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Detection of airborne bacteria and fungi in meat plants

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Abstract

The present work aimed to determine the total airborne bacterial and fungal loads in three meat plants, identify the isolated fungi, and determine the fungal diversity. The air samples were collected from 23 different sampling points within the meat plants using two different techniques: the Petri dish exposure (sedimentation) method and the impaction method (using Mas-100 Eco Air Sampler device). Using the sedimentation method, the total mesophilic aerobic bacterial and fungal counts were found to be 1 - 180 CFU/Petri-15 min (mean 48.49 CFU/Petri-15 min) and 1 - 105 CFU/Petri (mean 19.845 CFU/Petri), respectively. Using the impaction method, the counts ranged from 8 to 385 CFU/m^3 (mean 112.77 CFU/m^3) for bacteria, and from 2 to 320 CFU/m^3 (mean 57.45 CFU/m^3) for fungi. A total of 76 isolates were obtained. The most commonly isolated fungal species were *Mucor* spp. (*n* = 28, 36.84%), *Rhizopus* spp. (*n* = 22, 28.94%), and *Penicillium* spp. (*n* = 18, 23.68%). *Mucor racemosus* (*n* = 20) and *Rhizopus oryzae* (*n* = 14) were the most common species. Additionally, *Aspergillus* spp. (*n* = 3, 3.94%), *Geotrichum* spp. (*n* = 3, 3.94%), *Syncephalostrum* sp. (*n* = 1, 1.31%), and *Wallemia* sp. (*n* = 1, 1.31%) were detected. The present work demonstrated that fungi, which could be pathogens and spoilers, may be present in the ambient air of meat plants. Therefore, maintaining air hygiene in meat plants, from economic and health perspectives, is essential.

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Introduction

Air is a significant source of pollution in many fields, including medicine, pharmacy, agriculture, and food. Foods can become contaminated with physical, chemical, and microbiological contaminants during production, processing, storage, and distribution (Moracanin *et al.,* 2019). Sources of biological contaminants known as bioaerosols include bacteria, fungi, and viruses. These biological contaminants can produce toxins such as endotoxins and mycotoxins, metabolites and particles, and other organic allergens (Kandal, 2022). Airborne microbiota in food processing facilities can negatively affect the shelf life of products, leading to economic losses (Sandıkçı Altunatmaz *et al*., 2012; Masotti *et al.*, 2019). Additionally, their toxins and allergens can pose food safety and health issues (Cundith *et al*., 2002; Çöl and Aksu, 2007; Erol, 2007; Rico-Munoz *et al.,* 2019). In particular, the contamination of foods by spores of *Penicillium*, *Aspergillus*, *Fusarium*, and *Cladosporium*, which are present in the air, and the subsequent formation of mycotoxins, can lead to serious health problems and economic losses (Erol, 2007). Therefore, air can be considered a factor contributing to cross-contamination (Masotti *et al.,* 2019). Compared to other sources of contamination, facilities where excessive dust formation is not visible are assumed to have a lower likelihood of contamination, and the number of microorganisms in the air rarely exceeds 10^3 CFU/m^3 (Erol, 2007).

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In food processing facilities, airborne microorganisms can originate from air-conditioning systems, raw materials, food production systems, and humans and their activities (Masotti *et al*., 2019; Moracanin *et al.*, 2019). It has been noted that fungi can quickly spread through the air when contaminated raw materials enter the facility, leading to contamination as they settle on fresh products (Bernardi *et al*., 2019). Microorganisms can be present in the air as dust particles, or as free spores, or vegetative forms (Masotti *et al*., 2019). Among airborne microorganisms in food processing facilities, fungi are more commonly found compared to other microbial contaminants. Food processing facilities are typically humid environments. Temperature and relative humidity are critical factors for the survival of airborne microorganisms. When the humidity level inside the building exceeds 70%, the conditions become more favourable for fungal growth. Like fungi, bacteria also prefer moist and warm environments (18 - 25°C). Most airborne bacteria exhibit mesophilic characteristics, thriving at 20 to 35°C (Masotti *et al.,* 2019). However, in refrigerated environments, psychrophilic microorganisms are more prevalent (Sandıkçı Altunatmaz *et al.,* 2012). In slaughterhouses, Gram-negative bacteria primarily from the Enterobacteriaceae and Pseudomonadaceae families, and Gram-positive bacteria from the *Staphylococcus*, *Microbacterium*, *Bacillus*, and *Micrococcus* genera, are commonly found. The most significant species include *Bacillus megaterium*, *Bacillus brevis*, *Micrococcus luteus*, and *Micrococcus varians* (Moracanin *et al.,* 2019). Among pathogenic bacteria, *Listeria monocytogenes* is notable for its ability to remain viable in aerosols in the ambient air for over than 210 min (Masotti *et al.,* 2019). In indoor air, fungi such as *Cladosporium*, *Aspergillus*, and *Penicillium* species are frequently encountered, along with *Alternaria*, *Stachybotrys*, *Rhizopus*, *Mucor*, *Wallemia*, *Trichoderma*, *Chaetomium*, *Botrytis*, *Epicoccum*, and *Fusarium* species (Çöl and Aksu, 2007). Among airborne and mycotoxigenic fungi, *Aspergillus* (producing aflatoxins and ochratoxins), *Fusarium* (producing fumonisins, deoxynivalenol, and zearalenone), and *Penicillium* (producing patulin and citrinin) are significant (Anaya *et al*., 2019).

