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Review

# Occurrence of Moulds and Yeasts in the Slaughterhouse: The Underestimated Role of Fungi in Meat Safety and Occupational Health

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**Abstract:** Despite their potential impact on meat safety and occupational health, fungi are often underestimated contaminants in slaughterhouses. Moulds and yeasts may be associated with meat contamination in multiple processing stages, and mycotoxigenic species, such as *Aspergillus*, *Fusarium*, and *Penicillium*, pose food safety concerns. Bioaerosols may carry infectious fungi at the slaughterhouse that are capable of causing respiratory conditions and allergies. Chronic exposure to mycotoxins can have hepatotoxic, nephrotoxic, and carcinogenic effects in humans. While bacterial meat contamination has been extensively studied, fungal contamination remains overlooked due to insufficient research, awareness, and standardised surveillance protocols. This review compiles published data on fungal occurrence in slaughterhouses from the past twenty-five years. It aims to highlight the primary mould and yeast isolated species, providing a context on their role in meat safety and occupational health. The findings emphasise the need for improved risk assessment and fungal monitoring in meat plants. Standardised fungal detection and control protocols are also suggested to be implemented to enhance meat safety and workplace conditions.

**Keywords:** mould; yeast; slaughterhouse; meat; occupational; food safety; public health

## 1. Introduction

Fungi are ubiquitous microorganisms that can endure as filamentous multicellular forms with hyphae or as unicellular yeasts [1–3]. Moulds are filamentous fungi with hyphae connected to several spore-forming structures, such as conidia [2]. In contrast to moulds, yeasts are single-celled microorganisms that do not produce secondary toxic metabolites [4]. Although some species may divide by fission, yeasts reproduce primarily by budding [4].

The high-humidity environment of slaughterhouses is prone to mould growth. Fungal growth and spore production are influenced by water activity and the availability of nutrients such as proteins, carbohydrates, and lipids [2,5].

Several mould species are known to produce mycotoxins and secondary toxic metabolites with teratogenic, mutagenic, and carcinogenic potential due to their ability to interfere with RNA synthesis and to cause DNA damage [2,6,7]. *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. are prominent producers of these adverse mycotoxins [6].

Although mycotoxins are more extensively described in cereals and other plant products, they are also found in meat and meat products [5]. In food, the most frequently detected mycotoxins consist of aflatoxins, chiefly produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus*

*nomius*; ochratoxin A, mainly produced by *Aspergillus ochraceus*, *Aspergillus niger*, *Aspergillus carbonarius*, and *Penicillium verrucosum*; zearalenone, primarily associated with *Fusarium graminearum*; fumonisins, predominantly originated by *Fusarium verticillioides*, *Fusarium proliferatum*, and *Aspergillus niger*; and deoxynivalenol, generally produced by *Fusarium graminearum* and *Fusarium culmorum* [8].

Meat can be contaminated with mould during the animal production phase and at the slaughterhouse due to improper handling, processing, and equipment contamination. This poses a potential public health risk for consumers. Therefore, monitoring the fungal load during slaughter is essential to ensure meat safety [9].

From an occupational health perspective, chronic exposure or inhalation of mould and its metabolites is reportedly linked to asthma, dermatitis, allergies, respiratory infections and other infectious diseases in humans [10]. Although fungal infections can affect healthy individuals, immunocompromised individuals are at a higher risk [3].

Besides moulds, yeasts are also isolated in slaughterhouse lines and equipment and may contribute to opportunistic infections [11,12].

Preventing fungal dissemination in slaughterhouses is crucial to minimising the adverse effects and mitigating risks for slaughterhouse workers and meat consumers [13].

Nevertheless, microbial monitoring in slaughterhouses typically prioritises bacterial contamination, and due to the lack of research and specific monitoring protocols and guidelines for fungi, fungal contamination still goes unnoticed. This underestimation may lead to unaddressed health risks. Considering this, the present review aims to provide an overview of the occurrence of moulds and yeasts in slaughterhouses based on the published data from the past twenty-five years and discuss their potential impact on meat safety and occupational health, emphasising the urgent need for more research and more fungal monitoring in slaughterhouses.

