



Monitoring of food handlers and their influence on the preparation of ready-to-eat foods

Seguimiento a manipuladores de alimentos y su influencia en la preparación de alimentos listos para el consumo

Acompanhamento de manipuladores de alimentos e sua influência no preparo de alimentos prontos para consumo

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Abstract

Introduction: Transmission of foodborne pathogens is related to inadequate sanitary conditions, which are derived from either the food preparation process or the person who prepares it. Food handlers are essential to preserve food innocuousness and protect consumer's health. **Objective:** To evaluate demographic characteristics, hygiene practices, knowledge, work conditions and the presence of pathogenic microorganisms in food handlers at public school restaurants in Antioquia, Colombia. **Materials and methods:** The study evaluated 104 food handlers in a municipality in Antioquia. Demographic data and stool samples were collected to identify intestinal parasites, *Salmonella* spp., and *Staphylococcus aureus*. **Results:** The presence of intestinal parasites (e.g. *Entamoeba Complex* and *Trichuris trichiura*) was identified in 61% of female participants. *Staphylococcus aureus* was isolated from 49% of food handlers. **Conclusion:** Infection of food handler was confirmed, making them potential sources of food contamination. They are committed to their work and have the knowledge to preserve basic hygiene conditions. It is highlighted their importance and influence in adequate hygienic practices to minimize the risks of transmission, as well as the need for medical treatment and regular checkups of food handlers to counteract the risk to consumers.

Keywords: Food supply; *Salmonella*; *Staphylococcus aureus*; parasites; foodborne diseases. (Source: DeCS, Bireme).

Resumen

Introducción: La transmisión de patógenos a través de alimentos está relacionada con condiciones sanitarias inadecuadas, las cuales se derivan del proceso de elaboración de los alimentos o de quien los procesa. Los manipuladores de alimentos son esenciales para preservar la inocuidad de los alimentos y proteger la salud del consumidor. **Objetivo:** Evaluar las características demográficas, prácticas de higiene, conocimiento, condiciones laborales y la presencia de microorganismos patógenos en manipuladores de alimentos en restaurantes de escuelas públicas de Antioquia, Colombia. **Materiales y métodos:** El estudio evaluó 104 manipuladores de alimentos de un municipio de Antioquia. Se recolectaron datos demográficos y muestras fecales para identificar parásitos intestinales, *Salmonella* spp. y *Staphylococcus aureus*. **Resultados:** Se identificó la presencia de parásitos intestinales (*Entamoeba Complex* y *Trichuris trichiura*) en 61% de mujeres. *Staphylococcus aureus* fue aislada del 49% de participantes. **Conclusión:** Se confirmó la infección de los manipuladores, lo que los convierte en fuentes potenciales de contaminación. Ellos están comprometidos con su labor y tienen el conocimiento para preservar condiciones higiénicas básicas. Se resalta su importancia e influencia en prácticas higiénicas adecuadas para minimizar riesgos de contaminación y la necesidad de tratamiento médico y chequeos periódicos para contrarrestar riesgos para el consumidor.

Palabras clave: Abastecimiento de alimentos; *Salmonella*; *Staphylococcus aureus*; parásitos; enfermedades transmitidas por los alimentos. (Fuente: DeCS, Bireme).

Resumo

Introdução: A transmissão de patógenos através dos alimentos está relacionada às condições sanitárias inadequadas, que decorrem do processo de preparo dos mesmos ou de quem os processa. Os manipuladores de alimentos são essenciais para preservar a segurança alimentar e proteger a saúde do consumidor. **Objetivo:** Avaliar as características demográficas, as práticas de higiene, o conhecimento, as condições de trabalho e a presença de microrganismos patogênicos em manipuladores de alimentos de restaurantes de escolas públicas de Antioquia, Colômbia. **Materiais e métodos:** O estudo avaliou 104 manipuladores de alimentos de um município de Antioquia. Dados demográficos e amostras fecais foram coletados para identificação de parasitas intestinais, *Salmonella* spp. e *Staphylococcus aureus*. **Resultados:** A presença de parasitas intestinais (*Entamoeba Complex* e *Trichuris trichiura*) foi identificada em 61% das mulheres, *Staphylococcus aureus* foi isolado de 49% dos participantes. **Conclusão:** Foi confirmada a infecção dos manipuladores, o que os torna potenciais fontes de contaminação. Eles estão comprometidos com seu trabalho e possuem conhecimento para preservar as condições básicas de higiene. Destaca-se que é importante incentivar a práticas higiênicas adequadas para minimizar os riscos de contaminação para o consumidor, a necessidade de tratamento médico e custo de revisões periódicas.