In meat plants, contamination can occur at various points from slaughtering to processing to cold storage. Air can often be an overlooked potential source of contamination. Therefore, it is suggested that air should be considered a critical control point in meat processing plants (Cundith *et al.,* 2002). Meat plants are environments characterised by high humidity, temperature, and the presence of food substances. Under normal conditions, since food is not present in the air environment, growth and increase in the number of microorganisms are not expected. However, microorganisms suspended in the air, which adhere to particles, can be transported from one place to another, directly contaminating the food or the surfaces that come into contact with it. It has been noted that airborne contamination in meat plants can also vary with the seasons (Kandal, 2022).

Food safety has gained importance in recent years due to increasing consumer awareness, and the demand for healthy and reliable food has risen significantly. The present work thus aimed to identify airborne microbial contaminations at various points in three meat plants, and to establish their frequency and diversity. Results obtained from the present work would contribute significantly to achieving healthy and reliable foods.

Materials and methods

Sampling procedure

The air samples were collected from 23 different points (two samples for each point) across three meat processing plants during the summer. The sampling areas were designated as cold storage rooms, general production areas, meat-cutting sections, fermentation rooms, packaging units, and resting units. The sample collection was conducted using two separate methods: Petri dish exposure (sedimentation, N) and air sampler device (MAS-100 Eco Airsampler for food industry, Merck, Millipore, 109227 for impaction, C) (Çöl and Aksu, 2007). In the sedimentation method, Petri dishes were left exposed for 15 min. In the impaction method, all areas were sampled twice for 5 min using the device. The samples were immediately transported to the laboratory under refrigerated conditions (4 - 6°C), and Petri dishes were evaluated for total mesophilic aerobic bacteria (TMAB) and fungi.

Microbiological analysis

The TMAB and airborne fungi were determined using standard Plate Count Agar (PCA, Oxoid CM 463) and Dichloran Rose Bengal Chloramphenicol Agar (DRBC, Merck 1.00466), respectively, following the methods proposed by

American Public Health Association (APHA, 2001). Mesophilic aerobic bacteria were enumerated after two days of incubation at 35 - 37°C, and airborne fungal colonies were enumerated after five days at 22 - 25°C (APHA, 2001). The results were reported as colony-forming units (CFU), and expressed as CFU/m^3 and CFU/petri-15 min of air sampled.

The morphological (macro- and micro-) characteristics were determined for fungal identification according to taxonomic keys (Pitt, 1979; Samson and Pitt, 2000; Samson *et al*., 2002; Klich, 2002). For this purpose, the fungal colonies isolated from PCA and DRBC agar media were subcultured on Malt Extract Agar (MEA, Merck 1.05398), as recommended for the Mycological Examination of Foods as a medium for use in identifying fungi. The plates were incubated for five days at 22 - 25°C to obtain pure cultures. The pure cultures were then examined for colony morphology (shape, size, texture, growth, mycelium layer, and pigment production), macroscopically and microscopically. The fungal isolates were selected by identifying different colonies from each Petri dish. The colonies were examined following the methods described by Pitt (1979), Hocking *et al*. (1992), Samson and Pitt (2000), Samson *et al.* (2002), Klich (2002), and Pitt and Hocking (2009).

Temperature analysis

The temperature of the sampling areas was determined using a digital thermometer (Testo 110, Testo AG, Lenzkirch, Germany) with an accuracy of ± 0.2°C within the range of -25 to +75°C.

Statistical analysis

The results obtained from the analyses were statistically evaluated using the JMP IN 7.0.0 (Statistical Discovery from SAS Institute Inc. 2007) program. The data were analysed using one-way ANOVA. When significant differences ($p < 0.05$) were found, Duncan's multiple range test was used to determine the differences among means. Additionally, for some analyses, the "Hierarchical Clustering Method" (Cluster) using Ward's technique in the JMP program was utilised to create dendrogram charts. These charts helped identify groups that were close to each other in the analyses involving multiple variables. In the colour map of the graphs, the analysis results were shown from the lowest to the highest, with white and black colour tones ranging from light to dark.

Results and discussion

Evaluation of airborne bacteria and fungi in meat plants

The airborne microbial loads from three meat plants were meticulously examined using sedimentation (Petri dish exposure) and impaction (air sampler) methods, explicitly focusing on bacterial and fungal counts. From the 23 sampling points (Table 1), a total of 76 isolates were obtained, and the genera and species of the fungi were meticulously identified. Across all results, it was found that, except for the fermentation room and sausage production area, the numerical data obtained from vacuuming the air with the device were higher. The results showed bacterial counts ranging from 1 to 180 CFU/15 min using the sedimentation method, and 8 to 385 CFU/m^3 using the air sampler method, while fungal counts ranged from 1 to 105 CFU/15 min using the sedimentation method, and 2 to 320 CFU/m^3 (mean 57.45 CFU/m^3) using the air sampler method. Among the three different plants, the highest average results for bacterial counts were found in the third plant; and for fungal counts, the highest results

Table 1. Sampling points around meat plants' (MP1, MP2, and MP3).

