidad embrionaria que generan las micotoxinas que logran ingresar al huevo, además, se hace evidente la necesidad de desarrollar estrategias "orgánicas" para el control fúngico en la cáscara del huevo embrionado.

Palabras clave: Micotoxinas, Aflatoxina, Huevo embrionado

INTRODUCTION

Impact of the presence of toxigenic fungi in the embryonic egg

In practice, 100 % yield can seldom be achieved in egg incubation for "broiler" chicken production (Manders *et al.,* 2021). There are many factors that affect the incubation process and it is essential to know how to do a good analysis of all the available data to know what the problem is and to propose "organic" alternatives that do not modify the percentage yield of the product.

The commercial incubation rate is the number of births of "first-class chicks" (healthy, with good vitality and suitable for rearing), calculated on the number of eggs placed in the incubator, expressed in percent; this is affected by losses caused by different causes, the main ones are listed in Table 1. The authors point out that the frequency values that appear in Table 1 may increase as incubation conditions worsen (genetic quality, nutrition, management, conservation, climate, avian health, among others) (Manders *et al.,* 2021).

Table 1. Main causes of losses in the commercial incubation rate.
Tabla 1. Principales causas de pérdidas en la tasa de incubación comercial.

Cause	Frequency (%)
Broken shell / with movable inner tube	26.2
Presence of fungi / yeasts in the shell	22.6
Not fertilized	6.3
Dead embryo due to heterogeneous heat distribution in the incubation chamber	3.1
Other*	41.8

*Causes with frequencies less than 1.6 %. Source: Manders *et al.,* 2021.

Alternatively, the most common environmental factors that affect the industrial incubation process and cause losses in the commercial incubation rate are described in Table 2. In the egg shell it is common to find microorganisms, including non-pathogenic bacteria that are found in the oviduct and ovary of hens, such as *Lactobacillus* and *Micrococcus*, however, it is also possible to find pathogenic bacteria such as *Salmonella, Staphylococcus, Pasteurella, Listeria* and *Pseudomonas* of environmental origin and of the oviduct and ovary of hens, as well as toxigenic fungi (Neira-Solís, 2016).

Table 2. Most common environmental causes of alteration in the commercial incubation rate.
Tabla 2. Causas medioambientales más comunes de alteración en la tasa de incubación comercial.

Environmental cause	Signs
More 10 days in conservation at temperature > 18 °C of the eggs during the pre-incubation period (aging of the eggs).	1. High mortality from the first hours of incubation. Most embryonic eggs do not have blood rings. 2. Increases evaporation of water in eggs during weighing. 3. Embryonic development is uneven, some eggs delayed, others early.
High temperature (> 30 °C)	Increase in embryonic and hatched chick mortality. Most of the unhatched urchins are dead.
Low temperature (< 28.8 °C)	Extension of the incubation period (> 504 h). Unhatched semi-active live chicks.
High humidity (> 60 % RH)	High embryonic mortality due to poisoning due to the presence of pathogenic fungi in the cuticle, intermediate membrane and inner membrane of the egg shell.
Low humidity (< 56 % RH)	Live, semi-active chicks without hatching, due to dryness of the membranes and lung.
Alterations in the air circulation regime and in the turning of the eggs	Dramatically increases embryonic mortality due to anoxia.
Nutrient deficiency (vitamins and minerals) in the egg	If the feed of the laying hens is deficient in vitamins and minerals for some time prior to the evacuation of the egg (> 2 weeks), a high embryonic mortality occurs, in semi-active chicks without hatching and even after hatching, during the first periods breeding.

Source: Manders *et al.,* 2021.

The main genera of toxigenic fungi found as contamination of origin in the egg are: *Aspergillus, Fusarium* and *Penicillum,* as well as yeasts of the genus *Torula* (Neira-Solís, 2016).

The high humidity (56 – 60 % Relative Humidity, RH) and the incubation temperature (28.8 - 30 °C) at which embryonated eggs must be incubated favors the development of fungal microorganisms in their shell (Nyholm, 2020). From the moment of oviposition, the egg presents a high load of fungi and yeasts on its surface (> 10^3 "CFU" and "MFU", respectively per egg) (Bunker *et al.,* 2021). According to Chousalkar and McWhorter (2020), the horizontal microbial contamination of eggs (different from that of origin) depends on the cleanliness of the laying sites and the way they are handled after being obtained. If the shell remains intact, the only way for microorganisms and their mycotoxins to penetrate into the egg is through the pores (Flórez-Valencia, 2020).

In the storage of food of livestock origin, a major problem is the deterioration and contamination with mycotoxins produced by fungi such as *Aspergillus, Fusarium* and *Penicillium* that cause great economic losses throughout the world (Alonso *et al.,* 2013). In addition, these fungi produce allergenic spores and mycotoxins that cause serious potential health hazards (Egbuta *et al.,* 2017).

Impact of mycotoxins on the embryonic egg

Mycotoxins are considered toxic secondary metabolites produced by microscopic fungi during the stationary phase of their growth on food and often cause food poisoning (Peivasteh-Roudsari *et al.,* 2021; Ráduly *et al.,* 2020). In addition, the fungal allergens produced mainly by the genera *Alternaria, Aspergillus, Cladosporium* and *Penicillium* are ingested with foods such as cheeses processed by fungi, gross fungi, vegetables, dehydrated fruits, eggs, foods containing yeast, soy sauce or vinegar and produce respiratory allergies in susceptible subjects (Rodríguez-Orozco *et al.,* 2008). In embryonated eggs, it generates a high embryonic mortality due to intoxication, loss of osmoselective porous structure of the cuticle, intermediate membrane and internal membrane of the egg shell (Manders *et al.,* 2021).

The reduction of the fungal and yeast load in the eggshell is achieved by exposing the fertilized egg to a low penetration ultra-violet light system, with diluted solutions of 0.5 % formaldehyde, by exposing the water vapor at 40 °C and use of antifungal chemicals such as posaconazole, voriconazole, fluconazole and itraconazole, however, all strategies reduce the effect of altering the commercial incubation rate (Manders *et al.,* 2021). Adequate control measures to prevent the growth of fungi in the embryonic egg are of primary importance to avoid contamination with mycotoxins (Alaniz-De La O *et al.,* 2016).

Emerging technology for the control of fungi and their mycotoxins

The chemical and physical control of fungi on living and inherent surfaces is widely documented, recently, "organic" strategies have been described that can be used on living surfaces and foods, such as the use of bacteriocins from lactic acid bacteria, competitive yeasts such as *Streptomyces*, secondary metabolites of plants, among others (Takaya, 2002; Shah and Pell, 2003; Thines *et al.,* 2004; Prapagdee *et al.,* 2008; Smaoui, *et al.,* 2010). Regarding the control of the production of mycotoxins, the control of the temperature, relative humidity and biochemical environment of the substrate is the most effective strategy, however, there are novel methodologies for the destruction of the chemical structure of mycothixin such as ionizing and non-ionizing irradiation, high-pressure processing, pulsed electric field, pulsed light, cold plasma, and ultrasound (Magan and Olsen, 2004; Weaver *et al.,* 2020; Alizadeh *et al.,* 2020).

The rationale for this study

In the egg shell it is common to find microorganisms, including non-pathogenic bacteria, pathogenic bacteria and toxigenic fungi that are found as contamination of origin in the egg (*Aspergillus, Fusarium* and *Penicillum*), as well as yeasts of the *Torula* genus (Neira-Solís, 2016). In addition to the presence of these, the high humidity and the incubation temperature at which the embryonated eggs must be incubated favors the development of fungal microorganisms in their shells (Nyholm, 2020).

The presence of the fungus, its mycotoxins and its allergens in a food compromise the quality and safety of the food for human or animal consumption. Likewise, there is evidence of high embryonic mortality generated by mycotoxins that are introduced into the egg with fungal presence in the shell (Peivasteh-Roudsari *et al.,* 2021).

The purpose of this study

Determine the fungal load of origin and the presence of aflatoxins in an embryonated egg production lot.

MATERIALS AND METHODS

For the quantification of the total fungal load of egg origin in a production batch

From a batch of embryonated eggs (100 units), each egg was aseptically introduced one by one into a sterile bag with 15 mL of peptone diluent (DP, Difco™). Each of them independently rubbed the eggshell in circular motions for two minutes (by soft rub). Thee aliquots of 3.33 mL (for increase the method sensibility) and another of one milliliter were seeded in the potato dextrose agar culture medium (APD, Difco™), adding rose bengal (60 mg/L) and ampicillin (100 mg/L) using the technique of pouring in plate, to later be incubated for 5 d at 25 °C. Colonies with typical fungus and yeast morphology were counted. The report of the fungal load of origin was reported in MFU/egg.

To determine the presence of any of the four main aflatoxins (B_1, B_2, G_1, and G_2)

The ELISA (Enzyme-Linked Immunosorbent Assay) method was used, which is a direct competitive enzyme immunoassay in solid phase, provided by Sigma-Aldrich™. That it has an optimized specific antibody that allows the four subtypes of aflatoxin to be cross-determined. To carry out the method according to the supplier's recommendations, it is necessary to extract one milliliter of allantoic fluid from the embryonic egg and dissolve it in 2 mL of 70 % methanol. A 50 µL aliquot of this methanol solution is deposited into an antibody coated microwell on the ELISA plate. Subsequently, it had to be incubated for 15 min at 22 - 25 ºC. Once the time had elapsed, the methanol solution was decanted and 100 µL of the enzymatic substrate (Reagent A, Redoxy-specific immunoprotein conjugated with methylene blue) will be added. It was incubated for 60 min; the intensity of the blue color will decrease according to the concentration of any of the types of aflatoxin present. Therefore, the intensity of the color is directly proportional to the amount of conjugate bound and inversely proportional to the aflatoxin concentration in the sample or standard. Therefore, as the aflatoxin concentration in the sample or standard increases, the intensity of the blue color will decrease. The presence or absence of aflatoxin in the sample and its concentration with respect to the standard were reported.

Statistical data analysis

In this study the determinations are made in duplicate. The responses recorded as results have a dichotomous character (present or absent). The frequency is presented as a percentage of positivity or occurrence of the response variable. The T-student test (*alpha* = 0.05) was used to describe statistical differences between the replicas. Dispersion graphs of means and quartiles represented by box and whisker graphs were generated, both for the concentration of fungi present in the egg shell, and for the quantification of aflatoxins in the allantoic liquid.

RESULTS AND DISCUSSION

Microbial safety is an important factor contributing to the egg quality. During egg acquisition, there is significant risk of contamination of the eggshell surface with microscopic fungi. Mycelial hyphae may grow on the eggshell surface and penetrate into the egg content. However, there is no information on the populations of microscopic fungi on the eggshell surface and, consequently, on possible production of mycotoxins. Therefore, the aim of the study was to identify the species of microscopic fungi present on the eggshell surface acquired from different breeding systems and to measure the number of selected mycotoxins.

In our study, the composition of the fungal species in the egg shell was not determined, a total micellar count was carried out. The fungal load in the embryonic egg shell was determined, resulting in levels between 100 MFU/egg (limit of quantification) and 10 520 MFU/egg, with an average of 4 746 MFU/egg and a standard deviation of 2 058 MFU/egg (see Figure 1). All of the embryonated eggs presented the presence of fungi.

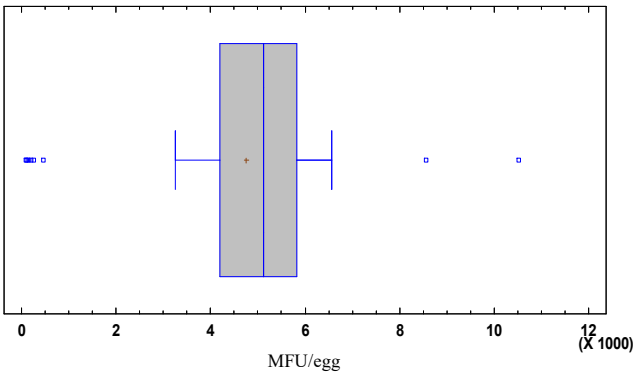

Figure 1. Distribution of the fungal load in the shell of the embryonic egg.
Figura 1. Distribución de la carga fúngica en la cáscara del huevo embrionado.

Tomczyk *et al.* (2018), reported that the composition of the species of fungi isolated from egg shells differed according to the housing system of laying hens, in such

a way that our study may show considerable differences against other similar. In the aforementioned study, a predominant prevalence of species of the genus *Alternaria* is shown, followed by *Fusarium, Scopulariopsis, Purpureocillium, Aspergillus, Botrytrichum* among others.