Palavras chave: Abastecimento de alimentos; *salmonella*; *staphylococcus aureus*; parasitos; doenças transmitidas por alimentos. (Fonte: DeCS, Bireme).

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Introduction

The School Feeding Program (SFP) is one of the oldest social programs in Colombia. It was created in 1941, but it was only until 2012 when it became a nationwide program that serves an average of 58,021 children in the country⁽¹⁾. Nowadays, the program faces various challenges, including guaranteeing the harmlessness of the food prepared in the school restaurants, as part of food security and nutritional quality, which contributes to the development of the child population.

Nowadays, it has been evidenced that there are different sources of food contamination, such as incorrect cooking, inappropriate temperature, inadequate instruments, and deficient hygiene and sanitary practices among food handlers. Food handlers constitute the main cause of foodborne diseases (FD), including virus transmission^(2,3). FD are an important cause of morbidity and mortality around the world, besides generating a huge economic impact due to health costs, and the economic activities related to the production of food, with children under five years of age as the most susceptible. The World Health Organization (WHO) reported that 125,000 children die per day due to foodborne diseases⁽⁴⁾.

In Colombia, according to the Sistema Nacional de Vigilancia en Salud Pública (SIVIGILA) data, 483 outbreaks of illness were reported in 2020, which involved 4550 cases. This showed a decrease of 52.1% compared to 2019, which could be explained by the closing of restaurants, educational institutions, and street vendors due to the SARS COV-2 pandemic. Thus, non-attendance to health institutions and frequent hand-washing might have affected the harmlessness of the food prepared at home⁽⁵⁾. Antioquia, Valle, Bolívar, and Bogotá were the places with the highest number of outbreaks, where the main causing agents were bacteria, including *Escherichia coli*, *Staphylococcus aureus*, coliforms and *Salmonella* spp⁽⁵⁾.

The WHO defines food handlers as those people that during the course of their daily routine work manipulate food or objects that could come into contact with it, becoming a potential source of microorganism transmission. Various studies have evidenced the presence of pathogenic microorganisms in food handlers^(6,7), reiterating the importance of maintaining continuous surveillance of their hygiene and health conditions, making it possible to implement the necessary measures regarding direct contact with food, in addition to the knowledge and compliance of the policies. This will in turn generate in them a favorable attitude concerning their behavior, and food security practices throughout the food chain⁽⁸⁾. Since these factors become a habit, which is fundamental for their work performance⁽⁹⁻¹¹⁾ "Colombian policy focuses on "...controlling dermal infections, injuries like wounds and burns, gastrointestinal, respiratory or other infections in food handlers, who could contaminate the food during its manipulation"⁽¹²⁾. Transmission of epidemiologically important microorganisms, including *S. aureus*, known for its FD production, systemic invaders like the identification of *Salmonella*

sp., *Shigella* sp., *E. coli*- STEC (especially if they belong to enterohemorrhagic infections)^(13,14).

Recent studies show that food handlers can carry a wide range of microorganisms and have been implicated in the transmission of many infections to the student community, which has become a common and persistent problem worldwide, associated with inadequate hygienic handling practices and lack of food safety training^(15,16).

The aim of this study was an evaluation of food handlers at public school restaurants in a municipality of Antioquia, Colombia, including their demographic characteristics, hygiene practices, knowledge, work conditions and the identification of pathogenic microorganisms, and its possible risks associated to the manipulation of food.