MP1	Sampling point	MP2	Sampling point	MP3	Sampling point
MP1/1	Meat processing unit	MP2/1	Sausage filling	MP3/1	Air conditioning
MP1/2	Frozen product packaging	MP2/2	Meat relaxation room	MP3/2	Packaging
MP1/3	Meatballs-dough	MP2/3	Packaging	MP3/3	Production
MP1/4	Meat-dough preparation section	MP2/4	Fermentation room	MP3/4	Cold storage
MP1/5	Meatballs *etc*. forming unit	MP2/5	Production location	MP3/5	Shredding meat
MP1/6	Packaged frozen product preservation (-18°C)	MP2/6	Cold storage	MP3/6	Meat warehouse
MP1/7	Fresh product packaging department	MP2/7	Meat cutting place		
MP1/8	Shock packaged products warehouse (-18°C)	MP2/8	Döner production		
		MP2/9	Bacon room		

using the sedimentation method were also in the third plant; while using the air sampler, the highest results were in the second plant. The air sampler detected higher bacterial counts in meatball forming, ham production, frozen product packaging, sausage filling, and cutting units. Using the sedimentation method, fungal results were highest in the fermentation room, while the device detected the highest counts in frozen product packaging.

Among the isolated fungal species, *Mucor* spp. (n = 28, 36.84%), *Rhizopus* spp. (n = 22, 28.94%), and *Penicillium* spp. (n = 18, 23.68%) were found to be the most prevalent. *Mucor racemosus* (n = 20 isolates) and *Rhizopus oryzae* (n = 14 isolates) were the most common species. Additionally, *Aspergillus* spp. (n = 3, 3.94%), *Geotrichum* spp. (n = 3, 3.94%), *Syncephalostrum* sp. (n = 1, 1.31%), and *Wallemia* sp. (n = 1, 1.31%) were also identified. These findings underscore the importance of our study in understanding and managing the airborne microbial loads in meat plants.

The air samples were collected from 23 different points across three different meat plants, and the bacterial and fungal counts were determined using the air sampler and sedimentation methods. The thoroughness of our research process was evident in the detailed cluster analysis results, presented in Figure 1, which utilised the Ward method (Murtagh and Legendre, 2014). This method minimises within-group variance to form clusters.