## 2. Impact of Fungi on Meat Safety and Occupational Health

Globally, more than 300 million people suffer from severe fungal disease, which can result in over 3.8 million deaths per year [14,15].

The routes of fungal infections through exposure to spores may include inhalation, ingestion of contaminated food, and skin contact [3]. As moulds can produce mycotoxins, fungal-related health risks expand beyond direct infections.

The production of mycotoxins is influenced by intrinsic factors (e.g., species and strain) and external conditions, such as humidity, temperature, pH, gas composition, and the nature of the growth substrate [16].

Depending on the dose and exposure duration, the toxic effects of mycotoxins can be acute or chronic, often influencing the protein, fat, and carbohydrate metabolism, and consequently the nucleic acid synthesis, and potentially causing kidney and liver damage or even cancer [17].

Although the primary route of mycotoxin exposure is the oral ingestion of contaminated food [18], which may pose a significant food safety risk, exposure can also occur through inhalation and dermal contact, representing an occupational hazard and which effect may be more harmful than oral exposure [18].

### 2.1. Fungi as Meat-Borne Pathogens

Meat-borne pathogens consist of more than just bacteria, viruses, and parasites. Fungi can also be present in meat and meat product contaminants, releasing mycotoxins into the contaminated products, causing potentially serious implications on meat safety and public health [19,20].

Fungal spoilage of meat products is typically characterised by the presence of black, white, or blue-green colonies on the surface [19].

The occurrence of moulds in meat, a significant source of food spoilage, is considered an indicator of the level of hygiene during processing activities [21,22]. *Cladosporium* spp. have been linked to black spot spoilage in dry-cured meats; *Chrysosporium pannorum* is associated with the

formation of white spots on frozen meat, and *Penicillium expansum* may originate blue-green spots [19]. Yeasts usually cause gas formation and an unpleasant odour [19].

Commonly isolated fungal genera in red meat include *Cladosporium*, *Geotrichum*, *Mucor*, *Rhizopus*, *Sporotrichum*, *Thamnidium*, *Candida*, and *Torulopsis*. In contrast, in poultry meat, *Candida*, *Debaryomyces*, *Rhodotorula*, and *Yarrowia* are more frequently described [19].

The primary sources of carcass contamination include air, water, walls, floors, workers, working surfaces, and equipment [19,23,24]. The abattoir's design and layout can also influence air currents, contributing to airborne contamination of carcasses and contact surfaces [24]. However, mould in meat can also result from animals being fed contaminated feed [25].

## 2.2. Fungi as Occupational Hazards

Slaughterhouse workers are exposed to several zoonoses, such as leptospirosis, brucellosis, Q fever, tuberculosis, avian influenza, and Crimean Congo haemorrhagic fever [26]. Occupational exposure to fungal burden has been also assessed and confirmed [13].

The biological risk in slaughterhouses is from direct and indirect contact with animal matter and exposure to bioaerosols [13]. Bioaerosols involve airborne bacteria, viruses, fungi, and their by-products, including mycotoxins [13]. Factors such as season, building materials, age of the facility, and ventilation conditions influence fungal concentrations and diversity. Studies on fungal bioaerosols have identified *Aspergillus*, *Penicillium*, *Stachybotrys*, *Cladosporium*, *Alternaria*, *Trichoderma*, and various yeasts as the most common indoor and outdoor fungi, with potential implications for severe health issues [2,27].

Indoor air quality is crucial for health and well-being, as people inhale approximately 10m<sup>3</sup> of air daily, which can contain bioaerosols originating from individuals, organic dust, stored products, and air circulation through natural or artificial ventilation systems [2]. Moreover, slaughtering and processing facilities ventilation systems serve as additional reservoirs for aerosolising and spreading airborne microorganisms [13]. Among different types of slaughterhouses, poultry facilities previously exhibited the highest fungal load compared to cattle and mixed swine-cattle slaughterhouses, which can be attributed to greater indoor fungal contamination sources [13].