The diversity of potentially pathogenic fungal species on the eggshell surface is related to the unique microclimate inside the henhouse with the deep litter system, that is, high air humidity and temperature, poor ventilation, exogenous contamination, (litter, feed) and endogenous contamination (dust) (Gros *et al.,* 2015). In addition, the possibility to control hen breeding conditions in the free-range system is limited. As hens have a free access to full-value feed or green forage, litter and the range, there is higher risk of contact with pathogenic organisms of different origin (Piskorska-Pliszczynska, *et al.,* 2014). However, adequate humidity and temperature reduces the risk of the extensive growth of pathogenic fungi (SCIENCE, 2021).

The presence of fungi on the eggshell surface involves the potential risk of their presence and production of mycotoxins in the egg content. Szablewski *et al.* (2010), showed that there were no fungi in the yolk after two weeks of storage at high relative humidity, however, they evidenced the presence of mycotoxins in the egg white. In addition, the study by Tomczyk *et al.* (2018), did not show any correlation between the mycotoxin content in the egg and the laying hen rearing system.

In this studio, the presence of any of the four main aflatoxins (B_1, B_2, G_1 and G_2) was determined, resulting in a positivity of 76 % (38/50) (Figure 2).

The aflatoxin concentration was determined by spectrophotometry (ELISA reader light/UV, Thermo-Scientific™ Multiskan™ FC) with respect to the standard provided by Sigma-Aldrich® (Figure 3). Estimates of aflatoxin concentration per egg were 5.4 ± 2.9 µg/egg (Figure 4). It should be noted that the level of mycotoxins in embryonated eggs is below the level allowed for human or animal consumption, it becomes relevant in the context of embryonic production of farm chickens. However, attention should be paid to the possibility of mycotoxin bioaccumulation in animal tissue that could be consumed by humans (Escrivá *et al.,* 2017). Our study reports on the new potential source of chemical and microbial hazards in the poultry farming industry.

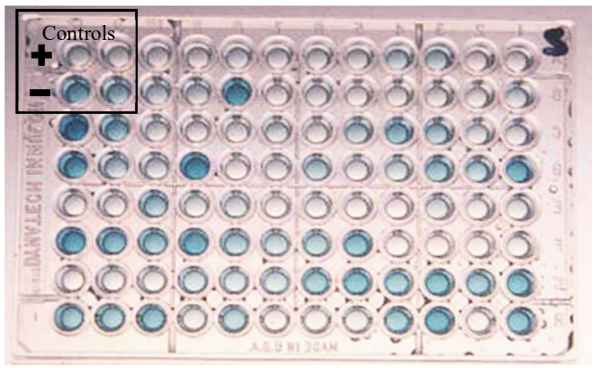

Figure 2. Results of the ELISA plate for the determination of the presence of any of the four main aflatoxins (B_1, B_2, G_1 and G_2).

Note: Wells not colored blue show positivity for the presence of aflatoxins.

Figura 2. Resultados de la placa de ELISA para la determinación de presencia de alguna de las cuatro aflatoxinas principales (B_1, B_2, G_1 y G_2).

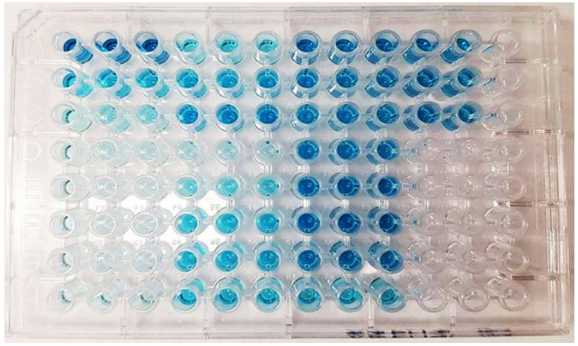

Figure 3. Results of the ELISA plate for the quantification of aflatoxins in embryonated egg samples.

Note: In rows A-C the absence of aflatoxin in the sample is confirmed. In rows D-H the concentration of aflatoxin in the sample was determined by dilution curve.

Figura 3. Resultados de la placa de ELISA para la cuantificación de aflatoxinas en las muestras de huevo embrionado.

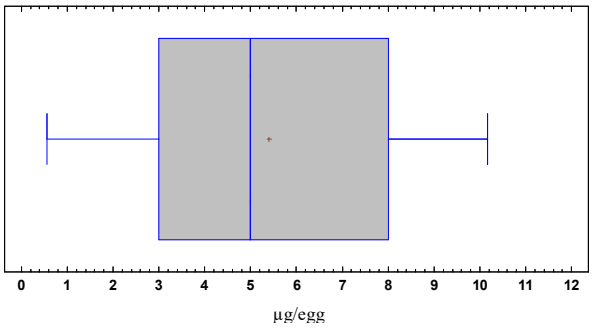

Figure 4. Distribution of aflatoxin quantification in the embryonic egg shell.

Figura 4. Distribución de la cuantificación de aflatoxinas en la cáscara del huevo embrionado.

CONCLUSION

It was possible to detect a fungal load of origin, all the units evaluated presented fungi in their shells. Definiteness, the quantitative analysis of the fungi isolated showed that the shell of embryonated eggs was a potential substrate for the growth of numerous fungi, including pathogenic and toxin-producing species, for example, *Aspergillus, Fusarium* and *Alternaria*. Moreover, the diversity of the fungal population differed according to the egg-laying hen housing system. The fungal species present on the eggshell surface may occur in the environment of the henhouse. The presence of aflatoxins in the embryonated egg production lot, compromises the quality and safety of the food, both for human or animal consumption, or for animal production, since there is evidence of the high embryonic mortality generated by the mycotoxins that manage to enter the egg, in addition, it is evident the need to develop "organic" strategies for fungal control in the embryonic eggshell.

Author Contributions

Conceptualization, C-F,TK, P-F,JJ, and N-V, CL; Methodology, C-F,TK, P-F,JJ, G-L. AY and O-P, LE; Investigation, C-F,TK, P-F,JJ, M-E,AL and R-G,MA; Data Curation, C-F,TK and P-F,JJ; Writing-Original Draft Preparation, C-F,TK and P-F,JJ.

Conflict of interest

The authors declare that they have no conflict of interest.

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JOURNAL OF MICROBIOLOGY & HEALTH EDUCATION, Vol. 3. No. 1, 2021, Pages 70-76.
http://journalmhe.org

ORIGINAL ARTICLE

Inhibition of *Candida albicans* by oregano (*Lippia* spp.) essential oil from Municipality of Rodeo, Durango, México.

Inhibición de *Candida albicans* por aceite esencial de orégano (*Lippia* spp.) del Municipio de Rodeo, Durango, México.

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Article history:
Received 24 Oct 2021
Received in revised from 22 Nov 2021
Accepted 24 Nov 2021
Available online 15 Dec 2021

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A B S T R A C T

One of the main opportunistic fungi in the world is *Candida albicans*, belong of the *Cryptococcaceae* family, it can present in an oval way with an average size of 2 to 4 microns, is the main cause of infections in the human body of candidiasis type. In Mexico in 2015 there were 177,394 cases of vaginal candidiasis between the ages of 19 to 29 years, 2,695 cases occur in the State of Durango. The essential oil of oregano (*Lippia* spp.) of the municipality of Rodeo, Dgo. Has a 70 % concentration of thymol, is one of the main natural antifungals, since it changes the permeability of the cell membranes causing the filtration of chemical constituents vital for metabolism. The experimental work was monitoring inhibition halos with three different concentrations (150 ppm, 250 ppm and 350 ppm), reading every 24, 48 and 72 hours to identify suitable time and concentration. A unifactorial analysis was used in a Tukey test and an F test for significance performed in the statistical program SAS (Statistical Analysis System), finding as a result a contact time of 48 hours with a suitable dosage of 250 ppm inhibiting 73.8 % to the fungus.
Keywords: Oregano oil, *Lippia* ssp., Thymol, Carvacrol and *Candida albicans*.

R E S U M E N

Uno de los principales hongos oportunistas en el mundo es *Candida albicans*, este es de la familia *Cryptococcaceae*, puede presentarse de forma ovalada con un tamaño promedio de 2 a 4 micrones, es la principal causa de infecciones en el cuerpo humano de tipo candidiasis. En México en 2015 hubo 177,394 casos de candidiasis vaginal entre las edades de 19 a 29 años, en el Estado de Durango se presentaron 2,695 casos. El aceite esencial de orégano (*Lippia* spp.) del municipio de Rodeo, Dgo. Tiene una concentración del 70 % de timol, es uno de los principales antifúngicos naturales, ya que cambia la permeabilidad de las membranas celulares provocando con ello la filtración de constituyentes químicos. Vital para el metabolismo. El trabajo experimental consistió en monitorear halos de inhibición con tres concentraciones diferentes (150 ppm, 250 ppm y 350 ppm), leyendo cada 24, 48 y 72 horas para identificar el tiempo y la concentración adecuado. Se utilizó un análisis unifactorial en una prueba de Tukey y una prueba F de significancia en el programa estadístico SAS (Statistical Analysis System), encontrando como resultado un tiempo de contacto de 48 horas con una dosis adecuada de 250 ppm inhibiendo el 73.8 % al hongo.
Palabras clave: Aceite de orégano, *Lippia* ssp., Timol, Carvacrol y *Candida albicans*.

INTRODUCTION

Oregano in Mexico is found in large proportions in the states of Durango, Chihuahua, Sonora, Zacatecas, etc. In general, its commercial value is due to its characteristics as spice, condiment and medicinal properties (Bonilla *et al*., 2011). It has greater importance in the industry and pharmaceutical for its essential oil, which is used as a fragrance in soaps, perfumes, cosmetics, flavorings; in addition, it preserves antibacterial, antifungal, antiparasitic, antimicrobial and antioxidant properties (Scazzocchio *et al*., 2016).

During the last years a dramatic increase in the incidence of infections caused by yeast has been reported, mainly the vaginal infections caused by *Candida albicans* L. called candidiasis. This opportunistic fungus is usually found in the body in a controlled manner, the problems are caused when it begins to proliferate due to the increase of humidity causing infections. In Mexico in 2014, 24 % of women have been victims of candidiasis, an infection caused by this proliferation of this fungus (IMSS, 2016).

Candida albicans, is a fungus and like most of them, its optimum temperature of growth is 37 ºC (body temperature), (Apares, 2016). Candidiasis is responsible for 80 to 90 % of vaginal yeast infections and the prevalence of these is between 5 and 20 % in non-pregnant and asymptomatic pregnant women (García, 2014). The development of new techniques of alternative organic medicines leads us to the study of essential oils, which contain physicochemical properties capable of inactivating microbial growth. In the leaves of oregano, we can find two main components, thymol that has the ability to decrease the spread of fungi and in the case of carvacrol that inhibits the growth of bacteria (Lara *et al*., 2016; SEMARNAT, 2015).

The information generated will contribute to the development in the social character due to the addition of information of different organic alternatives such as oregano oil, using it as a medical treatment, in the environmental character due to the decrease in production of inorganic antiseptics in order to use them to inactivate infections (ISP, 2010).

In addition, there are economic advantages due to the reduction of costs in the treatments, consequent to the low cost of the realization of the essential oil being another factor of economic development the increase in the production activity and obtaining local oregano where in the current region it is potential producer of this same plant ceasing to be a plant without exploitation (Menassé, 2016).

The present project aims to determine the optimal dose of essential oil of oregano (*Lippia* ssp.), from the municipality of Rodeo in the state of Durango, to inactivate the *Candida albicans* fungus causing vaginal infection.

MATERIALS AND METHODS

The present investigation was developed in the facilities of the Technological University of Rodeo of the municipality of Rodeo that is formed by a territorial extension of 1,389.3 km² and is located at a height of 1,340 meters above sea level and its geographic coordinates are 25.1705 °N, 104.5364 °W.

To determine the concentration and time of contact phase suitable for oil (*Lippia* ssp.), to inhibit *Candida albicans* a series of laboratory procedures was carried out in the general microbiology area of the Technological University of Rodeo. First the discs impregnated whith *Lippia spp.* essential oil were prepared, for this filter paper discs were impregnate with essential oil diluted with alcohol 96° at final concentration of 150, 250 and 350 part per million subsequently, blood agar was prepared replicating the methodology proposed by Rodríguez-Díaz *et al*. (2017); Lopez-Colombo *et al.* (2013), mention that it should be taken into account in the dilution of blood in the culture medium, it is necessary to neutralize the blood bactericidal properties and possible antimicrobial treatment received by the patient.