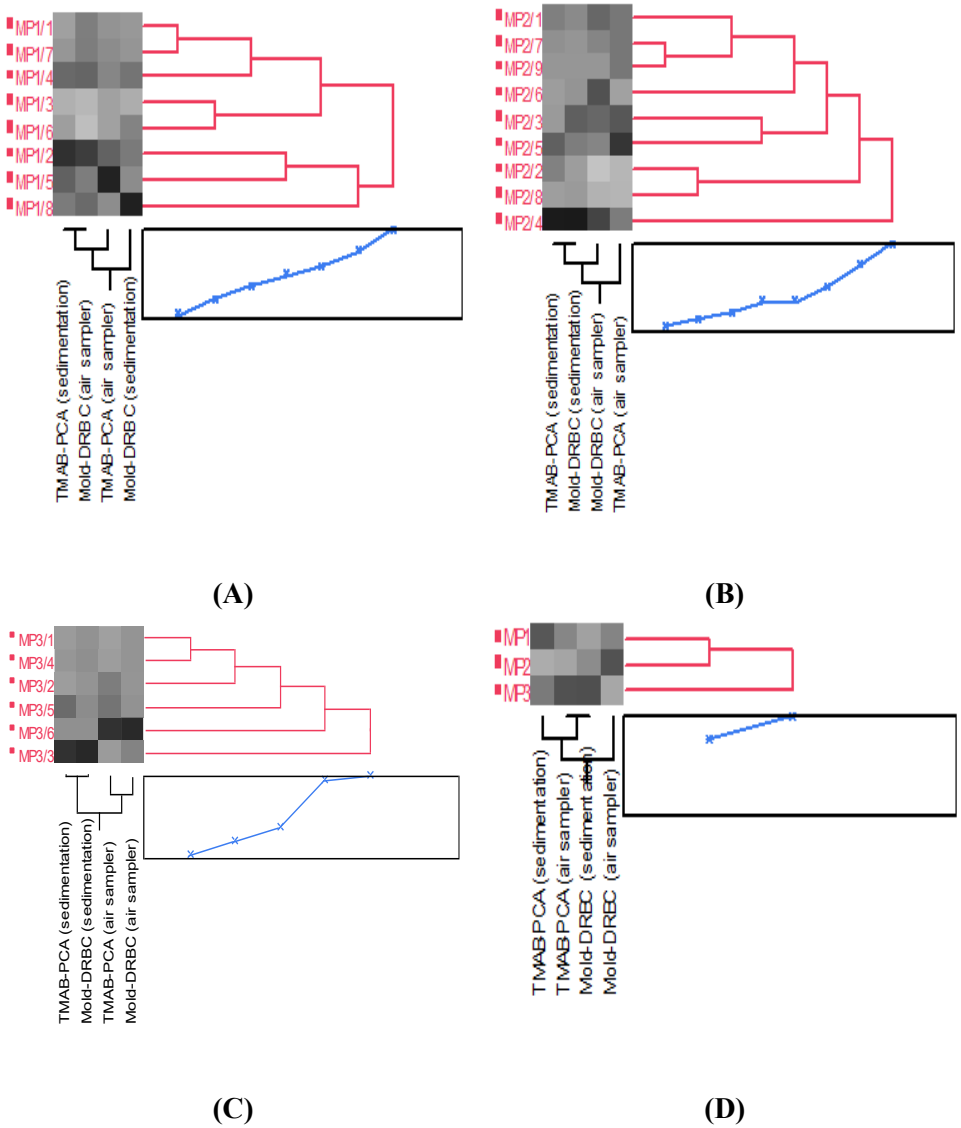

Figure 1. Hierarchical clustering analysis-dendrogram for all sampling points of meat plants (MP): **(A)** Meat Plant-1 (MP1), **(B)** Meat Plant-2 (MP2), **(C)** Meat Plant-3 (MP3), and **(D)** average results of bacteria and fungi on meat plant sampling points MP1-MP2-MP3. TMAB-PCA: Plant Count Agar; and DRBC agar: Dichloran Rose Bengal Chloramphenicol.

When examining the dendrogram in Figure 1A for MP1 meat plant, based on the sampling results from various points in the process, it was found that the meat processing unit and the frozen product packaging process points exhibited the most significant distance in terms of microbial counts. The highest microorganism counts were detected at sampling point MP1/5, located at the forming unit for products such as meatballs, as indicated by the TMAB-PCA (air sampler) method. The dark black colour in the dendrogram graph represents this.

Similarly, when examining the dendrogram in Figure 1B for MP2 meat plant, based on the sampling results from different points in the process, it was found that the meat cutting place (coded MP2/7) and the bacon room (coded MP2/9) were two closest groups in terms of microbial counts. The highest microbial counts were detected at process point MP2/4 (fermentation room), shown in dark black, based on the TMAB-PCA (sedimentation), Mould-DRBC (sedimentation), and Mould-DRBC (air sampler) methods. This point was the most distant from all other groups.

When examining the dendrogram in Figure 1C for MP3 meat plant, based on the sampling results from different process points, it was found that the process points coded MP3/1 and MP3/4 formed the closest group in terms of microbial counts. The highest microbial counts were detected at process point MP3/3 (production) based on the TMAB-PCA (sedimentation) and Mould-DRBC (sedimentation) methods, indicated by the dark black colour in the dendrogram. Similarly, the highest microbial counts, as determined per the TMAB-PCA (air sampler) and Mould-DRBC (air sampler) methods, were found at process point MP3/6 (meat warehouse), which is also indicated in dark black.

In Figure 1D, the dendrogram for all meat plants, based on the average bacterial and fungal counts at different sampling points, it was found that MP1 and MP2 clustered together. This clustering was supported because similar process points were generally sampled in both MP1 and MP2 meat plants, resulting in similar average microbial counts at the sampling points. Additionally, the PCA method yielded higher microbial counts than the DRBC method, accompanied by a corresponding shift in colour toward black in the dendrogram.

In the present work, when evaluating the numerical differences between the microbial counts obtained by the sedimentation and impaction methods, it was found that the microbial counts obtained by the impaction method were higher, as also reported in the literature (Çöl and Aksu, 2007). This is attributed to the device's ability to vacuum the entire ambient air. However, the bacterial and fungal counts obtained from the fermentation room and sausage production environment using the sedimentation method were higher than those obtained using the air sampler device. As noted, one disadvantage of the air sampler device is that excessive temperature and prolonged sampling times can cause the medium to dry out (Çöl and Aksu, 2007). This might be a contributing factor because the sampling was conducted during the summer. The higher bacterial counts detected in the meatball forming, ham production, frozen product packaging, sausage filling, and cutting units using the air sampler device, as well as the higher fungal counts detected in the fermentation room by the sedimentation method and in the frozen product packaging by the air sampler, are considered normal given the numerous variables that can influence the microbial load in these environments.

Using the Petri dish exposure method, the bacterial count ranged from 1 to 180 CFU/Petri dish-15 min (mean 48.49 CFU/Petri dish-15 min). In contrast when using the vacuum technique with the device, it ranged from 8 to 385 CFU/m^3 (mean 112.77 CFU/m^3). Figure 2 presents the average bacterial and fungal counts in different meat plants. Statistically, the highest results were observed for TMAB-PCA air sampler in MP1 meat plant, of 63.75 CFU/Petri-15 min, and for TMAB-PCA sedimentation in MP3 meat plant, of 133.0 CFU/Petri-15 min ($p < 0.05$). There were no significant differences ($p > 0.05$) in the average results for the Mould-DRBC sedimentation method between the MP1 and MP2 plants. The moulds and yeasts ranged from 1 - 105 CFU/Petri (mean 19.845 CFU/Petri) using the first technique, and 2 - 320 CFU/m^3 (mean 57.45 CFU/m^3) using the air sampling device. Overall, the numerical values detected by the device were higher, considering the general average values for each plant.