Materials and methods

Study design

A transversal descriptive study between September and November 2017, in all the restaurants (25 service units) affiliated to the School Food Program⁽¹⁾ in a municipality in the metropolitan area of Antioquia, Colombia, localized in the south of the Valle de Aburrá was carried out.

As inclusion criteria, all food handlers who permanently worked in the 25 school restaurants were taken, and people that worked sporadically at school restaurants were excluded from the study.

Survey design

For this study, a survey was developed keeping into account the variable reported in similar studies⁽¹⁷⁾. The reliability of knowledge questions was measured using Cronbach's alpha and the result was 0.82, indicating good consistency (quantification of the correlation between the items that make it up)^(8,11,17,18).

The survey was reviewed and approved by the restaurants' administrative personal, made up by a multidisciplinary team that included a food engineer, a quality engineer, a nutritionist and an external SFP auditor (food safety expert) who belonged to the mayor's office in the municipality where the study was conducted. The administrative staff, external auditor of the municipality's Mayor's Office (food engineering), were included as a participation mechanism for the collection of the necessary data to carry out the diagnosis, to have a look from experience and from the sanitary and compliance requirements of the regulations.

Subsequently, the survey was applied as a pilot with a group of manipulators corresponding to five restaurants (15 food handlers), randomly chosen, to determine the clarity of the questions, to identify additional options of responses, and to count the time needed to reply (the results are not shown). This activity made it possible to adjust the data collection and sample procedure, without interrupting activities of the food handlers, reducing risks of transmission of pathogens in the process. Therefore, the samples were taken on a non-working morning.

Finally, the survey was applied to the manipulators, alongside the professionals who were part of the study: bacteriologists and microbiologists, who helped read or reply in case of doubt. For the application of the survey and taking samples, an authorization of each food handler was taken, with their respective informed consent.

The survey evaluated aspects related to the knowledge of food handler regarding hygiene practices and cross contamination, high epidemiological-risk food handling, and continuing education. Questions focused on demographic items like educational level, age, and general health condition, and the variable of living with pets was included in this study⁽²⁰⁾. In this study, some HACCP aspects were consulted, to identify the level of knowledge of food handlers regarding this preventing system.

The project was endorsed by an ethics committee as a prerequisite for its financing, which was granted at the Colegio Mayor de Antioquia University Institution.

Sampling and sample processing for microbial inspection

For fecal sampling, participants received instructions and a sterile cup was provided. Within 24h, samples were recollected and transported to the laboratory, and were carried out in refrigeration conditions. Sample analysis included macroscopical conditions and an intestinal parasite investigation. Conventional microscopy was done by means of a wet-mount slide with physiological saline solution (0.85%) and Lugol's solution^(21,22), after a treatment with formaline. The search for parasites included the identification of protozoa and helminths, both commensals and pathogens⁽²³⁾.

For the identification of the presence or absence of *Salmonella* spp. the protocol described by the National Health Institute (INS) was used⁽²⁴⁾. Briefly, after suspending fecal sample in 2ml of saline solution (0.85%) in a proportion 1:1, 300µl were added to 5ml of pre-enrichment Rappaport broth and cysteine selenite broth (Oxoid®), each sample was incubated at 40°C for 24h and 37°C for 18h, respectively; after incubation, each broth was inoculated in two selective culture media: Hektoen Enteric Agar (HE) (Merck®), and Xylose Lysine Deoxycholate Agar (XLD) (Merck®), incubated in a Binder® incubator at 37+/-2°C, for 24-48 hours. For their interpretation, blue or blue-green colonies with or without a black spot were considered suspicious for *Salmonella* spp. when HE agar is used, and for XLD agar, pink colonies with or without a black center were considered suspicious for *Salmonella* spp⁽²⁴⁾. The suspicious colonies were tested for biochemical test (citrate, TSI, LIA, SIM, urea) to confirm their identities.