Subsequently, *Candida albicans* was cultured in human blood agar petri dishes using the diffusion disc method, it is a key tool to detect the presence of polymicrobial infections, subsequently 3 discs with the same dose were introduced to 3 Petri dishes and perfectly covered the edges with Parafilm^{TM} for labeling, then they were placed in the incubator calibrated at 37 °C., this process is repeated every 24, 48 and 72 h. At 72 hours, for the disposable of the culture dishes they were inactivated in autoclave at 15 pounds for 15 minutes wrapped in Kraft paper.

RESULTS AND DISCUSSION

The results of the monitoring data in the inhibition zones at three different times are presented in a unifactorial analysis with a Tukey test and a F test for significance performed in the SAS statistical program (Statistical Analysis System), (Stacciarini and Pace, 2017).

Table 1, shows the structure of the statistical analysis that was performed with the monitoring data of the inhibition zones, where it is realized that 108 data were read at each of the 3 levels (24, 48 and 72 hours) in the three different concentrations (150, 250 and 350 parts per million). A Tukey test was carried out, data shown below.

Table 1. Database information in SAS system.
Tabla 1. Información de la base de datos en el sistema SAS.

Procedure	Class	Levels	Values
ANOVA	B	3	150
			250
			350

An F-test of 0.0001 was obtained, which has shown in table 2 the significance of the monitoring of the data with a 95 % confidence interval showing that the levels of the treatment levels are different, discarding the null hypothesis focused on the similarity of the means in each treatment where I throw a mean of 1.667778, among the observed halos (Table 2). Demonstrating that the treatments studied are different by approving an alternative hypothesis.
In Table 3, we can observe the value of F for significance where a value of 0.001 was obtained by eliminating a null hypothesis and affirming an alternative hypothesis, implying that the treatments of the statistical model (150, 250 and 350) are different in a 95 % confidence interval.

Table 2. Tukey test in SAS (Statistical Analysis System).
Tabla 2. Prueba de Tukey en SAS (Sistema de análisis estadístico).

Source	DF	Sume of squares	Scuare of the mean	F-value	Pr > F
Model	2	20.58326667	10.29163333	167.70	<0.0001
Error	105	6.44360000	0.06136762		
Total, corrected	107	27.02686667			
	Rsquare	Coef. Variance	Root MSN	Media	
	0.761585	14.85359	0.247725	1.667778	

Table 3. F test with a confidence interval of 95 %.
Tabla 3. Prueba F con un intervalo de confianza del 95 %.

Process de ANOVA	
Alpha	0.05
Degrees of freedom error	105
Average of square error	0.061368
Critical value of the studentized range	3.36216
Minimum significant difference	0.1388

Based on the value of alpha is the difference between the means of the dosages by grouping them in two and thus obtaining the variabilities between these.
In Table 4 the alpha value of 0.05 is detected in our analysis, based on this the variability between treatments is determined. In this table, a greater significant difference is observed in the 150 ppm treatment, the other concentrations, no favorable response was observed (Figure 1).

It can be seen that at level 150 a minimum reading of 43 mm and a maximum reading of 129 mm was obtained, in the graph shown a mean, average and an equal median. Demonstrating the graph, at the 150 ppm level, a series of data with a standard deviation of 0.50285 and behavior in stable inhibition zones.

Table 4. Tukey Student's Range Test (HSD) for Y.
Tabla 4. Prueba de rango T-Student's de Tukey (HSD) para-Y.

b-Comparisons	Difference between means	Edge of confidence at al 95 % Simultaneus		
250 – 350	0.42000	0.28118	0.55882	***
250 – 150	1.06167	0.92285	1.20048	***
350 – 250	-0.42000	-0.55882	-0.28118	***
350 – 150	0.64167	0.50285	0.78048	***
150 – 250	-1.06167	-1.20048	-0.92285	***
150 – 350	-0.64167	-0.78048	-0.50285	***

Important comparisons of the 0.05 level are indicated by ***

Compared to the mustache graph of the level 150 ppm in the graph of 250 ppm has a mode, average and unequal medians, focusing the average of data an on the data of smaller size and obtaining a minimum reading of 178 mm and a maximum reading of 268 mm, which gives us a more extensive range of data than in the first level.
In the last 350 ppm level, a very deferential mustache plot has been developed since we obtain a larger interval between the minimum and maximum reading

of the data than in the other descriptions, with the minimum level being 113 mm and the maximum level being 211 mm. Unevenly, the mean and median drifted toward the higher data readings in the concentrations.
The diameters of the inhibition zones can be compared in the 48 and 72 hour readings. The first time the fungus was inhibited, it is noticed that contamination begins in the circumferences of the halos. Therefore, 72 hours is ruled out as a possible contact phase time for essential oil of oregano (*Lippia* ssp.).

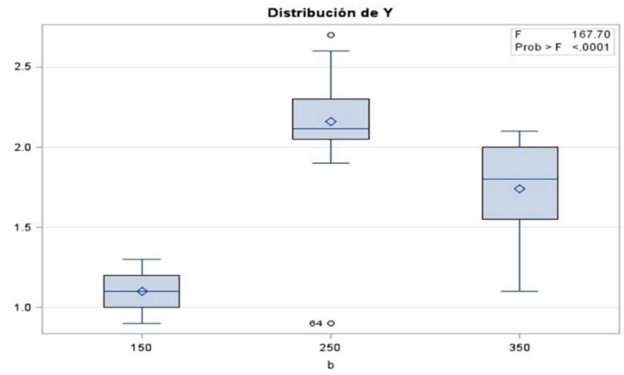

Figure 1. Distribution of the fungal load of the 3 concentrations of oregano oil used.
Figura 1. Distribución de la carga fúngica de las 3 concentraciones utilizadas de aceite de orégano.

It is possible to notice the behavior of the halos in the three different reading times where similar results are observed in the 24 and 48 hours in the concentrations of 15 and 25 %. In the dosage of 150 ppm (15 %) and 250 ppm (25 %), a difference of 10 mm was found in the first reading point at 24 hours, in the second reading point at 48 hours a difference is noticed 4 mm, demonstrating that there is no significant difference in the reading of data in question at 24 and 48 hours of monitoring in the inhibition halos, Hazen (2013), also showed that dimethyl sulfoxide (DMSO) inhibits the growth of *Candida* yeast, concludes that adding DMSO can provide misleading minimum inhibitory concentration (MIC), in the present study the essential oil of oregano (*Lippia* ssp.) was used, which consisted in diluting concentrations of 15 % and 25 % in a time of 24 and 48 hours, by means of contact.
The results of the tests of determination of the capacity of inhibition to the fungus *Candida albicans* by contact phase of essential oil of oregano in its main components (thymol and carvacrol), unlike de Benites (2015), it was observed that the ethanolic extract of *Caesalpinia spinosa* (Tara) had an inhibitory effect *in vitro* against *Candida albicans*, when using the different concentrations (25 %, 50 %, 75 % and 100 %), and this effect increases in relation directly proportional to the concentrations used in the study, giving as MIC 50 %.

This similarity in the reading of the radio of halos can be due to the similarity in the climatic characteristics of the two states and in turn the characteristics of the species of oregano.
In the work of a simple method for reading inhibition of *Escherichia coli* using an experimental work similar to the present study, they focused on demonstrating the results with a series of comparative graphs reading the monitors in mm as a unit of measurement and obtaining up to 50 % inhibition in *Candida albicans* being this a great sensitivity to the antibiotic rifampicin (Bonilla *et al*., 2011). Citing Saleh and Abu-Dieyeh (2021), they used PJ-WS-LE as extract where it showed the best efficacy, its various concentrations showed good inhibition of the four strains tested (*Escherichia coli, Proteus mirabilis, S. aureus and B. subtilis*). All bacteria showed a dose-dependent response against the extract with the largest zone of inhibition with 50 mg / ml of PJ-WS-LE extract.
In an investigation for Lopez-Rivera (2018), it showed that the inhibition halos for *Origanum vulgare* of 30.8 mm and for amikacin 30.0 mm, against *Escherichia coli*. The essential oil of *Origanum vulgare* with amikacin has a difference between both halos of inhibition of 0.8 mm. Finding a slightly higher value in favor of the combination of the oil of *Origanum vulgare* and amikacin, which is not significant compared to the present investigation with the inhibition of *Candida albicans* by the contact phase method (Jasso, 2011). In an investigation developed by Medina-De La Cruz *et al*. (2021), found that the statistical analysis showed that between the control without diluent and the one with DMSO at 1.25 % there is no statistically significant difference, therefore, the diluent does not affect the growth of the cells. An important data is that in all the strains there was a difference between the controls and the concentrations of essential oil of *C. mexicana* tested. Although for the *S. cerevisiae* strain, isolate NY66 and isolate NY62, the second concentration used, no longer shows statistical differences with respect to the previous concentration tested, this information was obtained somewhat similarly, but this is attributed to the fact that oregano oil develops high percentages of thymol and carvacrol, as shown in the present investing.
In a work realized by Acosta *et al*. (2019), made an aqueous extract of *Caesalpinia spinosa* (Tara) the

measurement of the halos, they were: 2 % (3.4 mm), 5 % (3.6 mm), 10 % (4.4 mm), 15 % (4.6 mm), and 20 % (5.2 mm). For the hydro-alcoholic extract of the rhizomes of *Polypodium picnocarpum* C. (Calaguala) the measure of the halos were 2 % (2.6 mm), 5 % (2.0 mm), 10 % (2.4 mm), 15 % (2.8 mm) and 20 % (2.2 mm). For the synergistic activity, the measurement of the inhibition zones were 2 % (3.2 mm), 5 % (3.0 mm), 10 % (4.4 mm), 15 % (3.8 mm) and 20 % (3.2 mm), similar to the present work obtaining a comparison of the mustache graph of the 150 mm level in the 250 mm graph has an unequal fashion, media and median (Cárdenas y Quintana, 2017).

In an investigation for Ismail *et al*. (2012), demonstrated that the crude extract of *Geranium wallichianum*, obtained 40 mm inhibition halo, twice the average of the halos obtained in our study with 50 % *Pelargonium graveolens* oil. It also determined the minimum inhibitory concentration of the crude extract of *Geranium wallichianum*, of 110.8 µm/ml (0.1108 mg/ml). In the present study a similar average of 43 mm was obtained from the halos obtained, but in essential oil of oregano (*Lippia* ssp.), by contact phase in its main components (thymol and carvacrol).

CONCLUSION

The essential oil of the leaf of *Lippia* ssp., from the community of Héroes de México in the municipality of Rodeo, Dgo., Showed important antifungal activity in the blood agar diffusion test against *Candida albicans*. Where he obtained an R squared of 0.761585 a coefficient of variance with 14.85359 and a mean of 1.667778, in the respective milestones.

Due to the results obtained by the F test of significance and Tukey, a difference was found between the three treatments and in turn a lower variance between the dose readings of 150 parts per million, having an average inhibition of fungus of 24 % in its circumference of the inhibition halo.

Comparing the percentages of inhibition of the two possible doses between 150 and 250 ppm, the latter obtained a greater amount of inhibition due to the characteristics of variance between data and amounts of concentrations, it was concluded that it is more advisable to use the concentration of 150 ppm , because there is no significant difference in the results of the means between treatments and especially the behavior of the readings is more stable, obtaining a smaller data reading interval in the treatment of 150 ppm and there is a variance between the results of the minor reads.

In obtaining an optimal time there was no problem since in Table 1, it is clearly observed the increase in the readings in 48 hours and a decrease in the follow-up at 72 hours, obtaining in this hour a visible contamination in the circumferences of the inhibition halos that eradicate the work of oregano essential oil.

Author Contributions

Full thanks to the authors for their contributions towards the methodologies used and the statistical analysis processes.

Acknowledgments:

The authors thank the Council of Science and Technology of the State of Durango, for the financing of said project and the Technological University of Rodeo for facilitating the facilities in the general microbiology laboratory area.

Conflict of interest

The authors declare that they have no conflict of interest.