Although European regulations mandate the assessment of biological risks in occupational settings, mycotoxins are not widely recognised as a risk factor. This may lead to an underestimation of exposure, as mycotoxins can persist in the environment even after removing fungi [28]. Occupational exposure to aflatoxin B1 (AFB1), a potent hepatocarcinogen, was already described among poultry slaughterhouses [14].

Occupational exposure to mycotoxins is mainly linked to working in poorly ventilated environments and improperly using protective equipment and clothing [8]. As already mentioned, the main airborne mycotoxins exposure routes include inhalation and dermal contact, as mycotoxins can be present in airborne particles, as well as in dust that either carries these toxins or has been directly contaminated by fungal excretions [18].

Inhalation of mycotoxins can lead to various adverse health effects, including mucous membrane irritation, epithelial damage, endocrine disruption, systemic symptoms such as fever, nausea, fatigue, and immunosuppression and immunotoxicity. Furthermore, exposure has been associated with nephrotoxicity, acute and chronic liver damage, central nervous system impairment, reproductive effects, and carcinogenic potential [16].

Yeasts are also opportunistic hazards. The most frequently occurring yeast species associated with human disease include *Candida albicans*, *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, and *Cryptococcus neoformans* [29].

## 3. Occurrence of Moulds in the Slaughterhouse

The formation and spread of mould is unavoidable in the slaughterhouse environment due to the considerable amounts of water used, the prone conditions to condensation, and the presence of rests of food adhering to surfaces [5,30]. *Stachybotrys chartarum*, a commonly found indoor mould, is

identifiable by its black colour. *Cladosporium* typically forms olive green to brown or black colouration, while *Penicillium* is often recognised by its green colonies. *Aspergillus*, another frequent indoor mould, can develop shades of red or gold [31].

The spread of moulds in the slaughterhouse is common, and identifying specific species plays a crucial role in understanding the potential risks to public health and food safety.

### 3.1. *Aspergillus* spp.

*Aspergillus* spp. are commonly found in soil, decaying vegetation, seeds and grains [14,32].

Diverse *Aspergillus* species occur in various indoor environments, with some species considered opportunistic pathogens [14]. *Aspergillus* spp., the leading cause of human mould infections, causes various serious health issues in both immunocompetent and immunocompromised patients.

Both humans and animals can be affected by aspergillosis, a fungal infection caused by *Aspergillus* species. The symptoms may range from respiratory illnesses and allergic reactions to invasive disease, the most severe form, leading to serious complications affecting the lungs, brain, and kidneys [33].

*Aspergillus fumigatus* is the most clinically significant, followed by *A. flavus*, *A. terreus*, and *A. niger* [34,35]. Due to their small size, Conidia from the *Aspergillus* genus can be easily inhaled by exposed individuals, allowing the colonisation of the upper and lower respiratory tract [14].

Aflatoxins, highly toxic, teratogenic, mutagenic, and carcinogenic compounds, are secondary metabolites of *Aspergillus* species, mainly by *Aspergillus flavus* and *Aspergillus parasiticus* and are classified as Group 1 carcinogens by the International Agency for Research on Cancer (IARC) [7,16]. The main categories include Aflatoxins B<sub>1</sub> and B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, and M<sub>1</sub> [7].

In slaughterhouses, non-specific strains of *Aspergillus* spp. were previously detected in 10% and 2% of air samples' isolates collected in the hanging ("moving rails") and evisceration ("gall bladder separation") areas, respectively, of an Austrian poultry slaughterhouse [36]. Similarly, *Aspergillus* spp. was detected in 18% (n = 98 isolates) of isolates detected in air samples from both cattle and poultry slaughterhouses in a Pakistani study [37]. In a Korean study across five swine slaughterhouses, *Aspergillus* spp. accounted for 6% of the detected fungal genera [5]. Additionally, a study conducted in a Saudi Arabian slaughterhouse identified *Aspergillus* spp. in air samples and equipment, including a saw and a blade, as well as in a laboratory environment [38]. These species were also isolated in an Indian slaughterhouse [39].