Samples were taken from the nasopharyngeal region of each manipulator with a sterile cotton swab, which was later deposited in a tube with BHI (Brain Heart Infusion) broth (Merck®) and transported in refrigeration at 4°C, for later inoculation on petri dishes that contained Baird Parker agar (Oxoid®) for identification of presutive colonies of

Staphylococcus aureus. Subsequently, they were incubated at 37°+/-2°C for 48 hours in aerobiosis conditions in a Binder® incubator. Rounded with smooth borders, convex, black, brilliant, fine white border, and double halo colonies were tested for coagulase and catalase. The coagulase test was done by inoculating the suspicious colony in 0.05 ml (BBL™ Coagulase Plasma, Rabbit with EDTA) prepared according to the specifications of the manufacturer. The presence of *S. aureus* was confirmed when any degree of coagulation was observed between 4-6 hours after incubation. The catalase biochemical test was carried out after previously selecting the suspicious colonies, deposited on slides and 500 µl of hydrogen peroxide was added. A positive test was confirmed by the generation of oxygen bubbles in the slide⁽²⁶⁾.

Data analysis

The data were analyzed using Excel (Office2019, Microsoft®). A univariate analysis of the qualitative variables, distribution of absolute and relative frequencies was carried out. The results were analyzed by means of Pearson's chi square with a 95% confidence interval, associating a qualitative variable with the obtained laboratory results. For this analysis, statistical software SPSS Version 21 was used.

Results

Demographic characteristics of food handlers

The demographic profiles of the food handlers who participated in the research study are presented in Table 1. According to the data obtained in the survey, 100% of the participants (104) were women, with ages ranging between 27-63 years and an average of 45 years (DS= 8.30). In total, 69% of the surveyed population lives in rural area. Regarding educational level, 2% of them finished primary school, most of them finished high school (86%), and 10.5% finished college. 73.4% of food handlers declared being free of any disease at that moment. Regarding living with pets, 31% declared having pets.

Food handler hygiene practices

In this respect, it was evidenced that all of the surveyed personnel (n=104) adhered to hand washing, before starting their work, after using the washroom, after handling waste, and when returning to work. Likewise, as part of the cultural practice in Colombia, all food handlers take a shower and brush their teeth daily.

As part of the regulation, all the staff complies with the periodical medical exam following the guidelines for food handlers⁽²⁰⁾.

When asked about changing their masks, 98% reported changing it daily, and 89% stated having frequent manicures. In total, 21% of the participants does not use gloves to handle the food (Table 2). Among the 9 variables studied, 7 variables presented a significant association with diagnosis of at least one parasite in food handlers (*p*-value <0.05). The variables manicure and glove change did not demonstrate a direct relationship with the diagnosis of at least one parasite.

Table 1. Sociodemographic profiles in food handler population and intestinal parasite infection

| n=104 Variable | Positive stool result | | Negative stool result | |
|--------------------------|-----------------------|------------|-----------------------|------------|
| | Frequency | Percentage | Frequency | Percentage |
| Age | | | | |
| 20-40 | 17 | 50% | 17 | 50% |
| >40 | 46 | 66% | 24 | 34% |
| level education | | | | |
| Primary | 2 | 100% | 0 | 0% |
| High school | 53 | 60% | 36 | 40% |
| College | 7 | 64% | 4 | 36% |
| Health insurance | | | | |
| yes | 63 | 61% | 41 | 39% |
| Type of household | | | | |
| Urban | 43 | 57% | 32 | 43% |
| Rural | 20 | 69% | 9 | 31% |
| Lives with pets | | | | |
| Yes | 16 | 50% | 16 | 50% |
| No | 47 | 65% | 25 | 35% |