According to the study by Çöl and Aksu (2007) on factors affecting the microbial load of ambient air in food processing facilities, it was reported that raw products such as fresh fruits, vegetables, and meat contained high levels of bacteria, which can be dispersed into the air through human activities and air

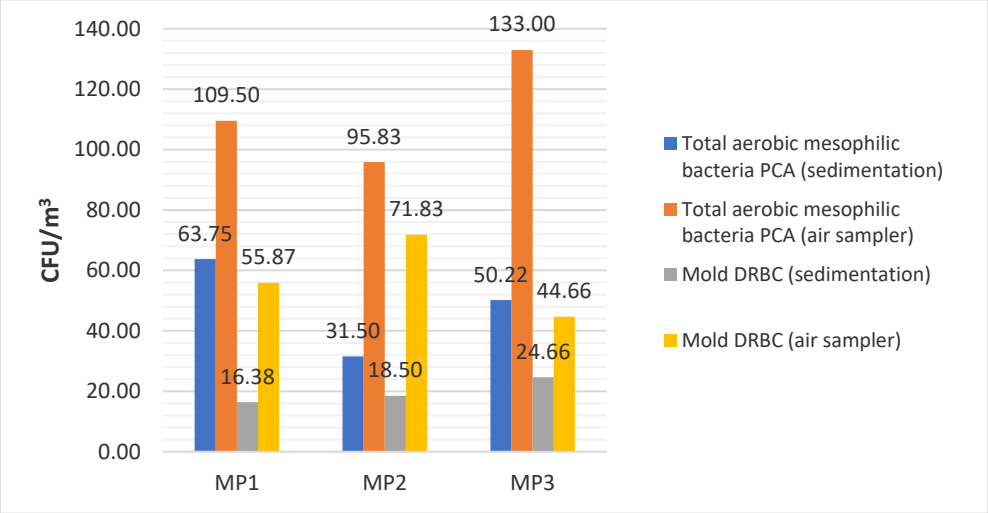

Figure 2. Average number of bacteria and fungi (CFU/m^3) from different meat plants.

movements in the environment. Therefore, when evaluating the results, differences between facilities could occur. These variations would depend on the contamination of the ambient air, the number of microorganisms present in the air outside the plant, the environment, the season, urbanisation, and weather conditions.

In the present work, the fungal colonies (76) purified from PCA and DRBC on Petri dishes from 23 areas were examined, both macroscopically and microscopically. The most common species were identified as *Mucor* spp. (*n* = 28, 36.84%), *Rhizopus* spp. (*n* = 22, 28.94%), and *Penicillium* spp. (*n* = 18, 23.68%). *Mucor racemosus* (*n* = 20), and *Rhizopus oryzae* (*n* = 14) were the most prevalent species. Additionally, *Aspergillus* spp. (*n* = 3, 3.94%), *Geotrichum* spp. (*n* = 3, 3.94%), *Syncephalostrum* sp. (*n* = 1, 1.31%), and *Wallemia* sp. (*n* = 1, 1.31%) were also identified.

There are various studies evaluating air quality in meat processing facilities. In a study by Kandal (2022), air samples were collected using an air sampler from various points, inside and outside a meat plant, during the autumn and summer seasons. Bacterial counts for the summer season ranged from 30 - 1,240 CFU/m^3, and fungal counts ranged from 0 - 20 CFU/m^3. During autumn, bacterial counts ranged from 40 - 80 CFU/m^3, and fungal counts ranged from 70 - 190 CFU/m^3. In spring, bacterial counts ranged from 40 - 220 CFU/m^3, and fungal counts ranged from 50 - 200 CFU/m^3. The same study indicated that the highest microbial load was found in slaughterhouse and sausage processing areas. In the present work, the higher counts in the sausage and meatball preparation units and carcass cutting area compared to the other areas agreed with this study (Figure 3). It is particularly thought that the particles from raw materials, additives, and spices used in powder form may increase the amount of bioaerosols in the air. Additionally, it can be considered that the personnel may also play a role in the transportation of aerosols.

In the present work, the fungal colonies identified through macroscopic and microscopic examination as *Mucor* spp., *Rhizopus* spp., *Penicillium* spp., *Aspergillus* spp., *Geotrichum* spp., *Syncephalostrum* spp., and *Wallemia* spp. are classified as indoor fungi (Çöl and Aksu, 2007). Various studies emphasise that *Penicillium, Aspergillus, Mucor,* and *Cladosporium* species are frequently found in food processing environments (Sørensen *et al*., 2008; Asefa *et al*., 2009). In a study investigating psychrophilic bacteria (40 - 82.3 CFU/m^3) and fungi (23.3 - 54.6 CFU/m^3) in coolers where various foods (meat, vegetable, mixed, and dessert) are stored, 17 different fungal genera were isolated from air samples. The most dominant species identified was *Penicillium italicum*, constituting 12.6% of the total, while the genus *Penicillium* comprised 29%. *Mucor racemosus* and *Botrytis cinerea* were each found at a rate of 5.8% (Sandıkçı Altunatmaz *et al.*, 2012). Similarly, studies by Sørensen *et al*. (2008) and Asefa *et al*. (2009) reported that *Penicillium* spp. were the predominant fungal species in food systems. In the present work, *Mucor* spp. were predominant at 36.84%, with *Mucor racemosus* alone accounting for 31.58% (24 isolates). These species were found at higher rates, particularly