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JOURNAL OF MICROBIOLOGY & HEALTH EDUCATION, Vol. 3. No. 1, 2021, Pages 77-84.
http://journalmhe.org

ORIGINAL ARTICLE

The general public's perceptions and use of antimicrobials in Mexico

Percepciones y uso de antimicrobianos por parte del público en general en México

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Article history:
Received 8 May 2021
Received in revised from 16 July 2021
Accepted 23 July 2021
Available online 15 December 2021

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ABSTRACT

Bacterial resistance to multiple antibiotics is a growing global problem, due in large measure to extensive and inappropriate usage of antibiotics. That resistance occurs in hospitals and their intensive care units and increasingly in the community setting as well. This prospective study surveyed 824 randomly selected households listed in the telephone directory, from November 2018 to January 2019. Through telephone interviews we determined knowledge about antibiotics and beliefs concerning their safety and efficacy. We studied the influence of age, gender, education, and having private or public health insurance on knowledge, self-medication, storing medication at home for emergency use ("hoarding"), and asking a private doctor to prescribe antibiotics ("demand prescribing"). For the 824 telephone calls that the interviewers completed, 753 of the households agreed to participate (91.4% response rate). Of those 753 participants, 699 of them (93 %) knew the term "antibiotic," 29 % (206/699) said it was a drug for bacterial infections, and 25 % (170/690) had asked a doctor for an antibiotic prescription. Penicillin was correctly identified as an antibiotic across age, gender, and education categories, but 36 % of respondents incorrectly said Benadryl (diphenhydramine), a common over-the-counter cough and cold formulation, was an antibiotic. Gender was not significantly associated with knowledge of antibiotic safety, with self-medication, or with hoarding antibiotics. On the other hand, completion of tertiary (university) education was significantly associated with correct knowledge of the safety of antibiotics and whether or not they could cure all infections. Of the various antimicrobials, beta-lactams were the ones that survey respondents had used most frequently in the preceding year, and 20% of antibiotics users had used multiple antibiotics in that period. In comparison to persons with private health insurance, more individuals without private health insurance said that antibiotics are safe and do not have side effects, and more of them also incorrectly called aspirin and Benadryl antibiotics. In México, inappropriate use of antimicrobials results from self-medication, over-the-counter availability at the community pharmacy, prescribing on demand, and lack of regulatory control. In order to contain antibiotic abuse, both the Drug Inspectorate of the Federal Commission for the Protection against Sanitary Risks (COFEPRIS, for its acronym in Spanish) should exert stricter control on the dispensing of antibiotics at private pharmacies. Further, education of the general public and of health care professionals on antibiotic misuse and appropriate use must be instituted, along with community-based surveillance of antimicrobial resistance trends.

Keywords: Antimicrobials, health education, professional education, self-medication

RESUMEN

La resistencia bacteriana a múltiples antibióticos es un problema mundial creciente, debido en gran medida al uso extenso e inadecuado de antibióticos. Esa resistencia se da en los hospitales y sus unidades de cuidados intensivos y, cada vez más, también en el ámbito comunitario. Este estudio prospectivo encuestó a 824 hogares seleccionados al azar que figuran en la

guía telefónica, desde noviembre de 2018 hasta enero de 2019. A través de entrevistas telefónicas, determinamos el conocimiento sobre los antibióticos y las creencias sobre su seguridad y eficacia. Estudiamos la influencia de la edad, el sexo, la educación y tener un seguro de salud público o privado en el conocimiento, la automedicación, el almacenamiento de medicamentos en el hogar para uso de emergencia ("acaparamiento") y la solicitud de un médico privado para recetar antibióticos ("prescripción a demanda "). De las 824 llamadas telefónicas que completaron los entrevistadores, 753 de los hogares aceptaron participar (tasa de respuesta del 91.4 %). De esos 753 participantes, 699 de ellos (93 %) conocían el término "antibiótico", el 29 % (206/699) dijeron que era un medicamento para las infecciones bacterianas y el 25 % (170/690) le había pedido a un médico un antibiótico. prescripción. La penicilina se identificó correctamente como un antibiótico en todas las categorías de edad, género y educación, pero el 36% de los encuestados dijo incorrectamente que Benadryl (difenhidramina), una formulación común para la tos y el resfriado de venta libre, era un antibiótico. El sexo no se asoció significativamente con el conocimiento de la seguridad de los antibióticos, con la automedicación o con la acumulación de antibióticos. Por otro lado, la finalización de la educación terciaria (universitaria) se asoció significativamente con el conocimiento correcto de la seguridad de los antibióticos y si pueden o no curar todas las infecciones. De los diversos antimicrobianos, los betalactámicos fueron los que los encuestados habían utilizado con mayor frecuencia durante el año anterior, y el 20 % de los usuarios de antibióticos había utilizado varios antibióticos en ese período. En comparación con las personas con seguro médico privado, más personas sin seguro médico privado dijeron que los antibióticos son seguros y no tienen efectos secundarios, y más de ellos también denominaron incorrectamente aspirina y antibióticos Benadryl. En México, el uso inadecuado de antimicrobianos se debe a la automedicación, la disponibilidad de venta libre en la farmacia comunitaria, la prescripción a pedido y la falta de control regulatorio. Para contener el abuso de antibióticos, tanto la Inspección de Medicamentos de la Comisión Federal de Protección contra Riesgos Sanitarios (COFEPRIS) debe ejercer un control más estricto sobre la dispensación de antibióticos en las farmacias privadas. Además, se debe instituir la educación del público en general y de los profesionales de la salud sobre el uso indebido y apropiado de los antibióticos, junto con la vigilancia comunitaria de las tendencias de resistencia a los antimicrobianos.
Palabras clave: Antimicrobianos, educación sanitaria, educación profesional, automedicación

INTRODUCTION

Bacterial resistance to multiple antibiotics is a growing global problem, due in large measure to extensive and inappropriate usage of antibiotics (Williams and Heymann, 1998; Levy, 1998). That resistance occurs in hospitals and their intensive care units and increasingly in the community setting as well.

Antibiotic resistance may be more widespread in lesser-developed countries, where there are fewer controls on the use of antimicrobial drugs (Hart and Kariuki, 1998) and where the higher incidence of infectious disease in the population fuels the demands for the drugs (Kunin, 1993.). In developing countries such major community pathogens as *Neisseria gonorrhoea*, *Streptococcus pneumoniae, Salmonella* Typhi, and *Shigella* spp. have already demonstrated their resistance to the first-line, less-expensive broad-spectrum antimicrobials (Brown *et al.,* 1982; Appelbaum *et al.,*1977; Sack *et al.,* 1997; Bennish *et al.,* 1992.). Gram-negative pathogens that cause hospital-acquired infections have developed resistance to extended-spectrum beta-lactam antimicrobials, which until recently demonstrated high cure rates for these infections (Jones, 1998; Jones and Pfaller, 1998). In the central and sud-america infections due to resistant pathogens are frequent in hospitals and also occur in the family practice setting. The reported rates of penicillin-resistant *Pneumococci* and chloramphenicol-resistant *Haemophilus influenzae* in the North America are low, but high rates of resistance to ampicillin, co-trimoxazole, and gentamicin by common gram-negative pathogens that cause urinary tract infections have been reported (Prabhakar, 2000).

Various factors contribute to this resistance problem in developing countries around the world (Hart and Kariuki, 1998). These include indiscriminate and widespread use of antimicrobials for community-acquired infections, self-medication, incomplete treatment courses, and the unregulated use of antibacterial drugs. Antibiotic use without physician consultation occurs not only in developing nations but also in the United States of America (Richman *et al*., 2001). In the United Kingdom, concerns have been raised regarding deregulation and shifting antimicrobials for topical or oral use from the "prescription-only medicine" status (to be dispensed only on a physician's prescription) to the "pharmacy medicine" category (pharmacists can dispense without a prescription) (Reeves *et al.,* 1999).

This study was undertaken to examine how the public at large in Mexico perceives and uses antibiotics. Mexico is a developing country with 128.9 million inhabitants. Divided into 32 states, between 2018 and 2020, the percentage of the population living in poverty went from 41.9% to 43.9%, Mexico is a developing country with 128.9 million inhabitants.

Between 2018 and 2020, the percentage of the population living in poverty went from 41.9% to 43.9%, and 34% of the population aged 15 or over will

not have completed secondary school in 2020, according to the INEGI census.

Medical care in Mexico is available free through public sector health care facilities. At these centers, patients can consult specialist physicians, undergo recommended investigations and procedures, and receive prescribed medication from the country's restricted drug list, all at no cost. Other than these centers, there are no free pharmacies. At these public centers, however, patients often encounter long waiting periods and find that drugs are available erratically or not at all. To receive expeditious advice and treatment, many patients consult a private doctor at their own expense, and that cost may be covered by private health insurance. Patients can also go to a private pharmacy and directly approach the pharmacist for advice and treatment. Talking to the pharmacist has the advantage of avoiding doctor consultation fees, and much of the population utilizes this approach.

The General Law of health classifies antibiotics as controlled drugs, to be dispensed only with a prescription. An anomaly in the law is its strict application only to those drugs defined by the term "antibiotics" per se and not to all antimicrobials; therefore, agents such as co-trimoxazole and the quinolones, which do not fall under this regulation, are available without prescription. Nevertheless, it is widely known that antimicrobial agents can be obtained at pharmacies without a prescription, and pharmacists simply dispense these drugs as over-the-counter medications in response to requests from customers.

Prior to this study, no data had been available on the general public's perceptions and use of antibiotics in Mexico.

Purpose of the study

We studied the influence of age, gender, education, and having private or public health insurance on knowledge, self-medication, storing medication at home for emergency use ("hoarding"), and asking a private doctor to prescribe antibiotics ("demand prescribing").

MATERIALS AND METHODS

Study design

We conducted a cross-sectional population-based study in Mexico by interviewing study subjects over the telephone. We calculated a sample size of 800 households with a working telephone, based on 80 % power to detect a difference of at least 3 % in perception and use of antibiotics, given an alpha of 0.05. As we did not know in advance what the telephone-survey participation rate would be for answered calls nor the number of busy or unanswered calls, we selected 1 600 households at random from the listed 22 million households in the telephone directory of the Telecommunication Services of Mexico. It is estimated that about 75 % of households in the country have landline phone (landline).

Interview

Six interviewers were trained to conduct structured interviews over the telephone. Prior to interviewing members of the general public, the interviewers practiced administering the questionnaire, to be certain that they were conveying identical meanings for the questions. The interviewers rehearsed with each other and also with some adult friends and relatives, both face-to-face and on the telephone. Based on that pilot-testing experience, the survey instrument was modified.

With the general public, the questionnaire was administered to a responsible adult household member who was at least 18 years old and who had to take care of family members when they were ill. The objective of the study and its format were explained to participants at the start of the interview. When persons refused to participate in the study, the interviewer asked if there was any specific reason for not complying, and recorded any reason that was given. Respondents who said they knew the term "antibiotic" were asked to explain their understanding of it, and those who had not heard the term were offered the following explanation: "Antibiotics are drugs that are prescribed for the treatment of diseases caused by germs."

Respondents were asked to identify antibiotics from a presented list of common drugs and also to answer questions on antibiotic safety, curative properties, possible common infectious conditions among household members, and any relevant action taken in those situations. Information was also obtained, for both adult and child patients, concerning storage of antibiotics at home for emergency use ("hoarding"); consulting a private sector physician for a fee; "prescribing on demand" for antibiotics, that is, with a doctor providing a prescription in response to a request from a patient; and self-medication. If antibiotics were consumed, participants were asked if they complied with all recommended instructions, and whether they shared these drugs with other family members or with friends.

In instances where individuals did not answer questions, the questions were repeated in order to prompt an answer. If the persons still failed to answer, that was classed as "no response."

Data analysis

Data were entered and analyzed using the Epi Info version 6.04 software program (Centers for Disease Control and Prevention, Atlanta, Georgia, United States). The chi-square test (Mantel-Haenszel test and Fisher's exact test) was used to compare categorical variables. Student t test analysis was used to compare the means of continuous data. Analyses for the linear trend in proportions were done by chi-square for trend using the STATCALC function in the Epi Info software.

RESULTS AND DISCUSSION

Out of 950 calls that the interviewers made, they received a busy signal or no answer in 126 of the cases, resulting in a sample of 824 respondents. Of those 824 persons, 753 agreed to participate, for a 91.4% response rate. Although the 753 was 5.9 % less than the original projected sample size of 800, an analysis showed that the small deficit did not alter the power of the study. The main reasons that persons gave for declining to participate were a lack of interest in the interview or being too busy to answer the questions. In the 32 states of Mexico the respondent participation rates ranged from 78 % to 100 %.

Table 1 shows the demographic characteristics of the survey respondents. All the demographic data were not available for all 753 survey participants; participants for whom particular data were not available are omitted from the respective descriptions shown in Table 1.

Among the survey participants there was a higher female than male representation, 67 % vs. 33 %. The age of the respondents ranged from 18 to 85 years, with 21 % being 51 years of age. Of the survey participants for whom the respective demographic information was available, 49 % (325/ 667) had salaried employee status and 56 % (385/683) had completed secondary education.