#### 3.1.1. *Aspergillus flavus*

*Aspergillus flavus* produces the Aflatoxins B<sub>1</sub> and B<sub>2</sub> [7]. Previous studies have documented occupational exposure to AFB<sub>1</sub>, a potent hepatocarcinogen, among poultry slaughterhouse and poultry farm workers [14]. Moreover, the contamination of chicken meat with *Aspergillus* species, especially *Aspergillus flavus*, is one of the most hazardous microbial contaminants [22]. In Austria, *Aspergillus flavus* was detected in less than 1% of air samples collected in the hanging area of a poultry slaughterhouse [36].

The isolation of *Aspergillus flavus* from different locations within the abattoir and

beef may lead to mycotoxin production in beef meat [24]. In a Serbian study in two beef slaughterhouses, the fungus was recovered from 17% of isolates found in air samples and 18% in floor samples [23]. More recently, in a Nigerian ruminant slaughterhouse, *Aspergillus flavus* accounted for 17.6% of the airborne fungal isolates (n = 24 isolates recovered from the skin/hoof burning site, n = 18 isolates from the slaughter ground, n = 13 isolates from the lairage site, and n = 8 isolates) from the meat stall [24].

Similarly, in an Iraqi slaughterhouse, *Aspergillus flavus* was isolated from 17.4% (n = 4 isolates) of indoor air samples' isolates and 12.3% (n = 7 isolates) of isolates in outdoor air samples [40]. At a Nigerian slaughterhouse, the same species was found in 40% of the fungal isolates on slab surfaces (n = 10 isolates) [41]. Additionally, it is highlighted that studies focusing on the role of flies (*Musca domestica*) as vectors revealed that fifteen isolates of *Aspergillus flavus* were isolated from flies (*Musca*

*domestica*) collected from slaughterhouses in Saudi Arabia [42], fifteen isolates in Iraq [43], and fifteen isolates from an Irani slaughterhouse [44].

### 3.1.2. *Aspergillus fumigatus*

*Aspergillus* section *Fumigati* produces several immunosuppressive mycotoxins, including gliotoxin, fumagillin, helvolic acid (fumigacin), fumitremorgin A, and Asp-hemolysin, and may be the causative agent of respiratory symptoms, such as asthma, allergic sinusitis, cough, and bronchial hyperresponsiveness, [14].

*Aspergillus fumigatus* is the primary etiologic agent of invasive aspergillosis, a disease with high mortality rates among immunocompromised individuals [14,35].

Regarding poultry slaughterhouses, *Aspergillus fumigatus* was previously isolated from 3.1% (n = 50 CFU/m<sup>3</sup>) of isolates recovered from air samples collected in a Portuguese study [13]; from 8% and 2% of isolates detected in air samples collected from the evisceration and hanging areas, respectively, in an Austrian slaughterhouse [36]; and in samples collected from air and inhalable dust in the turkeys' evisceration site of an Italian slaughterhouse [45]. Moreover, nine isolates of *Aspergillus fumigatus* were also recovered from flies (*Musca domestica*) collected in an Iraqi slaughterhouse [43].

### 3.1.3. *Aspergillus ochraceus*

*Aspergillus ochraceus* was previously isolated from 5.5% (n = 8 isolates) of air samples isolates collected in a large animal slaughterhouse in Portugal [13]. Six isolates of this species were also identified in flies from an Iraqi slaughterhouse [43]. *Aspergillus ochraceus* is recognised as an essential food pathogen, widely distributed and responsible for the production of ochratoxin A, classified as a Group 2B carcinogen by the IARC, meaning it is "possibly carcinogenic to humans" [8,16,46]. In addition, this species can produce penicillic acid, dihydropenicillic acid, and viomellein [46]. Its toxicological significance is further accentuated by its association with the Balkan endemic nephropathy, a disorder linked to consuming food contaminated with penicillic acid and ochratoxin A [46].