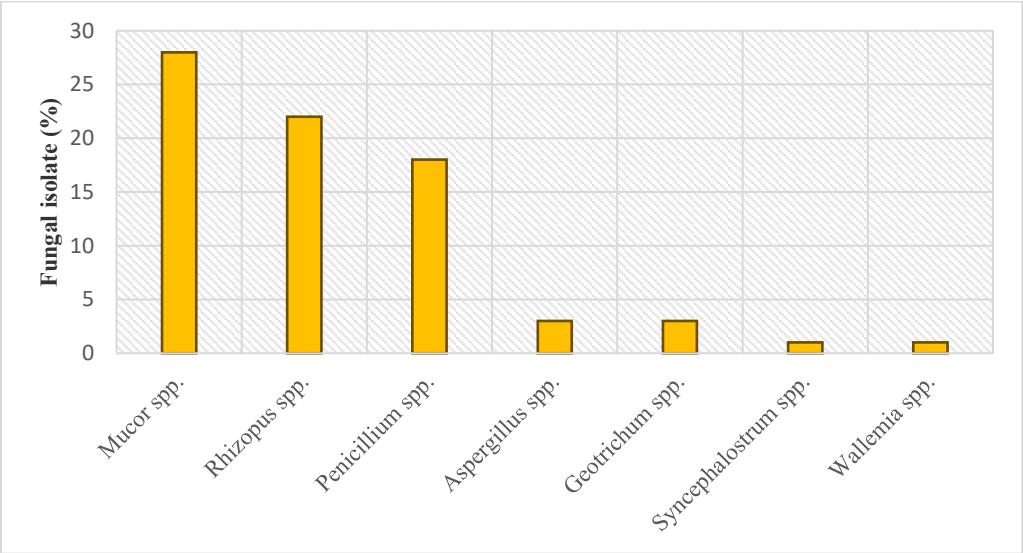

Figure 3. Frequency of fungal isolates from different meat plants (n = 76).

in production areas and cold storage units, than in other locations. Additionally, the fact that they were predominantly detected in all sampled regions of MP2 indicated a significant contamination situation, as evidenced by their detection in 19 out of 24 samples. Although MP2 had fewer samples, the greater diversity of fungi suggested the need for careful evaluation regarding aspects such as the ventilation system, personnel movement, particle density, and the careful use of raw materials. In the other two plants, MP1 had one *Mucor* sp. and one *Mucor racemosus,* while MP3 had three *Mucor* spp.; *Mucor racemosus* is a fungus that exhibits whisker-like spoilage of meat, and can slowly develop at low temperatures (Lund, 2000). Lowry and Gill (1984) reported that among five fungal species isolated from meat exhibiting mould spoilage other than black spots, *Mucor racemosus*, which was also frequently detected in the present work, was one of them, forming "whisker" colonies.

In the present work, *Rhizopus oryzae* was isolated in only one instance from the sausage filling unit in MP1, whereas it was detected eight times in MP2, and three times in MP3. Specifically, in MP2, it was found in the resting room (1), fermentation room (3), cold storage (2), meat cutting area (3), and doner production area (1). After *Mucor* spp., *Rhizopus oryzae* (18 isolates) was the second most frequently found isolate. The frequency of occurrence was observed to be notably higher in the production section. *Rhizopus oryzae* is classified as a GRAS (Generally Recognized as Safe) filamentous fungus widely used to produce certain traditional Eastern foods. It is primarily regarded as an excellent lactic acid producer. Presently, its potential for other biotechnological processes is also being explored (Nakashima *et al*., 1989; Londoño-Hernández *et al*., 2017). However, *Rhizopus oryzae* can cause mucormycosis and rhizopus rot (Dai *et al.,* 2024).

In the present work, *Aspergillus glaucus* was detected at a rate of 3.9%. *Aspergillus glaucus* is commonly found in nature, particularly in soil, plant material, air, and various food products. It is especially prevalent in food processing and moist, organic matter-rich environments. The dust, organic materials, temperature, and humidity created by the spices used in meat processing plants provide a suitable environment for this fungus. Among the *Penicillium* species (18 isolates), the dominant species was *Penicillium digitatum* (eight isolates), predominantly found in the packaging section. In a study by Mižáková *et al.* (2002), various moulds were detected in raw materials such as pork and beef, and in salami, indicating that spices can lead to fungal contamination. The study highlighted that fungi are most commonly found in ground black pepper, nutmeg, garlic powder, and crushed cumin. *Penicillium* sp., *Mucor* sp., and *Aspergillus* sp. detected in the present work suggested that these fungi can also be airborne in meat products.

In a study conducted by Parussolo *et al.* (2019) on raw materials and air in the production of fermented sausages in Brazil, *Aspergillus* and *Penicillium* spp. showed high levels of air contamination (> 10^4 CFU/m^3). These numbers were higher than those obtained in the present work. The

same study identified *Aspergillus westerdijkiae,* an ochratoxigenic species harmful to the meat industry, in both facilities. Additionally, environmental air in food processing areas was considered a critical point for product contamination and fungal spread. In the same study, *Cladosporium* sp. and xerophilic fungi were found in raw materials; xerophilic species of *Aspergillus* and *Wallemia* were found in spices, and in the air, the dominant species were *Aspergillus ruber* (in the first collection), *Aspergillus pseudoglaucus*, *Cladosporium*, *Penicillium glabrum*, *Penicillium brevicompactum*, *Penicillium chrysogenum*, *Aspergillus montevidensis*, and *Penicillium griseofulvum.* In the present work, the absence of such a diverse fungal flora in all three facilities could be attributed to the implementation of a disinfection program, a measure that the operators, as food safety professionals, can actively contribute to.