Knowledge and use of antibiotics

Of the 753 persons participating in the study, 699 of them (93 %) had heard of the term "antibiotic." Of those 699 persons, 193 respondents (28 %) provided a nonspecific description such as "something the doctor tells you to buy," leaving a total of 506 persons who offered a more-specific explanation for the term antibiotic. Out of those 506, 455 persons provided a definition of the term, which we grouped into the following categories: a drug for bacterial infections (206 responses), just a drug (138), and a drug prescribed by the doctor (58), a drug for virus/cold (31), and a drug for pain, fever, or stimulating antibodies (22).

Table 1. Demographic characteristics of the survey respondents.
Tabla 1. Características demográficas de los encuestados.

Characteristic	No.	%
Gender		
Male	248	33
Female	495	67
Age (years)		
18–30	199	27
31–40	235	32
41–50	137	19
≥ 51	156	21
Refused	12	2
Highest educational level completed		
Primary school	137	20
Secondary school	385	56
Tertiary institution	161	24
Employment status		
Employed for wages	325	49
Self-employed	98	15
Housewife	121	18
Retired or unemployed	123	18
Private health insurance		
Have private health insurance	202	28
No private health insurance	532	72

[a] The total number of participants was 824; 753 of them (91.4%) agreed to participate in the survey; the figures in the table omit missing data (e.g., no answer was given).

The knowledge and use of antibiotics was analyzed using the number of participants who had heard of the term "antibiotics," which was 699. Below we first present information on the overall knowledge and use of antibiotics; later we will present similar information for the various demographic subgroups of the respondents who had heard of the term "antibiotics" and who provided an answer to the respective question. Knowledge was assessed based on the list of common drugs that the telephone interviewers presented to the participants. Penicillin was correctly identified as an antibiotic by 83 % of the respondents, while 80 % could correctly identify tetracycline, and 78% could do that for Augmentin (amoxicillin/clavulanic acid). Ten percent of the respondents incorrectly said that Tylenol (paracetamol, acetaminophen) was an antibiotic, and 9 % did that for aspirin, 36% made that mistake for Benadryl (diphenhydramine), a common over-the-counter cough and cold formulation.

Of the participants answering the question, 24 % of them said they believed that antibiotics could cure all infections. Fifteen percent of responding participants

felt all antibiotics are safe, and only 12 % of respondents said that antibiotics were free from side effects. Twenty-one percent of responding participants said that they hoarded antibiotics, storing them at home for emergency purposes.

Based on recall, 31 % of households (220 of 699) said they had used an antibiotic during the past year, and 20 % of those people (44 of 220) had used more than one antibiotic in that period. The beta-lactams were the most frequently used antibiotics, and amoxicillin was consumed by 37 % (81 of 220) of users. The penicillins were used more frequently than the cephalosporins, 7 % (15 of 220) vs. 4 % (8 of 220), respectively. Among the cephalosporins, only Ceclor (cephalothin) and Zinnat (cefuroxime) had been used.

Out of the 699 respondents, 136 of them (19 %) reported they self-medicated with antibiotics obtained from private pharmacies, without a doctor's prescription. Out of 690 persons, 170 of them (25 %) admitted to demanding a prescription, which is, asking a doctor to provide a prescription even if the physician felt the antibiotic was unnecessary. Although 74 % (520 of 699) of the people said they did not share antibiotics with family and/or friends, 17 % (122 of 699) refused to answer this question.

Table 2 and Table 3 provide details on antibiotic knowledge and use for the various demographic groupings included in the survey. The numbers of respondents shown in those two tables differ from the demographic groupings shown in Table 1 because some respondents did not answer all the questions on knowledge or use and/or because information for those who did answer was not available on gender, age, education, or insurance status. Those respondents for whom data were not available were excluded from the analysis.

Table 2. Demographic variables associated with antibiotic identification in study of the general public`s perceptions and use of antibiotics.

Tabla 2. Variables demográficas asociadas a la identificación de antibióticos en el estudio de la percepción y el uso de antibióticos por parte del público en general.

	Drugs that respondents said were antibiotics																	
	Penicillin			Tetracycline			Augmentin			Benadryl			Aspirin			Tylenol		
Variable	*n*	No.	%	*n*	No.	%	*n*	No.	%	*n*	No.	%	*n*	No.	%	*n*	No.	%
Gender																		
Male	180	146	81	105	83	79	102	80	78	156	58	37	177	16	9	185	24	13
Female	386	321	83	238	193	81	212	164	77	346	121	35	400	36	9	400	32	8
Age																		
18–30	145	109	75	92	68	74	87	67	77	120	53	44	154	17	11	156	25	16
31–40	191	155	81	122	105	86	122	95	78	174	66	38	183	11	6[b,c]	200	10	5[b,c]
41–50	114	103	90[d]	64	56	88	56	45	80	100	27	27	120	6	5[b,c]	117	7	6[b,c]
≥ 51	106	93	88	58	41	71	55	33	60	230	30	13[b]	107	16	15	109	12	11
Highest education completed																		
Primary	113	89	79	52	33	64	51	25	49	58	23	40	121	17	14	115	15	13
Secondary	290	235	81	191	157	82	176	139	79	192	73	38	325	26	8	318	35	11
Tertiary	137	125	91[b]	93	79	85[b]	77	69	90[b]	81	17	21[b]	150	6	4[b]	133	4	3[b]
Private health insurance																		
Have	165	137	83	104	94	90[b]	99	81	82	148	43	29	175	7	4	171	12	7
Don't have	374	314	84	219	164	75	203	148	73	315	123	39[d]	380	38	10[d]	411	37	9

[a] This table shows the demographic variables for the respondents who said that the respective drug was an antibiotic. The total numbers of persons in the various groups differ from the ones in Table 1 since the respondents who refused to answer or who did not know were excluded in this analysis.
[b] Chi-square test, $0.01 < P < 0.05$.
[c] Chi-square test was significant when comparing 18–30 years versus 31–40 and 41–50 years.
[d] Chi-square test, $P < 0.01$.

Table 2 shows the demographic characteristics of respondents who said -correctly or incorrectly- that the drugs from the list that the interviewer provided were antibiotics. Significantly more respondents between 41 and 50 years old correctly identified penicillin as an antibiotic. Also, while a significantly higher proportion of the persons in the oldest age group (> 51) correctly said Benadryl was not an antibiotic, this oldest age group was somewhat more likely than those 31 - 50 years old to incorrectly classify aspirin and Tylenol as antibiotics. Subjects with tertiary education were more likely to correctly identify the antibiotics from among the various drugs presented in the list. In comparison to persons who had private health insurance, a significantly higher proportion of subjects without such insurance wrongly classified Benadryl and aspirin as antibiotics. Significantly more respondents with private health insurance accurately classified tetracycline as an antibiotic as compared with those who did not have the benefit of private health insurance.

Table 3 shows demographic details on respondents answering questions concerning knowledge and use of antibiotics. Gender was not significantly associated with knowledge of their safety, with self-medication, or with hoarding antibiotics. Tertiary education was significantly associated with correct knowledge of antibiotics regarding their safety, their ability to cure infections, and their having no side effects. Relatively more people without health insurance said antibiotics are free of side effects, as compared with persons who had health insurance.

Education was not a predictor of hoarding (Table 3). However, age was significantly associated with storing antibiotics at home, with older persons being less likely to keep antibiotics at home for emergency purposes. Self-medication was less likely to be undertaken by those with a secondary or tertiary education. Significantly more individuals without health insurance said they do self-medicate with antibiotics.

Table 3. Sociodemographic variables associated with antibiotic knowledge, self-medication, and hoarding in study of the general public`s perceptions and use of antibiotics.
Tabla 3. Variables sociodemográficas asociadas al conocimiento de antibióticos, automedicación y acaparamiento en el estudio de la percepción y uso de antibióticos por parte del público en general.

	Cures all infection[b]			Safe[c]			No side effects[d]			Self-medicates[e]			Hoards[f]		
Factor	*n*	No.	%	*n*	No.	%	*n*	No.	%	*n*	No.	%	*n*	No.	%
Gender															
Male	196	45	23	200	30	15	200	24	12	195	39	20	183	44	24
Female	424	106	25	429	64	15	417	50	12	436	96	22	420	84	20
Highest education completed															
Primary	127	42	33	126	26	21	126	29	23	126	34	27	124	26	21
Secondary	320	80	25	320	48	15	308	37	12	323	71	22[g]	308	74	24
Tertiary	150	18	12[g]	156	14	9[h]	133	4	3[h]	147	25	17[g]	147	28	19
Age (yrs.)															
18–30	165	38	23	164	23	14	167	15	9	167	45	27	163	49	30
31–40	204	51	25	206	33	16	200	20	10	200	44	22	204	45	22
41–50	125	25	20	125	15	12	123	16	13	121	23	19	121	17	14[h]
≥ 51	124	36	29	122	22	18	118	13	11	124	21	17[h]	121	17	14[h]
Private health insurance															
Have	185	37	20	177	23	13	187	15	8	187	30	16	184	35	19
Don't have	422	114	27	412	66	16[h]	407	57	14[h]	422	97	23[h]	400	88	22

[a] The demographic variables are presented and analyzed for those respondents who answered "Yes" to the respective statement. Respondents who refused to answer or who gave no answer were excluded in this analysis. Thus, the totals in the respective groups (e.g., gender) differ from those in Table 1.
[b] Cures all infections = respondents said that antibiotics cure all infections.
[c] Safe = respondents said that antibiotics are safe.
[d] No side effects = respondents said that antibiotics are free from side effects.
[e] Self-medicates = respondents said that they self-medicate, using antibiotics obtained from a private pharmacy without a doctor's prescription.
[f] Hoards = respondents said they store antibiotics at home for emergency use.
[g] Chi-square test $P < 0.01$.
[h] Chi-square test $P < 0.05$.

CONCLUSION

In México, inappropriate use of antimicrobials results from self-medication, over-the-counter availability at the community pharmacy, prescribing on demand, and lack of regulatory control. In order to contain antibiotic abuse, both the Drug Inspectorate of the Federal Commission for the Protection against Sanitary Risks (COFEPRIS, for its acronym in Spanish) should exert stricter control on the dispensing of antibiotics at private pharmacies. Further, education of the general public and of health care professionals on antibiotic misuse and appropriate use must be instituted, along with community-based surveillance of antimicrobial resistance trends.

Conflict of interest:

The authors declare that they have no conflict of interest.

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JOURNAL OF MICROBIOLOGY & HEALTH EDUCATION, Vol. 3. No. 1, 2021, Pages 85-91.
http://journalmhe.org

ARTÍCULO ORIGINAL

Empleo de levaduras nativas como agentes de biocontrol de hongos fitopatógenos en uva (*Vitis vinifera* subsp. *vinifera* L.)

Use of native yeasts as biocontrol agents for phytopathogenic fungi in grapes (*Vitis vinifera* subsp. *vinifera* L.)

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Información del artículo:
Recibido el 21 de octubre de 2021
Recibido el revisado el 18 de noviembre de 2021
Aceptado el 19 de noviembre de 2021
Disponible on-line el 15 de diciembre de 2021

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R E S U M E N

La vid es susceptible de ataque por enfermedades fúngicas ocasionadas por diversas especies de hongos filamentosos, levaduras y algunas bacterias. En Baja California Sur, México, la incidencia y la severidad de las infecciones fúngicas en la vid se incrementaron en los dos últimos ciclos vegetativos. El control biológico mediante el uso de microorganismos encontrados naturalmente que antagonizan los patógenos poscosecha ha sido desarrollado como una alternativa al uso de fungicidas químicos. Objetivo de este estudio fue es estudiar los efectos antagónicos de aislamientos de levadura nativas sobre hongos filamentosos de interés agronómico y económico. Se aislaron hongos filamentosos y levaduras de granos de uva. Se determinó la actividad antagónica de las levaduras aisladas, contra los hongos filamentosos aislados. El ensayo se realizó primero *in vitro* a 25°C y luego *in vivo* a 25°C y a 2°C (solamente *Botrytis cinerea*, debido a su capacidad de crecer a bajas temperaturas). En total se aislaron 16 levaduras y 6 hongos filamentos: *Botrytis cinerea, Aspergillus caelatus, Aspergillus carbonarius, Penicillium comune*, *Rhizopus setolonifer* y *Plasmopara viticola*. Los ensayos de antagonismo *in vitro* e *in vivo* a 25°C mostraron que los mostos en fermentación fueron el principal ambiente vitivinícola a partir del cual se aislaron levaduras antagonistas (en su mayoría especies *S. cerevisiae*) capaces de suprimir a los hongos fitopatógenos aislados. En el caso de *B. cinerea*, el ensayo *in vivo* también se realizó a 2°C, pero ningún aislamiento antagonista redujo el porcentaje de incidencia de la pudrición gris más del 50%. *B. cinerea, A, carbonarius* y *R. stolonifer* presentaron sensibilidad a la acción de 8 aislamientos de levaduras antagonistas (5 pertenecientes al género *Saccharomyces* y 3 a no-*Saccharomyces*). Los aislamientos de la especie *S. cerevisiae* (aisladas principalmente de mostos en fermentación) mostraron una alta capacidad antagónica para suprimir los distintos hongos filamentosos en condiciones *in vitro*, como *in vivo.* Algunas levaduras biosupresoras fueron capaces de presentar un amplio espectro antagónico contra hongos filamentosos.