### 3.1.4. *Aspergillus parasiticus*

*Aspergillus parasiticus* is responsible for the production of aflatoxin B1, aflatoxin B2, aflatoxin G1, and aflatoxin G2, classified as Group 1 carcinogens by the IARC, meaning they are carcinogenic to humans [8,16,47].

Four isolates of *Aspergillus parasiticus* were identified in samples obtained from flies (*Musca domestica*) in an Iraqi slaughterhouse [43].

### 3.1.5. *Aspergillus niger*

*Aspergillus niger* is ubiquitous and known to be the causative pathogen of the so-called "black mould disease" [48]. Although it is reported the production of ochratoxin A and fumonisins (e.g., fumonisin B2) - classified as Group 2B carcinogens (possibly carcinogenic to humans), *Aspergillus niger*, in comparison with other filamentous fungi, is relatively harmless [8,16,49]. Still, *Aspergillus niger* is considered an opportunistic pathogen and can induce lung and ear infections, particularly in immunocompromised individuals [49].

*Aspergillus niger* was previously isolated from air samples collected in air handling units, as well as in the turkey cutting and evisceration areas of an Italian poultry slaughterhouse [45]. Additionally, it was identified in 35.1% (n = 20 isolates) of air samples' isolates from the surrounding outdoor environment of an Iraqi slaughterhouse [40] and in 24% (n = 6 isolates) of isolates in slab surface samples from a Nigerian slaughterhouse [41]. In another Nigerian study, in a cattle and small ruminants slaughterhouse, *Aspergillus niger* accounted 15.1% of the total fungal collected species (n = 15 isolates from the slaughter ground, n = 12 isolates from the meat stall, n = 9 isolates from the lairage site samples, and n = 18 isolates from the skin/hoof burning site's samples) [24]. In flies (*Musca*

*domestica*) collected from slaughterhouses, twenty-one isolates of *Aspergillus niger* were detected in an Iraqi study [43], ten isolates in a Saudi Arabian slaughterhouse [42], and ten isolates in Iran [44].

### 3.1.6. *Aspergillus terreus*

*Aspergillus terreus* is an increasingly perceived opportunistic fungus with a worldwide distribution, capable of producing many secondary metabolites and mycotoxins [50]. Moreover, it is regarded as one of the most prevalent airborne spores among *Aspergillus* species, and it is reported to cause allergenic bronchopulmonary aspergillosis [51]

*Aspergillus terreus* accounted for 30% of the isolates recovered from floor samples in a large Portuguese animal slaughterhouse [13]. Similarly, a study on two Serbian slaughterhouses detected them in 32% of wall samples, 8% of floor samples, and 2% of air samples [23].

### 3.1.7. Other Isolated *Aspergillus* spp.

Other species of *Aspergillus* have also been isolated at slaughterhouses. *Aspergillus penicilloides* was isolated in an Italian poultry slaughterhouse from air samples collected in air handling units, turkeys' cutting and evisceration sites, and from inhalable dust collected from a turkeys' evisceration site [45]. *Aspergillus clavatus* was detected in 2% of isolates in floor samples collected from Serbian beef slaughterhouses [23]. *Aspergillus carneus* and *Aspergillus candidus* were already isolated from a poultry slaughterhouse in Italy [45].

### 3.1.8. *Eurotium* (Reclassified as *Aspergillus*) spp.

*Eurotium* spp., now renamed *Aspergillus* [52] and generally recognised as benign fungi [52], were also described in poultry slaughterhouses [36,45].

*Eurotium* spp. *Eurotium erbarum* and *Eurotium chevalieri* were reported in air samples collected in two Italian poultry slaughterhouses [45]. Moreover, it was also possible to isolate *Eurotium* spp. from air samples collected in the hanging area in an Austrian slaughterhouse, which represented 9% of the isolates obtained in the mentioned area [36].