In a study by Vinayananda *et al.* (2018), air samples were collected using the impaction method from various locations in a meat plant, including the slaughterhouse, processing room, cooling room, and further processing room. The samples were evaluated for total microorganisms, psychrophilic count, yeast count, and mould counts. The study found that the slaughterhouse had significantly higher counts of aerobic microorganisms, followed by the processing room, further processing rooms, and cooling room. Yeast and mould counts were also significantly higher in the slaughterhouse. In another study conducted in Konya, air samples were taken from the cold storage rooms of six supermarkets, butcher shops, and slaughterhouses. The average bacterial counts were determined to be 158, 1821, 700, 1763, 770, and 636 CFU/m^3, respectively (Akan, 2009).

The first report of mould growth on meat stored in cold storage dates back to 1923. In a study that systematically examined fungi on cold-stored meats from the Southern Hemisphere to England, the identified fungal species included *Cladosporium herbarum* (causing black spot on meat), *Thamnidium chaetocladioides*, *Thamnidium elegans, Mucor racemosus, Mucor mucedo, Mucor lusitanicus, Penicillium expansum, Penicillium anomalum, Saccharomyces* spp.*, Sporotrichum carnis, Torula botryoides,* and *Wardomyces anomala* (Brooks and Hansford, 1923). The same study reported that *Mucor* spp. did not grow at -6°C, but could grow at 0°C or slightly above. Many of the detected fungi were commonly found in plant debris and animal extracts, indicating that the sources could be substrates occurring in and around slaughterhouses. Airborne spores settle on carcasses before and during storage, transforming into fungal growth under suitable conditions. Controlling temperature and humidity conditions in cold storage and avoiding unnecessary prolonged storage can prevent the growth of these fungi. In our study, *Mucor racemosus* was detected twice in -18°C storage. Its presence is considered to be momentary and coincidental. In a study evaluating the food safety of dry-aged meat, it was stated that *Penicillium* sp., *Aspergillus* sp., *Cladosporium* sp., *Chrysosportim* sp., *Thamnidium* sp., *Rhizopus* sp., *Mucor* sp. and *Aureobasidium* sp. moulds could be found at low temperatures (Savini *et al*., 2024).

In a study conducted to identify sources of microbial contamination during the stages of salami production, samples were taken and examined from three different facilities. According to the findings, the yeast and mould counts in air samples taken from the product preparation unit ranged between 1.30 and 2.44 log10 CFU/Petri (Önen, 2020).

In a study examining fungi in the air of three meat processing plants, 119 fungal isolates were obtained from the first plant, with *Aspergillus* spp. being the most dominant at 72.3% (*Aspergillus candidus*, 16.0%; *Aspergillus pseudoglaucus*, 14.3%; *Aspergillus versicolor*, *Aspergillus glaucus*, and *Aspergillus tonophilus* each at 13.4%), and *Penicillium species* at 14.3%. In the second meat processing plant, 148 isolates were obtained, with *Penicillium* spp. being dominant at 43.2% (*Penicillium solitum* and *Penicillium griseofulvum*, 35%), followed by *Aspergillus* species. Among other genera, *Aspergillus niveus* was the most common at 12.8%, followed by *Scopulariopsis* spp. (*Scopulariopsis candida and Scopulariopsis brumptii)* at 6.8%, and *Sporendonema* sp. (*Sporendonema casei*) at 4.1%. In the third plant, 64 fungal isolates were collected, with *Aspergillus* spp. being dominant at 59.4% (the most common species were *Aspergillus cristatus*, 17.2%; *Aspergillus ruber*, 15.7%), similar to the first plant. Other fungi included *Penicillium* at 23.5%, *Scopulariopsis brevicaulis* at 10.9%, and *Sporendonema casei* at 4.7%. The contamination levels detected in the same plant during the winter and summer were 4.15 and 4.26 log CFU/m^3 for plant 1; 5.15 and 5.11 log CFU/m^3 for plant 2; and 4.15 and 4.20 log CFU/m^3 for air/room. The study highlighted that fungal species detected in the air were also found in the products during the

maturation process, indicating normal contamination (Scaramuzza *et al.,* 2015).

In a study investigating the natural mycobiota in dry-cured hams in two production facilities, and specifically the incidence of mycotoxin-producing fungi, a total of 338 fungal colonies were isolated from three production stages: post-salting, maturation, and aging. The results indicated that fungi were more frequently isolated during the aging phase, with *Penicillium* being the dominant filamentous fungus genus. Out of 74 fungal strains, 59 were identified as *Penicillium* strains. Sixteen *Penicillium* species were identified, with *Penicillium commune* (24 strains) and *Penicillium chrysogenum* (13 strains) being the most common. Fungi producing ochratoxin A (OTA) included different *Penicillium* species such as *Penicillium chrysogenum*, *Penicillium commune*, *Penicillium polonicum*, and *Penicillium verrucosum* (Alapont *et al*., 2014). Additionally, the relative risk of OTA presence in dry-cured meat products was shown to be 75% (Sánchez-Montero *et al.,* 2019).