Palabras clave: *Vitis vinifera*, Enfermedades fúngicas, Biocontrol, Levaduras nativas

A B S T R A C T

The vine plant is susceptible to attack by fungal diseases caused by various species of filamentous fungi, yeasts and some bacteria. In Baja California Sur, Mexico, the incidence and severity of fungal infections in grapevines increased in the last two vegetative cycles. Biological control through the use of naturally found microorganisms that antagonize postharvest pathogens has been developed as an alternative to the use of chemical fungicides. Objective of this study was to study the antagonistic

effects of native yeast isolates on filamentous fungi of agronomic and economic interest. Filamentous fungi and yeasts were isolated from grape grains. The antagonistic activity of isolated yeasts against isolated filamentous fungi was determined. The test was performed first *in vitro* at 25 ° C and then *in vivo* at 25 ° C and 2 ° C (only *Botrytis cinerea*, due to its ability to grow at low temperatures). In total, 16 yeasts and 6 filamentary fungi were isolated: *Botrytis cinerea, Aspergillus caelatus, Aspergillus carbonarius, Penicillium comune, Rhizopus setolonifer* and *Plasmopara viticola. In vitro* and *in vivo* antagonism tests at 25 ° C showed that fermenting musts were the main viticultural environment from which antagonist yeasts (mostly *S. cerevisiae* species) capable of suppressing isolated phytopathogenic fungi were isolated. In the case of *B. cinerea*, the *in vivo* test was also carried out at 2 ° C, but no antagonist isolation reduced the percentage of incidence of gray rot by more than 50%. *B. cinerea, A, carbonarius* and *R. stolonifer* showed sensitivity to the action of 8 antagonist yeast isolates (5 belonging to the genus *Saccharomyces* and 3 to non-*Saccharomyces*). The isolates of the species *S. cerevisiae* (isolated mainly from fermentation musts) showed a high antagonistic capacity to suppress the different filamentous fungi under in vitro and in vivo conditions. Some biosuppressive yeasts were able to present a broad antagonistic spectrum against filamentous fungi.

Keywords: Vitis vinifera, Fungal diseases, Biocontrol, Native yeasts

INTRODUCCIÓN

La vid es susceptible de ataque por enfermedades fúngicas ocasionadas por diversas especies de hongos filamentosos, levaduras y algunas bacterias. En los campos agrícolas de la zona costa de Baja California Sur, México (BCS) existen una superficie de 11 mil 500 hectáreas de cultivo perennes, los cuales permanecen sembrados todo el año, 9 mil 899 hectáreas trabajan bajo la modalidad de riego; mientras que las restantes mil 601 son de riego de temporal, según informó el encargado del Despacho de la Representación Estatal de la Secretaria de Agricultura y Desarrollo Rural (2019) (1). El cultivo de vid encabeza la lista de perennes, con 4 mil 46 hectáreas tanto de uva de mesa como para vino. Las principales zonas de cultivo se encuentran en Valle de Guadalupe, San Antonio de las Minas, Real del Castillo, Santo Tomás y San Vicente (1).

En BCS, así como en diferentes zonas de cultivo de la vid en México, la incidencia y la severidad de las infecciones fúngicas en la vid se incrementaron en los dos últimos ciclos vegetativos (2).

Las enfermedades fúngicas son una de las principales causas de la pérdida de productividad en cultivos perennes. Las pérdidas ocasionadas por las enfermedades causadas por hongos y virus son muy limitantes en el cultivo de la vid, porque disminuye hasta un 80% de cada cosecha, reducen la calidad, incrementan los costos de producción y reducen el vigor y longevidad de los viñedos, existiendo enfermedades causadas por hongos que son endémicas, esto es, son prevalentes y están bien establecidas (3,4). Para su control, una de las líneas a seguir es la utilización de métodos predictivos, que a través de la observación de distintas variables ambientales pueden pronosticar la aparición de enfermedades, permitiendo la aplicación de los fungicidas antes de que aquellas se establezcan. La observación directa de los cultivos también es un método eficaz para determinar los agentes fitopatógenos debido a que cada uno de ellos presentan ciertas características distintivas sobre el tallo y el fruto de la vid (ver Figura 1), sin embargo, entre otros casos la aparición de los síntomas obedece a un asentamiento avanzado y de difícil regresión, o aparece después de haber causado daños. De entre las patologías más comunes de origen fúngico se encuentran: El mildium causado por *Plasmopara vitícola*, es el principal problema de la producción de la vid, especialmente en las variedades que pertenecen a *Vitis vinifera*. Es el principal limitante de la producción de uva para vino a nivel mundial, debido a que ataca todas las partes verdes de la planta, principalmente las hojas, donde se presentan manchas amarillentas de apariencia aceitosa. El oidium, conocido también como "cenicilla", causado por el hongo *Uncinula necátor*, es otra micosis importante en los cultivos de vid. Ataca todos los tejidos verdes y penetra sólo las células epidermales, pero afecta también las células vecinas, dando una apariencia polvosa de color gris blanquecino a los órganos atacados. El haz y el envés son igualmente susceptibles a la infección en cualquier edad de la hoja, las que se deforman y detienen su crecimiento. Por otro lado, la causada por el hongo *Botrytis cinérea*, la llamada "pudrición gris" ocasiona una considerable pérdida en la calidad y rendimiento de la cosecha. El hongo invade la inflorescencia antes de la caída de las cubiertas florales. Desde estos tejidos ataca el pedicelo y el raquis del racimo, formando pequeñas lesiones de color café, que luego se tornan oscuras, casi negras. Hacia la época de cosecha estas lesiones rodean el pedicelo y el raquis y las porciones del racimo por debajo de la lesión se secan. Y finalmente, la roya, causada por el hongo *Phakopsora uva*, también aparece entre las enfermedades más comunes de la vid. Es muy destructiva cuando no se controla oportunamente.

Hongo patógeno encontrado	Infección en fruto	Infección en tallos y hojas
Botrytis cinerea (pudrición gris)		
Aspergillus caelatus		
Aspergillus carbonarius		
Plasmopara viticola		
Rhizopus spp.		
Penicillium spp.		

Figura 1. Características de la infección fúngica en la vid.
Fuente: Adaptado de Giralt *et al.,* 2005 y Pancher *et al*., 2012
Figure 1. Characteristics of fungal infection in the vine plant.
Source: Adapted from Giralt *et al.,* 2005 and Pancher *et al*., 2012

Los primeros síntomas aparecen en forma de pequeñas manchas esparcidas o densamente distribuidas de color amarillo en el envés de las hojas y ocasionalmente aparece en los peciolos, brotes jóvenes y raquis. Más tarde, en el haz aparecen manchas de tejido muerto. Generalmente, las primeras lesiones aparecen sobre las hojas maduras, aproximadamente, unos 60-70 días después de la poda. Las infecciones severas de roya causan una defoliación prematura de la planta que ocasionan deficiencias en el llenado y madurez de los frutos. La defoliación prematura, también ocasiona la brotación de las yemas durante el periodo de descanso, que es muy detrimental para la cosecha siguiente, al utilizar la planta las reservas que utilizarán 5, 6, 7 y 3).
El control biológico mediante el uso de microorganismos encontrados naturalmente que antagonizan los patógenos poscosecha ha sido desarrollado como una alternativa al uso de fungicidas químicos. Objetivo de este estudio fue es estudiar los efectos antagónicos de aislamientos de levadura nativas sobre hongos filamentosos de interés agronómico y económico.

MATERIALES Y METODOS

Recuento y aislamiento de hongos y levaduras

Diez gramos (peso seco) de semillas de uva recuperados de la maceración del fruto de mala calidad (desperdicio) se suspendieron en 90 mL de diluyente de peptona al 1 % estéril en bolsas plásticas. Posteriormente se procedió a una homogenización a 10 rpm por 5 minutos, en un equipo agitador (Stomacher® 400 Circulator®). Por triplicado, empleando diluciones decimales seriadas se realizó el recuento y aislamiento de hongos filamentosos y levaduras en agar extracto de malta al 2 % (AEM) adicionado de 30 ppm de clorhidrato de tetraciclina.
Se aislaron hongos filamentosos y levaduras de granos de uva. Se determinó la actividad antagónica de las levaduras aisladas, contra los hongos filamentosos aislados. El ensayo se realizó primero *in vitro* a 25°C y luego *in vivo* a 25°C y a 2°C (solamente *Botrytis cinerea*, debido a su capacidad de crecer a bajas temperaturas).

Preparación de levaduras para el test de antagonismo *in vitro*

Para el ensayo del antagonismo se partió de un inoculo inicial de levaduras de 10^8 cel/mL. Para esto se realizó un pre inoculo que contenía 20 mL de YEPD líquido, con agitación constante a 100 rpm a 25 °C durante 24 h. Luego de la incubación, las células se separan del medio por centrifugación a 400 g durante 15 min.
El pellet resultante de las levaduras se resuspendió en suero fisiológico y se volvió a centrifugar en las mismas condiciones, para asegurar que no quedaran restos del medio YEPD. El segundo pellet obtenido se resuspendió por segunda ocasión en suero fisiológico y se hicieron los recuentos en camada de Neubauer. En función del recuento microbiológico obtenido, se realizaron diluciones necesarias para obtener un inoculo de 10^8 cel/mL para la realización del ensayo (Carrau Magariños, 2005).

Preparación del inoculo de esporas fúngicas para el test de antagonismo *in vitro*

Se partió de un inoculo fúngico de 7 días en medio PDA. Se separaron las esporas y se emplearon en el ensayo de antagonismo. Para desagregar las esporas del micelio del hongo se trató el inoculo con agua destilada estéril con el agregado de 0.1 % de Tween 80, y se raspo suavemente el cultivo con una asa estéril. Luego se filtró la suspensión de conidios a través de 2 capas de gasa estéril. Lo obtenido del filtrado se centrifugo en suero fisiológico estéril a 400 g, durante 15 minutos para eliminar los restos del medo donde estaba. Finalmente se resuspendió en agua destilada estéril y se ajustó la concentración a 10^6 conidios/mL mediante diluciones (Carrau Magariños, 2005).

Determinación de la capacidad antagónica *in vitro*

Se analizaron un total de 30 cepas de levaduras, Para la realización de esta determinación se prepararon las suspensiones de esporas y levaduras de acuerdo a lo descrito anteriormente. Se realizaron perforaciones de 7 mm de diámetro por 5 mm de profundidad en el centro de las placas con medio símil jugo de uva con agregado de agar (50 g/L de glucosa, 60 g/L de fructosa, 2.5 g/L de ácido tartárico de potasio, 3.0 g/L de ácido L-Málico, 0.2 g/L de ácido cítrico, 0.5 g/L de fosfato de amonio y 10 mg/L de ergosterol) (Carrau Magariños, 2005).
El fondo de la perforación se celo con agar agua, con el objetivo de asegurar que los inóculos no se fueran hacia la parte de abajo del medio y crecieran hacia afuera de la perforación. Luego se inocularon en la perforación 50 µL de suspensión de levaduras a partir de una población de 10^8 CFU/mL del microorganismo a probar potencialmente antagónico. Luego transcurridos 30 min, se inocularon 50 µL de la suspensión de esporas de los hongos a probar en una concentración de 10^6 conidios/mL.