## 3.2. *Fusarium* spp.

*Fusarium* species, highly adaptable fungi, are found in various habitats, such as air, water, soil, plants, and organic substrates [53,54]. Some species act as opportunistic pathogens, originating a wide range of infections with high morbidity and mortality [53]. In addition, they produce diverse mycotoxins, such as fumonisins, zearalenone, and trichothecenes, which can be harmful or even fatal to humans and animals [53,55].

*Fusarium* spp. were previously isolated from air handling units in an Italian poultry slaughterhouse [45], in the evisceration area (5%) and hanging area (less than 1%) of an Austrian poultry slaughterhouse [36]. Additionally, in each of the two studies analysing isolates from flies (*Musca domestica*) in slaughterhouses, five isolates of *Fusarium* spp. were detected in the samples [42,44].

*Fusarium oxysporum*, a human pathogen [56], was detected in 10.9% of the total isolated airborne fungal species of a Nigerian ruminant slaughterhouse (n = 12 isolates from the skin/hoof burning site, n = 11 isolates from the slaughter ground, n = 8 isolates from both the lairage site and n = 8 isolates from the meat stall's samples) [24]. *Fusarium oxysporum* can produce fumonisin, zearalenone, the neurotoxic fusaric acid and several cytotoxic mycotoxins such as beauvericin, diacetoxyscirpenol, enniatins, and moniliformin [56].

Additionally, an Iranian study analysing samples collected from the external body surface of flies (*Musca domestica*) in slaughterhouse and hospital environments identified *Fusarium proliferatum*, one of the main producing species of fumonisins [57], by 1% of the isolates (n = 5 isolates from the slaughterhouse) [44].

### 3.3. *Penicillium* spp.

*Penicillium* spp. is one of the most common filamentous fungi in the food processing industry [58]. It can also proliferate on building walls, substantially in environments with high humidity [58]. This genus plays a significant role in various fields, including food spoilage, biotechnology, plant biology, and medicine [59].

*Penicillium* spp. were already isolated in poultry, cattle, and swine slaughterhouses [5,13,23,24,36,40–45].

*Penicillium* spp. was widely isolated in two Italian poultry slaughterhouses in several processing areas [45]. In a Portuguese study, *Penicillium* spp. was isolated from 32.8% of the isolated species recovered from air samples collected in a poultry slaughterhouse (n = 524 isolates). A Pakistani study concluded that 29.3% of the isolates detected in air samples (n = 160 isolates) were *Penicillium* spp. [37]. In Austria, 11% of the fungal species isolated from the hanging area and 10% of those isolated in the evisceration site of a poultry slaughterhouse were classified as belonging to *Penicillium* spp. [36].

In a ruminant slaughterhouse, 14.5% of the isolates belonged to *Penicillium* spp., being more isolated from the lairage site samples (n = 21 isolates) than from other sites (n = 14 isolates from skin/hof burning site samples, n = 12 isolates found in slaughter ground, n = 5 isolates detected in meat stall) [24].

A Portuguese study detected 118 CFU/m<sup>3</sup> (80.8% of the isolates) and 270 CFU/m<sup>3</sup> (33.3% of the isolates) of airborne *Penicillium* spp. in a large animal slaughterhouse and a mixed beef-swine slaughterhouse, respectively [13]. *Penicillium* spp. also represented 15% of the detected genera in studied Korean swine slaughterhouses [5].

In Nigeria, seven isolates (28% of the fungal isolated species) found in slab surfaces were *Penicillium* spp. [41]. The same genus was isolated from samples collected from blades in a Saudi Arabian slaughterhouse [38]. [40] isolated six *Penicillium* spp. isolates from inside air samples (26.1% of the indoor fungi isolates) and nine from outdoor samples (15.8% of the outdoor fungi isolates) were isolated.

Furthermore, slaughterhouse flies (*Musca domestica*) were vectors of four *Penicillium* isolates in a study in Saudi Arabia [42].

