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Original Article

Evaluation of Microbial Load in Oropharyngeal Mucosa from Tannery Workers

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ABSTRACT

Background: Animal skin provides an ideal medium for the propagation of microorganisms and it is used like raw material in the tannery and footware industry. The aim of this study was to evaluate and identify the microbial load in oropharyngeal mucosa of tannery employees.
Methods: The health risk was estimated based on the identification of microorganisms found in the oropharyngeal mucosa samples. The study was conducted in a tanners group and a control group. Samples were taken from oropharyngeal mucosa and inoculated on plates with selective medium. In the samples, bacteria were identified by 16S ribosomal DNA analysis and the yeasts through a presumptive method. In addition, the sensitivity of these microorganisms to antibiotics/antifungals was evaluated.
Results: The identified bacteria belonged to the families Enterobacteriaceae, Pseudomonadaceae, Neisseriaceae, Alcaligenaceae, Moraxellaceae, and Xanthomonadaceae, of which some species are considered as pathogenic or opportunistic microorganisms; these bacteria were not present in the control group. Forty-two percent of bacteria identified in the tanners group are correlated with respiratory diseases. Yeasts were also identified, including the following species: *Candida glabrata*, *Candida tropicalis*, *Candida albicans*, and *Candida krusei*. Regarding the sensitivity test of bacteria identified in the tanners group, 90% showed sensitivity to piperacillin/tazobactam, 87% showed sensitivity to ticarcillin/clavulanic acid, 74% showed sensitivity to ampicillin/sulbactam, and 58% showed sensitivity to amoxicillin/clavulanic acid.
Conclusion: Several of the bacteria and yeast identified in the oropharyngeal mucosa of tanners have been correlated with infections in humans and have already been reported as airborne microorganisms in this working environment, representing a health risk for workers.

1. Introduction

Mexico is ranked among the 10 largest leather manufacturers in the world; the Mexican state of Guanajuato is responsible for 65% of the tanning and finishing of leather products. The tannery industry is one of the largest economic activities in the city of León, Guanajuato. Approximately 700 leather tanneries are located in this city, which range significantly in sophistication; some consisting of small family-run businesses, with $<$ 10 employees and having minimal infrastructure, to modern manufacturing facilities with $>$ 100 employees. The health condition of tannery employees is adversely affected by the working environment, which includes physical, chemical, and biological factors, and dust and fumes present in the atmosphere [1]. The severity of the effect on health varies on individual physical characteristics including allergen sensitivity, immune capacity, and exposure to the contaminants (frequency, duration, and type).

The unprocessed animal hides, the raw material used in the tannery industry that has a high moisture content as well as growth facilitating nutrients (carbohydrates, fats, and proteins), provide an

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ideal medium for the rapid reproduction of microorganisms [2]. Prior research shows a significant correlation between microbial concentration and temperature and relative humidity [3], because most of the bacteria and fungi require specific environmental conditions to proliferate. The processing of the animal hides requires significant water usage; causing a high-humidity working environment. When combined with low-oxygen concentration, elevated temperatures, and low air circulation, the high-humidity environment acts as a catalyst for microorganism propagation [4].

The most frequent bacteria in unprocessed hides include: *Escherichia coli*, *Staphylococcus epidermidis*, *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *Bacillus anthracis*, *Bacillus subtilis*, and *Bacillus mycoides* [2]. Tetanus, anthrax, leptospirosis, epizootic aphtha, Q fever, and brucellosis are examples of diseases that workers have contracted during the tanning process because of contaminated hides [5].

In addition to bacteria, filamentous fungi were also identified, belonging to the species: *Penicillium commune*, *Penicillium glaucum*, *Penicillium wortmannii*, *Penicillium frequentans*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus oryzae*, and *Aspergillus fumigatus.* Other genera found include: *Alternaria*, *Cladosporium*, *Trichoderma*, *Fusarium*, *Aureobasidium*, and *Scopulariopsis* [2].

The possibility of contracting an infection is a *Aspergillus fumigatus* constant hazard for the tannery employees, because the hide serves as a medium for numerous microorganisms; several of these organisms also have been identified as airborne microorganisms [6,7], related to the development of respiratory disease contracted by inhalation [8]. Several antibiotic resistant bacteria have been strongly correlated with respiratory and diarrhea illnesses. In addition, yeasts belonging to the genus *Candida* were also identified. Normally found in the oral cavity, these bacteria have also been correlated with autoimmune diseases and immunosuppressed patients [9]. Respiratory diseases caused by inhalation of mold spores include atopic asthma, rhinitis, hypersensitivity pneumonitis, allergic bronchopulmonary aspergillosis, allergic fungal sinusitis, and other detrimental health effects like infections and allergic reactions are well documented [8,10,11].