Se incluyeron 3 controles: a) placas sembradas con la levadura, b) placas sembradas con las esporas del patógeno y c) placa con una levadura control M26 (*M. pulcherrima*). Cada ensayo, incluidos los controles se realizaron por duplicado.
Las placas con los inóculos se incubaron por 7 días a una temperatura de 20-24 1C. Los resultados del antagonismo de las levaduras frente al hongo se expresaron como índice de efectividad (IE) del crecimiento del hongo. Dicho índice se calcula de la siguiente manera:

$$\%\mathrm{I}\,\frac{\mathrm{DCF}-\mathrm{DCE}}{\mathrm{DCF}}\,x\;100$$

Donde;
DCF: Diámetro de crecimiento del hongo fitopatógeno en placa control.
DCE: Diámetro de crecimiento del hongo fitopatógeno en placa del ensayo.

Determinación de la capacidad antagónica *in vivo*
El ensayo se realizó en un lote de cultivo al aire libre de uva (*Vitis vinifera* subsp. vinifera L.), se reservó un área de cultivo particular que no tenía contacto directo con el resto de vid para producción comercial, cuyo cultivo presento una temperatura media de 24.6 °C. Así mismo, en el laboratorio de biotecnología se trabajó con un lote de vid a 2 °C.
Los ensayos se realizaron mediante exposición del inoculo fúngico y de levaduras en la planta y fruto. Se incluyeron controles negativos. Se comparó la presencia/ausencia del hongo en las plantas y frutos inoculados con la levadura y los controles.

RESULTADOS Y DISCUSIÓN

Aislamiento y diferenciación de los microrganismos
En total se aislaron 16 levaduras que luego del análisis microscópico y de los crecimientos sucesivos en el medio Agar Lisina, se confirmó que pertenecían al género *Saccharomyces,* sin embargo, también desarrollaron algunos no-*Saccharomyces.* Fue posible también aislar a 6 hongos filamentos: *Botrytis cinerea, Aspergillus caelatus, Aspergillus carbonarius, Penicillium comune, Rhizopus setolonifer* y *Plasmopara vitícola* (ver Figura 1).
Los ensayos de antagonismo *in vitro* e *in vivo* a 25°C mostraron que los mostos en fermentación fueron el principal ambiente vitivinícola a partir del cual se aislaron levaduras antagonistas (en su mayoría especies *S. cerevisiae*) capaces de suprimir a los hongos fitopatógenos aislados.

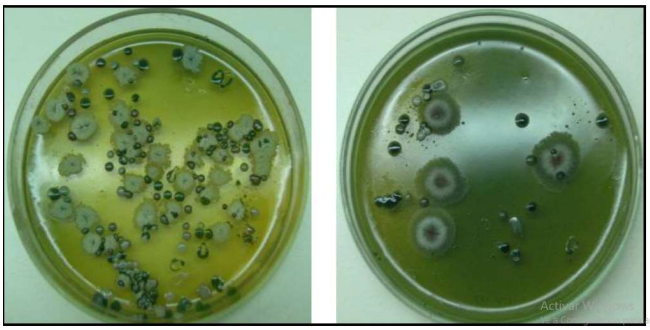

Figura 1. Microorganismos aislados de las muestras de la vid (de uva *Vitis vinifera* subsp. Vinifera L.).
Figure 1. Microorganisms isolated from grapevine samples (*Vitis vinifera* subsp. Vinifera L.).

En el caso de *B. cinérea*, el ensayo *in vivo* también se realizó a 2°C, pero ningún aislamiento antagonista redujo el porcentaje de incidencia de la pudrición gris más del 50%. *B. cinérea, A, carbonarius* y *R. stolonifer* presentaron sensibilidad a la acción de 8 aislamientos de levaduras antagonistas (5 pertenecientes al género *Saccharomyces* y 3 a no-*Saccharomyces*)
Se clasificaron tres grupos de levaduras, según el porcentaje de inhibición sobre el crecimiento de patógenos expresado como el IE:

1. Las que tienen un IE entre 0 – 10 % y comprende a las levaduras con acción nula o casi nula frente al aptoeno. En este grupo se encuentran las identificadas como CHW09, AR09, CS09, CS09/11, CS09/18, ME09/14 y representan el 23% del total de las levaduras.
2. Las que tienen un IE de 11 – 50 %. Estas levaduras tienen acción frente al patógeno que va de baja a moderada. En este grupo se encuentran las levaduras CS09/08 y ME09/15 y representan el 7 % de las levaduras totales.
3. Las que tienen un IE de 51 – 100 %, que tienen un franco efecto antagónico contra los fitopatogenos, consideran a las levaduras identificadas como 02/19ª, 02/25ª, 02/5ª, 00/09, 00/19, 000/23, 00/8 y MED09/17, todas del genero *Saccharomyces.* La cuales representan el 70 % del total de las levaduras aisladas.

En la Figura 2 se muestran algunos resultados del ensayo de inhibición *in vitro.*

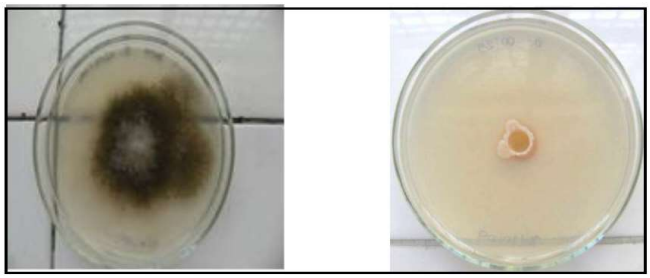

Figura 2. Ensayo de inhibición *in vitro.*
Figure 2. *In vitro* inhibition assay.

El objetivo de este trabajo fue la búsqueda de levaduras, con actividad antagónica contra fitopatógenos de la vid. A partir de estos resultados se puede inferir como en los trabajos de Carrau Magariños (2005) y Rabosto *et al.,* (2006) que los racimos de uvas constituyen un nicho muy interesante para el aislamiento de microorganismos con actividad biocontroladora frente a los fitopatógenos. Es necesario seleccionar e incrementar la población de estos microorganismos flora normal en la vid para que su número contrarreste los efectos colonizantes de los fitopatógenos.
Estos resultados también coinciden con lo expuesto por Chalutz y Wilson (1900) los cuales encontraron que al sembrar placas en un concentrado del lavado de la superficie de las frutas cítricas, solo crecían levaduras y bacterias, no hongos. Sin embargo, al diluir el concentrado y sembrándolo en placas se pude apreciar la aparición del crecimiento fúngico. Esto sugiere que las bacterias y las levaduras que se encontraban como flora normal en la fruta podían suprimir o impedir el crecimiento del fitopatógeno que estaba presente solo pudo crecer cuando baja la carga de bacterias y levaduras nativas de la fruta mediante la dilución del agua de lavado. Este resultado estaría indicando que cuando frutas y vegetales son lavados previo a su almacenamiento son más susceptibles a desarrollar enfermedades. Debido a que se eliminan los antagonistas frente a patógenos que naturalmente se encuentran en frutas y vegetales.

CONCLUSIÓN

Algunas levaduras biosupresoras fueron capaces de presentar un amplio espectro antagónico contra hongos filamentosos. En el viñedo se encuentra un buen ecosistema para el aislamiento de microorganismos levaduriformes con espectro antagónico frente a los fitopatógenos. Al concentrar el número de las levaduras antagónicas en la superficie de la planta y el racimo de uvas se faculta una protección pre y pos cosecha contra los fitopatógenos. El empleo del biocontrol con microorganismos del mismo ecosistema regula y mantiene el flujo de poblaciones microbianas a nivel de campo

Conflicto de intereses:
Los autores declaran no tener ningún conflicto de intereses.

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JOURNAL OF MICROBIOLOGY & HEALTH EDUCATION, Vol. 3. No. 1, 2021, Pages 92-96.
http://journalmhe.org

ORIGINAL ARTICLE

Effect of a clinical pathway to reduce hospitalizations in nursing home residents with pneumonia

Efecto de una vía clínica para reducir las hospitalizaciones en residentes de hogares de ancianos con neumonía

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Article history:
Received 15 May 2021
Received in revised from 9 July 2021
Accepted 12 July 2021
Available online 15 December 2021

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A B S T R A C T

Nursing home residents with pneumonia are frequently hospitalized. Such transfers may be associated with multiple hazards of hospitalization as well as economic costs. To assess whether using a clinical pathway for on-site treatment of pneumonia and other lower respiratory tract infections in nursing homes could reduce hospital admissions, related complications, and costs. A cluster randomized controlled trial of 680 residents aged 65 years or older in 22 nursing homes in Cartagena, Colombia. Nursing homes began enrollment between January 2, 2001, and April 18, 2002, with the last resident follow-up occurring July 4, 2005. Residents were eligible if they met a standardized definition of lower respiratory tract infection. Treatment in nursing homes according to a clinical pathway, which included use of oral antimicrobials, portable chest radiographs, oxygen saturation monitoring, rehydration, and close monitoring by a research nurse, or usual care. Hospital admissions, length of hospital stay, mortality, health-related quality of life, functional status, and cost. Thirty-four (10 %) of 327 residents in the clinical pathway group were hospitalized compared with 76 (22 %) of 353 residents in the usual care group. Adjusting for clustering of residents in nursing homes, the weighted mean reduction in hospitalizations was 12% (95% confidence interval [CI], 5 %-18 %; p = 0.001). The mean number of hospital days per resident was 0.79 in the clinical pathway group vs 1.74 in the usual care group, with a weighted mean difference of 0.95 days per resident (95 % CI, 0.34-1.55 days; p = 0.004). The mortality rate was 8 % (24 deaths) in the clinical pathway group vs 9 % (32 deaths) in the usual care group, with a weighted mean difference of 2.9% (95% CI, –2.0 % to 7.9 %; p = 0.23). There were no significant differences between the groups in health-related quality of life or functional status. The clinical pathway resulted in an overall cost savings of US $1016 per resident (95 % CI, $207-$1824) treated. Treating residents of nursing homes with pneumonia and other lower respiratory tract infections with a clinical pathway can result in comparable clinical outcomes, while reducing hospitalizations and health care costs.

Keywords: Nursing home, pneumonia, clinical pathway, health care costs

R E S U M E N

Los residentes de hogares de ancianos con neumonía son hospitalizados con frecuencia. Tales transferencias pueden estar asociadas con múltiples peligros de hospitalización, así como con costos económicos. Evaluar si el uso de una vía clínica para el tratamiento *in situ* de la neumonía y otras infecciones del tracto respiratorio inferior en hogares de ancianos podría reducir las admisiones hospitalarias, las complicaciones relacionadas y los costos. Un ensayo controlado aleatorio grupal de 680 residentes de 65 años o más en 22 hogares de ancianos en Cartagena, Colombia. Los hogares de ancianos comenzaron a inscribirse entre el 2 de enero de 2001 y el 18 de abril de 2002, y el último seguimiento de los residentes se realizó el 4 de julio de 2005. Los residentes eran elegibles si cumplían con una definición estandarizada de infección del tracto respiratorio

inferior. Tratamiento en residencias de ancianos según una vía clínica, que incluyó el uso de antimicrobianos orales, radiografías de tórax portátiles, monitorización de la saturación de oxígeno, rehidratación y seguimiento estrecho por parte de una enfermera investigadora o cuidados habituales. Ingresos hospitalarios, duración de la estancia hospitalaria, mortalidad, calidad de vida relacionada con la salud, estado funcional y coste. Treinta y cuatro (10 %) de 327 residentes en el grupo de vía clínica fueron hospitalizados en comparación con 76 (22 %) de 353 residentes en el grupo de atención habitual. Ajustado por la agrupación de residentes en hogares de ancianos, la reducción media ponderada de las hospitalizaciones fue del 12% (intervalo de confianza [IC] del 95 %, 5 % -18 %; p = 0.001). El número medio de días de hospitalización por residente fue 0.79 en el grupo de vía clínica frente a 1,74 en el grupo de atención habitual, con una diferencia media ponderada de 0.95 días por residente (IC del 95 %, 0.34-1,55 días; p = 0.004). La tasa de mortalidad fue del 8 % (24 muertes) en el grupo de vía clínica frente al 9% (32 muertes) en el grupo de atención habitual, con una diferencia de medias ponderada del 2.9 % (IC del 95 %, –2.0 % a 7.9 %; p = 0.23). No hubo diferencias significativas entre los grupos en la calidad de vida relacionada con la salud o el estado funcional. La vía clínica resultó en un ahorro general de costos de US $ 1016 por residente (IC del 95 %, $ 207- $ 1824) tratado. Tratar a los residentes de hogares de ancianos con neumonía y otras infecciones del tracto respiratorio inferior con una vía clínica puede dar como resultado resultados clínicos comparables, al tiempo que reduce las hospitalizaciones y los costos de atención médica.
Palabras clave: Residencia de ancianos, neumonía, vía clínica, costos de atención de la salud.