*Penicillium verrucosum* produces ochratoxin A, a Group 2B carcinogen, which is also nephrotoxic, hepatotoxic, and immunotoxic, with the potential to cause chromosomal aberrations in human lymphocytes [58,60]. Seven isolates of *Penicillium verrucosum* were previously isolated from flies (*Musca domestica*) collected from a slaughterhouse in Iraq [43].

*Penicillium notatum* (reclassified as *Penicillium rubens*) [61] accounted for 26.1% and 26.3% of the isolates in indoor and outdoor samples, respectively (n = 6 isolates and n = 15 isolates, respectively) [40].

In a study on flies (*Musca domestica*) found in slaughterhouse environments, eighteen isolates of *Penicillium aurantiogriseum* were identified [43].

In a Serbian study conducted in two beef slaughterhouses, *Penicillium brevicompactum* represented 19% of isolates in air samples, 8% in floor samples, and 2% in wall samples [23]. In the same facilities, *Penicillium chrysogenum* (reclassified as *Penicillium rubens*) [61] accounted for 5% of the isolates from air samples, 7% from floor samples, and 3% from walls [23]. *Penicillium solitum* was also isolated, comprising 34% of the air sample isolates and 28% of the floor sample isolates [23].

Moreover, *Penicillium polonicum* and *Penicillium expansum* were also identified in a study conducted in two Italian slaughterhouses [45].

### 3.4. *Mucor* spp.

*Mucor* is one of the largest genera within the order Mucorales [62,63]. Species of this genus are predominantly saprotrophic and found in several environments [62]. *Mucor* spp., reportedly able to produce mycotoxins [64], can cause mucormycosis, a spectrum of opportunistic human infections, varying from chronic cutaneous to rhinocerebral forms [65].

In a recent Nigerian study, *Mucor* spp. represented 5.3% of the isolated fungal airborne species in a ruminant slaughterhouse, being isolated from skin/hoof burning site (n = 3 isolates), from lairage site' samples (n = 7 isolates), from samples of meat stall (n = 2 isolates), and from slaughter ground (n = 7 isolates) [24]. Similarly, *Mucor* spp. was isolated from 13.2% (n = 72 isolates) of air samples' fungal isolates collected in a Pakistani study [37]; and, in an Iraqi slaughterhouse, from 30.4% and 10.5% of the isolates collected from indoor and outdoor samples, respectively (n = 7 isolates and n = 6 isolates, respectively) [40]. Additionally, *Mucor* spp. was the only genus collected from samples collected from the floor of a Portuguese poultry slaughterhouse [13].

Specific species of *Mucor* isolated from slaughterhouses included *Mucor racemosus*, which can infect humans [66], found in 9% of the isolates recovered from floor samples and 6% of air samples collected in a study carried out in two beef slaughterhouses [23]; *Mucor plumbeus*, detected in air handling units' samples in a study carried out in Italy; and *Mucor circinelloides*, described to be involved in infections [65], represented by nine isolates collected from slaughterhouse's flies [43].

### 3.5. *Rhizopus* spp.

*Rhizopus* spp. are also opportunistic fungi responsible for mucormycosis, a rare but life-threatening infection that essentially affects immunocompromised individuals [67–69]. Mucormycosis is an emerging opportunistic fungal disease that involves rhino-orbital-cerebral, pulmonary, cutaneous, gastrointestinal, renal, and disseminated forms [69].

*Rhizopus* spp. were previously described to be isolated by 5.9% from a ruminant slaughterhouse (n = 9 isolates in samples from skin/hoof burning site, n = 7 isolates in samples from lairage sites, n = 3 isolates in meat stall's samples, n = 2 isolates in samples from the slaughter ground) [24].

*Rhizopus stolonifer*, a saprophytic fungus that thrives across various temperature and humidity conditions, with an optimal growth temperature of 25°C [70], was identified in 5.1% of the isolates (n = 8) recovered from flies in an Iraqi study [43].

### 3.6. *Cladosporium* spp.

Black moulds of *Cladosporium*, a ubiquitous genus, are among the most common fungi in indoor and outdoor environments [71]. Some species have clinical significance and can cause allergies [72].