Table 2. Correlation analysis associated with the presence of microorganisms and hygienic practices of food handlers

| n=104 Variable | Positive stool result | | | Positive pharyngeal swab | | |
|--|-----------------------|------------|---------|--------------------------|------------|---------|
| | Frequency | Percentage | p-value | Frequency | Percentage | p-value |
| Handwashing before starting their work | | | | | | |
| Always | 63 | 61% | <0.001 | 50 | 48% | <0.001 |
| Handwashing after going to the restroom | | | | | | |
| Always | 63 | 61% | <0.001 | 26 | 25% | <0.001 |
| Handwashing after handling waste | | | | | | |
| Always | 63 | 61% | <0.001 | 49 | 47% | <0.001 |
| Shower | | | | | | |
| Always | 63 | 61% | <0.001 | 26 | 25% | <0.001 |
| Entry medical exam | | | | | | |
| Yes | 62 | 60% | <0.001 | 49 | 48% | <0.001 |
| No | 1 | 100% | | 1 | 100% | |
| Mask changing frequency | | | | | | |
| Daily | 64 | 63% | | 24 | 24% | <0.001 |
| N/A | 2 | 100% | | 0 | 0% | |
| Tooth brushing frequency | | | | | | |
| Always | 63 | 61% | <0.001 | 50 | 48% | <0.001 |
| Manicure | | | | | | |
| Always | 54 | 58% | | 23 | 25% | |
| Usually | 7 | 78 | | 3 | 33% | |
| Never | 2 | 100% | | 0 | 0% | |
| Glove change | | | | | | |
| Daily Per | 32 | 63% | | 5 | 10% | |
| Shift | 20 | 63 | | 9 | 28% | |
| Weekly | 18 | 58% | | 8 | 26% | |
| Do not use it | 13 | 59 | | 4 | 18% | |

Regarding hygiene practices, 56.1% of the participants (n=60) declared sneezing, smoking, coughing, eating, chatting, using their cellphone, while preparing food. On the other hand, 43.9% indicated they did at least one of these activities, highlighting chatting, handling their cellphones, and

handling waste and food simultaneously while preparing the food.

Food handler knowledge and work conditions

More than half of the food handlers (55%) had been working for the company between zero and five years. The majority of them (96%) had attended the food handling course when they started working for

the company and received continuing education regarding Good Handling Practices. Similarly in this study, it was identified that more than 60% of food

handlers performed different tasks within the same process increasing the risk of cross-contamination (Table 3).

Table 3. Correlation analysis associated with the presence of microorganisms and knowledge of food handling

| Variable | Positive stool result | | | | | |
|---|------------------------------------|------------|---------|--------------------------|------------|---------|
| | Diagnosis of at least one parasite | | | Positive pharyngeal swab | | |
| | Frequency | Percentage | p-value | Frequency | Percentage | p-value |
| Years in the company (Experience) | | | | | | |
| 0-5 | 34 | 60% | | 30 | 53% | |
| 0-15 | 25 | 60% | | 19 | 45% | |
| >15 | 4 | 80% | | 1 | 20% | |
| Food handling course before starting the job | | | | | | |
| Yes | 60 | 60% | | 48 | 48% | |
| No | 3 | 75% | | 2 | 50% | |
| Good handling practices contining training | | | | | | |
| Yes | 61 | 62% | | 48 | 48% | |
| No | 2 | 40% | | 2 | 40% | |
| Task performed in the process | | | | | | |
| Waste handling | 56 | 63% | <0.001 | 26 | 29% | <0.001 |
| Food handling | 56 | 62% | <0.001 | 40 | 44% | <0.001 |
| Food handling process | 55 | 62% | <0.001 | 44 | 49% | <0.001 |
| Finished product handling | 54 | 61% | <0.001 | 42 | 47% | <0.001 |
| Cleaning and disinfection | 59 | 61% | <0.001 | 46 | 47% | <0.001 |
| Supply reception | 54 | 62% | <0.001 | 41 | 47% | <0.001 |

To corroborate the knowledge of the employees with regard to perishable food handling, we asked some questions related to the management of the process temperature, storing of the product, and use of exclusive tools to prevent cross contamination in two foods: meat and salad. It is reported that 96.3% of the food handlers use an exclusive chopping board, both for meat and salad. However, the knife is not used exclusively for these two types of food (44% and 53% for meat and salad, respectively). Food handlers considered that for meat, the riskier danger was the presence of microorganisms, while for salad, they considered it critical to manage the temperature after processing it.