INTRODUCTION

Pneumonia and other lower respiratory tract infections are common among residents of nursing homes (Jackson *et al.,* 1992; Marrie, 2002) These infections are one of the most frequent reasons for transferring residents to hospital (Kerr and Byrd, 1991, Teresi *et al.,* 1991) Hospitalization may be associated with a reduction in quality of life, a decline in functional status, falls, and other hazards (Creditor, 1993). The economic costs associated with such hospital transfers are substantial (Kayser-Jones, Wiener and Barbaccia, 1989).

Given the potential hazards to residents and the burden on the acute care health system, a strategy for treating residents with pneumonia on-site in the nursing home may be beneficial. However, the effectiveness of providing care for residents with pneumonia and other lower respiratory tract infections on-site in the nursing home is uncertain.

Purpose of the study

We developed a clinical pathway, or algorithm, for treating nursing home residents with pneumonia and other lower respiratory tract infections on-site in the nursing home. We conducted a cluster randomized controlled trial to test the hypothesis that a clinical pathway would reduce hospitalizations. The effect of the pathway on clinical outcomes and health care costs was also assessed.

MATERIALS AND METHODS

Study design

Nursing homes were paired by the number of occupied beds to help ensure similar rates of pneumonia and other lower respiratory tract infections between study groups. One member of each pair was randomized to a clinical pathway and the other member to usual care by a statistician independent of the study team using a random numbers table. Outcomes were measured in individual residents but the nursing homes served as the unit of allocation, intervention, and analysis.

Study Nursing Homes

A research coordinator (L.M.) contacted potentially eligible nursing homes in the Cartagema region, Colombia. Nursing homes provide medical, nursing, and personal care to residents. To reside in these facilities, individuals must require 24-hour nursing services, daily personal assistance, or be at risk of harm in their current home. Most residents are admitted from home or hospital and spend the rest of their lives in the nursing home. To be eligible for the study, nursing homes had to have at least 100 residents and have no stated policies for pneumonia treatment. Typically 1 registered nurse provided care for residents on each unit, which ranged in size from 30 to 50 beds. Personal care was provided by health care aides, with an average ratio of 1 registered nurse for every 7 health care aides in the nursing home. Nursing homes located on the campus of tertiary care centers were excluded.

Study Participants

Residents aged 65 years or older were eligible if they met a standardized definition of lower respiratory tract infection, which consisted of having at least 2 of the following: new or increased cough, new or increased sputum production, temperature of more than 38 °C, pleuritic chest pain, or new or increased findings on chest examination. Pneumonia was defined by the presence of 2 or more symptoms or signs of lower respiratory tract infection along with a new or increased infiltrate on chest radiograph. Residents who were not expected to live more than 30 days from the date of enrollment (as judged by their attending physician and

nurse), those residents with a history of anaphylactic or serious allergic reaction to fluoroquinolones, or those residents with advance directives precluding transfer to hospital were excluded. All participants or their designated surrogate decision makers gave informed consent.

Interventions

Nurses in both study groups were asked to contact the study nurse if residents were potentially eligible. Study nurses made routine visits to the nursing home to assess resident eligibility, discuss the trial, obtain informed consent, and enroll residents. Residents' physicians were not involved in recruitment or in the consent process.

Clinical Pathway: Residents were assessed clinically by study nurses according to the study protocol. The study nurse measured vital signs and assessed whether the resident was eating and drinking. Care was provided in the nursing home if residents met all of the following criteria: pulse of 100/min or less, respiratory rate of less than 30/min, systolic blood pressure of at least 90 mm Hg, oxygen saturation of at least 92 % (or ≥ 90 % if the resident had chronic obstructive pulmonary disease), and ability to eat and drink. If any 1 of these criteria was not met, the resident was transferred to the hospital. The nurse determined oxygen saturation by using a portable pulse oximeter initially without supplemental oxygen. If oxygen saturation was below the cutoff level, the nurse would administer oxygen and wait for 30 minutes. If upon remeasurement oxygen saturation was above the cutoff level, criterion for on-site treatment in the nursing home was met.

Chest radiographs were performed in the nursing home by a mobile unit within 12 hours of enrollment. However, presence of an infiltrate compatible with pneumonia was not a criterion with respect to site of care. The research nurse administered hypodermoclysis in the nursing home to residents who were dehydrated. This was performed by inserting a 21-gauge butterfly needle subcutaneously infusing saline at a rate of 30 mL per hour initially; if tolerated, it was increased to 60 mL per hour. The insertion site was checked hourly for the first 2 hours, then every 2 hours thereafter. Levofloxacin, administered as one 500-mg tablet orally once daily for 10 days, an antibiotic on the Colombia Drug Formulary and therefore paid for by the provincial government, was prescribed empirically as recommended in the Colombian pneumonia treatment guidelines. The dose was reduced to 250 mg for residents with known renal insufficiency. Residents who were initially treated in the nursing home but subsequently deteriorated such that they no longer met criteria for nursing home treatment were transferred to hospital. For residents who were transferred to hospital, the pathway specified that they be transferred back to the nursing home once criteria for nursing home treatment were met. The research nurse informed the physician that the resident had been enrolled and informed him/her of any major change in the resident's clinical status. However, physicians were not involved in the implementation of the various components of the clinical pathway. For residents taking warfarin, international normalized ratios were ordered and monitored by the resident's primary care physician who was made aware that the resident was taking levofloxacin administered by the study nurse.

Usual Care: Care for residents allocated to usual care treatment was left up to the resident's physician (the physician and nursing home staff made treatment decisions, including antimicrobial use and transfer to hospital). A chest radiograph was requested within 48 hours of enrollment to assess the number of residents with radiological infiltrates compatible with pneumonia in both study groups. Study nurses recorded vital signs, oxygenation, and ability of the resident to eat and drink at each visit to assess comparability with the intervention group but did not implement any interventions.

Statistical Analysis

To compare mean differences in outcomes between clinical pathway and usual care nursing homes, we used an analysis appropriate for cluster randomized trials; namely, a t test weighted by an inverse binomial variance weight for binary outcomes and a *t* test weighted by an inverse variance for continuous outcomes. These weights were proportional to the inverse of the variances of the cluster (nursing home) means or proportions. A weighted analysis of covariance, using a minimum variance weight, was used to evaluate changes in health-related quality of life and functional status, in which we assessed whether mean resident changes from baseline in nursing homes were significantly different between study groups. All outcomes were analyzed on an intention-to-treat basis. A subgroup analysis using data from residents with radiologically confirmed pneumonia was planned a priori. SAS version 9.1.3 (SAS Institute, Cary, NC) was used for all analyses and all p values were 2-sided with $p<0.05$ considered statistically significant.

We also collected and valued health care resource utilization for residents from both the clinical pathway and usual care nursing home groups. The perspective taken for the economic analysis was that of a third-party payer. Included in the analysis were assessment costs and additional diagnosis and treatment resources, such as nursing time and portable chest radiographs, required by residents receiving the clinical pathway.

RESULTS AND DISCUSSION

A total of 680 residents were enrolled (327 in the clinical pathway group and 353 in the usual care group). Characteristics of participants in the 2 groups were similar. There was complete data at follow-up of hospitalization for 661 participants (97 %), with 14 withdrawals (9 in the clinical pathway group and 5 in the usual care group) due to palliative care or a change in advance directives, 3 transfers from the nursing home (2 in the clinical pathway group and 1 in the usual care group), or adverse reactions to antimicrobial therapy (2 in the clinical pathway group). No residents met eligibility criteria for enrollment in 1 usual care nursing home. One of 77 blood cultures yielded *Streptococcus pneumoniae.*

Primary Outcome Measure

Thirty-four residents (10 %) in the clinical pathway group were hospitalized compared with 76 (22 %) in the usual care group. Adjusting for the clustering of residents in nursing homes, the weighted mean admission rate was 8 % in the clinical pathway group vs. 20 % in the usual care group, with a weighted mean difference of 12 % (95 % confidence interval [CI], 5 %-18 %; p = 0.001). The weighted mean number of hospital days per resident was 0.79 in the clinical pathway group vs 1.74 in the usual care group, with a weighted mean difference of 0.95 days per resident (95 % CI, 0.34-1.55 days; p = 0.004).

Of the residents in the clinical pathway group who were hospitalized, 4 were admitted for reasons other than pneumonia or lower respiratory tract infection, 1 for each of the following: elective surgery, fecal impaction, vertigo (at the family's insistence), and high international normalized ratio. In the usual care group, 2 residents were transferred for reasons other than pneumonia (1 due to stroke and 1 due to gastrointestinal bleed). None of the 31 residents hospitalized for pneumonia and other lower respiratory tract infections in the clinical pathway group were stable based on our criteria. In contrast, 18 (24 %) of the 74 residents in the usual care group hospitalized for these indications would have been considered stable (p = 0.003).

The results were similar when the analysis was restricted to residents with radiographically confirmed pneumonia. Eighteen (18 %) of 98 residents in the clinical pathway group vs. 43 (30 %) of 142 residents in the usual care group with pneumonia were hospitalized. The weighted mean admission rate was 9 % for the clinical pathway group vs 29 % for the usual care group, with a weighted mean difference of 19 % (95 % CI, 7 %-32 %; p = 0.005).

Seven residents (2 %) in homes randomized to the clinical pathway group made an emergency department visit with no admission compared with 14 residents (4 %) in the usual care group. All such visits were for pneumonia or lower respiratory tract infection. The weighted mean visit rate was 1.2 % in the clinical pathway group and 1.6 % in the usual care group, with a weighted mean difference of 0.4% (95 % CI, –1.9 % to 2.8 %; p = 0.72).

Secondary Outcome Measures

The mortality rates in both study groups were similar. There were 24 deaths (8 %) among residents enrolled in the clinical pathway group and 32 (9 %) among residents in the usual care group. Adjusting for clustering of residents in nursing homes, the weighted mean mortality rate in the clinical pathway group was 3.1 % and in the usual care group was 6.0 %, with a weighed mean difference of 2.9 % (95 % CI, –2.0 % to 7.9 %; p = 0.23). There were no significant differences between study homes in change in scores in health-related quality of life or functional status. Similarly, there were no differences in time to stabilization of vital signs, urinary or skin and soft-tissue infections, or falls. There were no catheter-related urinary infections in the clinical pathway group and only 1 (0.3 %) in the usual care group (mean difference, 0.3 %; 95 % CI, −0.94 % to 1.61 %; p>0.99). There were 8 skin and soft tissue infections (2.5 %) in the clinical pathway group and 5 (1.4 %) in the usual care group (mean difference, −1.1 %; 95 % CI, −1.2 % to 3.8 %; p = 0.30).

Adverse Events

Between clinical pathway and usual care groups, there were no significant differences in residents who experienced nausea (6 [2 %] vs. 11 [3 %]; p = 0.33), vomiting (13 [4 %] vs. 22 [6 %]; p = 0.23), diarrhea (13 [4 %] vs. 16 [5 %]; p = 0.85), or rash (2 [0.6 %] vs. 2 [0.6 %]; p = 0.93). One resident in a clinical pathway nursing home developed tendinitis and another resident

developed hives, both leading to early discontinuation of levofloxacin.

Resource Utilization and Cost

The initial up-front cost of oxygen and hydration therapy, mobile radiographs, and clinical pathway administration was higher for residents in the clinical pathway group by US $87 per resident (95 % CI, $ 83- $ 91). However, these up-front costs were more than offset by reduced professional billings, resident transport, and hospitalization costs ($ 1103), resulting in an overall cost savings, on average, of $ 1016 per resident (95 % CI, $ 207 - $ 1824). When US costs of hospitalization, therapy, consultations, diagnostic imaging, and professional fees were used, the resulting savings were even larger, with an overall cost saving for clinical pathway residents of $ 1517 (95 % CI, $ 601 - $ 2433). We found that a clinical pathway to treat residents of nursing homes with pneumonia and other lower respiratory tract infections reduced hospitalizations by more than half compared with usual care, resulting in substantial cost savings, on average, of $1016 per resident.

A limitation of our study is that we enrolled nursing homes with 100 or more beds, such that the results may not be generalizable to smaller nursing homes. Although the study was not blinded, the clinical pathway was a standardized protocol in that the nurses or members of the investigative team played no role in the decision to admit residents to hospital or obtain chest radiographs.

CONCLUSION

Treating residents of nursing homes with pneumonia and other lower respiratory tract infections with a clinical pathway can result in comparable clinical outcomes, while reducing hospitalizations and health care costs.

Conflict of interest:

The authors declare that they have no conflict of interest.

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