In the present work, *Penicillium digitatum*, which was most frequently found in the packaging unit, is known as blue-green mould. It has been reported to cause the most economically significant post-harvest diseases of citrus fruits in all production areas with low summer rainfall, and can occasionally be detected in meat (Holmes and Eckert, 1999; Pitt and Hocking, 2009).

In a study examining the fungal diversity in commonly used spices, *Aspergillus* was identified as the most common contaminant. *Rhizopus* was the second most common with 25 isolates, followed by *Penicillium* with 11 isolates. Other fungi contaminating spices included *Rhizopus oryzae*, *Mucor racemosus*, and *Syncephalastrum racemosum*, along with genera such as *Mucor*, *Fusarium*, *Alternaria*, *Absidia*, *Eurotium*, *Cochliobolus*, and *Scopulariopsis* (Uygun, 2002). In the present work, the detection of specific fungal isolates (*Rhizopus oryzae*, *Mucor racemosus*, and *Syncephalastrum racemosum*) commonly found in spices suggested that the handling, addition, or various processing stages of spices could lead to airborne contamination *via* particles.

In meat processing plants, the contamination of meat products can lead to significant economic and health problems. One of the most overlooked routes, through which pathogenic and spoilage microorganisms can contaminate products, is airborne. Air, as a potential source of contamination, should be considered a critical control point in the facility for both pathogenic and spoilage organisms (Kang and Frank, 1989; Franco *et al.*, 1995). It has been reported that a microbicidal air purification system can be beneficial in controlling airborne contamination in meat processing plants, and significantly reducing the risk of microbial contamination of meat products in a small meat processing plant (Cundith *et al*., 2002).

In a study investigating fungal contaminations in a beef slaughterhouse and on beef carcasses, a total of 34 fungal genera, represented by 62 species and one variety, were collected from beef carcasses (23 genera, 44 species, one variety) and their environment (25 genera, 49 species, one variety) (Ismail *et al*., 1995). It was emphasised that *Aspergillus* was the only genus frequently isolated from the air, water, floors, walls, and beef carcasses. Thirteen species of *Aspergillus* were identified, with *Aspergillus flavus* and *Aspergillus niger* found at moderate levels on beef carcasses, and at high levels in their environment. Additionally, *Aspergillus alutaceus*, *Aspergillus fumigatus*, *Aspergillus sydowii*, *Aspergillus terreus*, and *Aspergillus versicolor* were consistently isolated from the environment, but very rarely from beef carcasses (Ismail *et al*., 1995). *Cladosporium* (two species) and *Penicillium* (eight species) were also found at high levels in the environment, and at low levels on beef carcasses (Burfoot *et al*., 2003). Similarly, a study on the air of three quail farms identified 13 fungal species, with *Aspergillus* being the most dominant. *Aspergillus niger* was the highest at 22.7%, while *Mucor racemosus* was the lowest at 1.5% (Nwadiaro *et al*., 2015). This study supported the importance of airborne contamination, as demonstrated in our study, by showing that the environment and related factors are significant sources of fungal contamination for beef carcasses (Ismail *et al*., 1995). It was also noted that the slaughterhouse environment, carcass cutting, and related practices had aerosol formation and microbial dispersal effects (Burfoot *et al.,* 2003). However, a study determining the relationship between microbial loads in the air and on carcasses, in two beef slaughterhouses, stated that the correlation between air and carcass contamination for each bacterial group on the slaughter line was weak. The data highlighted the difficulty of definitively assessing the relationship between air and carcass contamination levels (Prendergast *et al.*, 2004).

The hygiene procedures implemented by each plant, the raw materials used (such as spices), the various methods of using these materials (mixing, bulk storage, and aerosols generated during ventilation), and the attitudes and behaviours of personnel can all contribute to differences in the fungal flora present in the plant's air. These factors may explain the presence of various fungal species in the air of meat processing plants, as reported in different studies.

The present work suggested that ambient air could be a route through which pathogenic and spoilage microorganisms can contaminate products. As a potential source of contamination that should not be overlooked, the ambient air in plants should always be included in hygiene procedures within the framework of good hygiene practices.

Conclusion

In the present work, two different techniques were used: the Petri dish exposure method (sedimentation) and the impaction method (using the Mas-100 Eco Air Sampler device). Since the air within the area could be examined thoroughly using the impaction (vacuum) method, both the number and diversity of microorganisms detected were high. The most common fungal species were *Mucor* spp., *Rhizopus* spp., and *Penicillium* spp., while *Mucor racemosus* and *Rhizopus oryzae* were the most prevalent species. Additionally, *Aspergillus* spp., *Geotrichum* spp., and *Syncephalostrum* spp. were identified. The present work also demonstrated that fungi, which might be pathogens and spoilers, could be present in the ambient air of meat plants. Airborne contaminations should not be taken lightly since they can cause health and economic losses. Therefore, it is recommended to develop quality standards to mitigate airborne contamination at meat plants.

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