The factors associated with the presence of microorganisms and knowledge in food handling were compared through an univariate analysis of the qualitative variables, distribution of absolute and relative frequencies. Variables with $p \leq 0.05$ included the different tasks performed during the process such as waste management, raw food handling, food-in-process handling, finished product handling, cleaning and disinfection, and supply reception. Other variables did not demonstrate a direct relationship with the diagnosis of at least one parasite of interest.

Microbial inspection

In the microbiological tests for intestinal parasites, we found that 63 samples out of the 104 analyzed identified five species of intestinal protozoans (*Endolimax nana*, *Entamoeba coli*, *Iodamoeba butschlii*, *Entamoeba Complex*, *Blastocystis hominis*) and an intestinal helminth (*Trichuris trichiura*), with a overall presence rate of 61%. *E. nana* was the parasite with the highest presence rate with 62.12%, followed by *B. hominis* with 46.96%, both are commensal parasites. A low infection rate was

detected for *E. histolytica/dispar* or *Entamoeba complex* (10.28%), and *T. trichiura* (0.93%). In the fecal cultures, we did not observe the presence of *Salmonella* spp., while for the laryngeal swab, the frequency of *Staphylococcus aureus* was 49% (Table 4).

Factors associated with the presence of microorganisms

A contingency 2x2 table was carried out independent tests separately to verify, if there is a correlation or dependency among the various levels of factors of interest, and the presence of microorganisms (presence of parasites and presence of coagulase positive *S. aureus*).

Regarding the presence of intestinal parasites and the the sociodemographic factor there was no relation among the studied variables and the presence of intestinal parasites. As for hygiene practices, the statistically significant ($p < 0.001$) variables were hand washing, daily shower, frequent teeth brushing, and entry medical exams. In the knowledge and work conditions factor, the only variable that was statistically significant ($p < 0.001$) corresponded to the task developed, since all the levels are statistically different.

Sociodemographic factor and presence of *S. aureus* in the pharyngeal swab, those was not related among the studied variables. In the other hand, for the hygienic practices factor all variables were statistically different ($p < 0.001$) except having a manicure and changing gloves. For the knowledge and work conditions factor, the behavior of the variable was similar to that observed for the evaluation of intestinal parasites.

Table 4. Description and distribution of the presence of microorganisms in food handlers

| | Number of infected people | Global percentage (%) | Infection percentage (%) |
|---|------------------------------|--------------------------|-----------------------------|
| Stool test | 63 | 61 | 100 |
| <i>Blastocystis hominis</i> | 31 | 29 | 47 |
| <i>Entamoeba coli</i> | 10 | 9.3 | 15.2 |
| <i>Entamoeba Complex</i> (<i>histolytica/dispar</i>) | 11 | 10.3 | 16.7 |
| <i>Endolimax nana</i> | 41 | 38.3 | 62.1 |
| <i>Iodamoeba butschlii</i> | 2 | 1.9 | 3 |
| <i>Trichuris trichiura</i> | 1 | 0.9 | 1.5 |
| Wet smear | | | |
| <i>Salmonella</i> spp | 0 | 0 | 0 |
| Pharyngeal swab | | | |
| <i>Staphylococcus aureus</i> | 51 | 49 | 100 |