During the period from 1998 to 2001, a study conducted by the Guanajuato Secretary of Health identified 58 locations in the city of León, with increased mortality risk due to diarrhea and respiratory diseases. These locations were included in industrial zones and areas with local water sources heavily contaminated with tannery effluent. In addition, the Guanajuato Secretary of Health also reported 146,930 cases of respiratory infections and 27,530 cases of acute diarrhea, which constituted the two predominant symptoms of disease in the State during 2012 [12].

Our department carried out previous studies in 23 tanneries and a control site in the city of León, Mexico [13]. In this study the microbiological quality in the indoor air was evaluated following the methodology described by the National Institute of Safety and Hygiene at Work NTP-409 and NTP-299 of the Ministry of Labor and Social Affairs of Spain [14,15]. The average fungi concentration by tannery ranged from 100 colony-forming units (CFU)/m^3 to 10,000 CFU/m^3, and in the case of bacteria, the average load ranged from 400 CFU/m^3 to 6,000 CFU/m^3. The fungi and bacterial loads found in the control site were significantly lower, at $\leq$ 300 CFU/m^3 and $\leq$ 120 CFU/m^3, respectively. Because of the lack of Mexican guidelines that limit values of airborne microorganisms for indoor environments, these results were compared with particular European countries' guidelines, specifically the Swedish requirements [16]. The results showed that 87% of the studied tanneries had a bacteria load $<$ 500 CFU/m^3 and 83% of these had a fungi load $<$ 300 CFU/m^3. The bacteria and fungi loads in the control site were lower than the Swedish limits. The indoor/outdoor ratio (I/O), an indicator of air quality [17] revealed that 43.5% of the studied tanneries had a poor air quality indoors due to bacterial load, similarly 52.2% of the studied tanneries, had a poor air quality indoors due to fungal load. Additionally, this study revealed the presence of airborne pathogens hazardous to humans in the indoor tannery environment.

The objective of the current study was to quantify and identify the microorganisms present in the oropharyngeal mucosa of a group of tannery employees versus those present in the oropharyngeal mucosa of a control group, in order to investigate a possible correlation between the work environment and the health status of workers.

2. Materials and methods

2.1. Sampling

This study took place at the tannery industrial zone in León, México. The study was developed during September 2012 to May 2013. The selected tanneries were operational during the study period. One study group of tannery workers (tanners) and one control group of automotive industry workers were analyzed. Oropharyngeal samples from the tanners group ($n = 19$) were taken during October–November 2012. Samples from the control group were taken during December–February 2012. The samples were taken in a clinical environment, in the Laboratory of Public Health, Guanajuato State.

Oropharyngeal mucosa samples were taken through a sterile hyssop, using a Stuart medium and preserved in cold for transport to the laboratory.

2.2. Processing of samples

The samples were then cultured on solid medium in Petri dishes, for which each hyssop corresponding to each worker was streaked on solid medium by massive striae technique. The solid media used were Brilliant Green Bile Agar (BGBA, Becton, Dickinson and Company), Tryptic Soy Agar (TSA, Becton, Dickinson and Company), and Sabouraud Dextrose Agar (SDA, Becton, Dickinson and Company) (BD, Becton, Dickinson and Company), Cuautitlán Izcalli, Estado de México, México, to enterobacteria, bacteria, and yeast growth, respectively. Enterobacteria and bacteria were then incubated at 37°C for 48 hours, and yeast were incubated at 25°C for 72 hours. At the conclusion of the incubation period, a counting was conducted and reported as CFU/mL. Each bacterium and yeast was macroscopically differentiated, based on the following parameters: whole shape, size, edge/margin, color, opacity, elevation, surface, and consistency.

2.3. Differentiation of microorganisms

Each bacterium and yeast macroscopically differentiated was isolated and identified; in this process each bacterium was streaked on a plate containing TSA and the yeasts were streaked on SDA plates. Bacteria were incubated at 37°C for 48 hours, and yeast at 25°C for 72 hours. Then, each isolated bacterium was inoculated in 5 mL of Tryptic Soy Broth (TSB, Becton, Dickinson and Company) and incubated at 37°C through shaking (250 rpm) all night. Each yeast was inoculated in 5 mL of Sabouraud Dextrose Broth (SDB-Becton Dickinson and Company) and incubated at 25°C through shaking (250 rpm) during a 48–72-hour period.

At the end of the incubation period, the identification of bacteria was performed by DNA extraction, which was carried out using the kit ZR Fungal/Bacterial DNA Kit (Zymo Research, Irvine, USA). Amplification of the 16S ribosomal DNA (rDNA) gene was conducted using the primers: Fd1: 5′- CCG AAT TCG ACA GAG TTT GAT