Discussion

Within the demographic data analysis, we established that the age range of the manipulators in our study was similar to studies by Dorotiková *et al.*⁽⁷⁾, and Engdaw *et al.*⁽¹⁴⁾. Nevertheless, other studies in Eastern countries report lower age range, compare with study (20-30 years), and an active participation of men (around 50%) in food preparation. In this study, all food handlers were women, which evidences that the role of women in food services is possibly related to sociocultural aspects in each region. In this study, it is shown that food handlers had an educational level above primary school, that coincides with findings from other authors, and highlights that the educational level^(8,10) is an important factor for the good performance of employees, since there is a tendency in those manipulators with low educational levels to present high levels of stress and anxiety during their daily tasks. In this study, some of food handlers explain that they would like more support from the administration when they desired to improve their academic level. Likewise, it is important that those people working in food services receive regular training in aspects related to food safety, under a baseline scheme on previous knowledge, skills, and attitude^(8,28). Da Cunha *et al.*⁽²⁹⁾ explain a model to identify knowledge, attitudes and practice (KAP model) in order to improve hygienic conditions for food preparation, on which many studies have focused. However, Cunha *et al.*⁽²⁹⁾ insist that if there is a relationship between the knowledge of the handlers and their attitudes, it is essential to validate in practice the conditions of food preparation, which account for the hygienic-sanitary quality of the prepared foods, and if it is important to identify other conditions that affect food preparation.

On the other hand, the variable related to living with pets did not present a direct relationship with respect to the presence of parasites in the handlers, however, it is important to take into account that according to the findings identified by Acosta *et al.*⁽²⁸⁾, in their study, it is discussed the presence of intestinal parasites associated with living with pets. In their study it was explained that specifically in canines, *Ascaris lumbricoides* was reported in 22% and *Entamomeba histolytica* in 10% of the population studied. That investigation determined the potential infection of handlers with zoonotic agents that cause

intestinal parasitosis can be generated by direct contact with the fecal matter of the canine or by the owner's contact with some parts of the infected animal that have been in contact with parasite eggs⁽²⁷⁾.

Employee work motivation and satisfaction are determining factors for good performance in routine tasks and the implementation of standards related to food hygiene and harmlessness^(13,26). This is confirmed by the analysis of hygiene practices, commitment, knowledge, and practice of habits and conditions that was established as a mandatory condition for food handlers [Resolution 2674 of 2013, chapter III]⁽¹⁹⁾ and as parameters to decrease the risk of becoming the vehicle of a foodborne disease. Labović *et al.*⁽²⁶⁾, demonstrated the influence of training handlers, and how it affects performance, improving the hygienic quality of food and its environment.

It is important to highlight that studies like Clark *et al.*⁽³²⁾ who define that there is a high correlation between the commitment of the leaders with food handlers responses⁽²⁸⁾. In this study, a key factor was identified and evidenced related to the satisfaction of the staff regarding the relation with the leaders of the restaurants and their good hygiene conditions.

In this study it was possible to show evidence that there are clear protocols on safe practices, which are known by the food handlers. Similar results are shown by Fanta *et al.*⁽¹⁶⁾, who reported approximately 46% of the population maintains and follows the basic safety and hygiene practices without fail. However, we want to note that compliance with hygiene practices and the attitude of the employee are of the utmost importance, given the fact that when there is a positive attitude, safe handling of the food is achieved⁽³²⁾. One of the main control activities is handwashing, and Dorotiková *et al.*⁽⁷⁾ stated that regular hand washing reduces the risk of diarrheal diseases in children care institutions. A key finding in this study is mainly associated with the fact that even when the manipulator washes their hands when they have to, when referring to the use and change of gloves there is no clarity whether these activities are equally relevant for the prevention of cross contamination^(32,33).

Additionally, in this study, the manipulators' knowledge about the implementation of a system of Hazard Analysis Critical Control Points (HACCP) was evaluated, and if they were capable of identifying the most critical control points within the process, as a crucial point to develop a safe environment for food preparation. Dzwolak⁽³⁵⁾ has explored the importance of implementing this type of preventive systems in small establishments like restaurants, and he concludes how relevant this program is in the improvement of food handler and hygienic surface as possible vehicles of pathogenic microorganisms that could reach the food. As such, manipulators have the ability to identify the main dangers in two processes: protein and salad preparation, in the menu. For meats, the critical control point is associated with the control of the cooking temperature that guarantees the elimination of pathogenic microorganisms that could be there. Contrastingly, for the salad process and depending on the type of process it has undergone, fresh or cooked. In the case of fresh salad, its critical control point is the concentration of disinfectant and the time of contact needed for its sanitization. In the other hand, cooked salad, it is a similar process for meat, the critical control point is associated with the cooking temperature that guarantees the elimination of pathogenic microorganisms⁽³²⁾.

our study reported findings for parasites in stool, such as *B. hominis*, *Entamoeba Complex* and *Trichuris trichiura*, it is contrasted with those by Barros *et al.*⁽³⁹⁾ and Bastidas *et al.*⁽⁴⁰⁾. *B. hominis* is considered to be an opportunistic and emerging parasite with a high prevalence in developing countries⁽³³⁾, and its infection has been associated with low nutritional status in school-age children, and that affectation is synergistic when associated with *E. nana*, *I. bustchlii*, and *E. coli*⁽³⁸⁾. These species have been reported in other studies performed in manipulators in food services^(33,42,43), reflecting that parasite species found in our study frequently affect the population of food manipulators and their control is a worldwide concern. Yimam *et al.*⁽⁴⁷⁾, reported in their systematic research, the presence of parasites in foodhandlers from Ethiopia restaurants, they explain that healthy and hygienic conditions play an important role in their control, and establish what the colonization of foodhandlers, with parasites, represent a risk for the contamination of beverages and prepared foods. The presence of commensal parasites are reported, including opportunistic parasites such as *B. hominis* and *Entamoeba complex*, a fact that suggests greater attention to colonized food handlers. *Trichuris trichiura* is a geohelminth, and requires part of its life cycle being developed in soil to be an infectious agent. However, for all cases in which the presence of at least one parasite was identified, a deworming activity was suggested in food handlers.

On the other hand, the absence of *Salmonella* sp. in manipulators coincides with other studies⁽³⁰⁾, and it can be inferred that it could indicate efficient control by regulating entities, and that is it thanks to the implementation of the systems of good manufacturing practices within school restaurants. Nevertheless, even though in this study manipulators are not the direct source of this pathogen, other studies keep reporting its prevalence in food,

especially ready-to-consume products^(29,44). Additionally, Smith *et al.*⁽⁴⁶⁾, in their study were reported an analysis of whole genome sequence of *Salmonella enteritidis*, sample that came from a patient, and they suggested the possibility of a common contaminated food source, which could be contaminated with eggs strongly implicated, this type of finding allows us to infer that food continues to be the greatest source of contamination for this type of pathogen.

Regarding the presence of coagulase positive *S. aureus* reported in this study, similar data was found in studies by Dorotiková *et al.*⁽⁷⁾, who showed 27% of *S. aureus* in food handlers. These data highlight the importance of searching asymptomatic carriers of *S. aureus* in food services, as evidenced in this study since there was no correlation with the respiratory symptoms, nor with the personal who consulted the doctor in the last six months, since they are a direct source of food contamination, and could even generate outbreaks of food poisoning in the population they serve^(9,10,34,44). However, the correlation analysis showed that good and adequate hygiene practices directly impact this parameter, and as such, they should be emphasized in terms of cross contamination control through manipulators. For all cases in which the coagulase positive *S. aureus* was reported, an antibiotic treatment was suggested in food handlers, and an increase in hand washing.

Conclusions

The results of this study reveal risk factors related to the presence of microorganisms linked to foodborne diseases in manipulators. This study found intestinal parasites and the presence of coagulase positive *S. aureus* in healthy population. The presence of these microorganisms indicates the need for permanent clinical control and surveillance, along with training programs focused on the perception of the risk, which could lead to a positive impact on preventive practices. In addition, it is clear that hygiene practices are care and control activities in which each food handler has to be aware of performing them adequately to minimize the risk of transmission any pathogen. For future studies, sensitive and specific analysis techniques could be proposed to compare the negative results, for example, as it was for *Salmonella* spp. In the case of *S. aureus* healthy carriers, it is relevant to confirm positive results through molecular techniques, if these isolates belong to strain that produce important exotoxins, that can contribute to increase risk factors of transmission foodborne illness.

Conflicts of interest: The authors declare no conflicts of interest.

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