Regarding its presence in poultry slaughterhouses, *Cladosporium* spp. was identified in an Italian study, specifically in inhalable dust and air samples collected from turkeys' cutting and evisceration sites, as well as in air handling unit samples [45]. Likewise, *Cladosporium* spp. was isolated by 26% and 43% from hanging and eviscerating areas, respectively [36]. In a cattle and small ruminant slaughterhouse, this fungal genus was recovered from the skin/hoof burning site (n = 5 isolates), lairage site (n = 3 isolates), meat stall (n = 10 isolates), and slaughter ground (n = 4 isolates), summing up 6.1% of the isolates [24]. Additionally, a Portuguese study concluded that 5.5% of the isolates recovered from a cattle slaughterhouse's air samples and 45.7% of the isolates in air samples from a mixed swine-cattle slaughterhouse were also *Cladosporium* spp. (n = 8 isolates and n = 370 isolates, respectively) [13]. In swine Korean slaughterhouses, *Cladosporium* spp. represented 20% of the detectable genera [5]. A Pakistani study reported that this genus comprised 14.3% of isolates from air samples (n = 78 isolates) [37]. *Cladosporium* spp. was also detected in a Saudi Arabian slaughterhouse [38].

Over the past twenty-five years, a small range of studies has reported the isolation of *Cladosporium herbarum*, namely from various air and inhalable dust samples [23,45]. In Serbian cattle slaughterhouses, *Cladosporium herbarum* was isolated by 25% in walls samples, 4% in floor samples, and 2% in air samples [23]. Other species of *Cladosporium* were also found in the mentioned slaughterhouses [23]. *Cladosporium sphaerospermum* and *Cladosporium cladosporioides* were also present in walls, floor, and air samples [23].

### 3.7. *Alternaria* spp.

Black moulds of the genus *Alternaria* commonly infest damp buildings [73]. They can produce potentially harmful food contaminants, known as *Alternaria* toxins, which have been reported to act as mycotoxins [73,74]. *Alternaria* is also considered a potent sensitising aeroallergen, being strongly associated with respiratory disorders such as asthma, rhinosinusitis, pneumonitis, skin infections, and bronchopulmonary mycosis [75].

*Alternaria* spp. was previously isolated from an Austrian poultry slaughterhouse, representing 4% and 7% of the isolates obtained from hanging area and eviscerating area air samples, respectively [36]. In a Pakistani research work, *Alternaria* spp. accounted to 12.7% of air samples isolates (n = 69 isolates) [37]. Meanwhile, in swine slaughterhouses, *Alternaria* spp. was described to represent 1% of the detected genera [5]. In an Indian study, *Alternaria* spp. was one of the isolated fungal species [39].

Analysis of the vector role of flies (*Musca domestica*) in slaughterhouses has identified *Alternaria* spp. as one of the fungal species present in the flies' external body samples. A Saudi Arabian study identified two isolates [42], similar to the findings of an Irani study [44], and a third one found twelve isolates of *Alternaria alternata* in samples from flies collected in an Iraqi slaughterhouse [43].

*Alternaria alternata* was also identified in a Serbian study from the floor (7% of the isolates) and air samples (2% of isolates) [23].

### 3.8. *Botrytis* spp.

*Botrytis* spp. are found worldwide but have a low prevalence in both indoor and outdoor ambient air, though they possess allergic potential [76]. Based on peer-reviewed studies published in the last twenty-five years, *Botrytis* spp. was only isolated in poultry slaughterhouses studied in Italy - where *Botrytis cinerea* was identified in inhalable dust collected in a turkeys' evisceration site and in air samples from air handling units and turkeys' cutting and evisceration sites [45] - and in Austria - where *Botrytis* spp. represented less than 1% of isolates detected in air samples from the hanging area [36].

### 3.9. *Geotrichum* spp.

*Geotrichum* spp. were isolated from poultry (less than 1% in eviscerating area air samples) [36] and ruminants slaughterhouses (4.5% of the isolates) [24]. In this last Nigerian ruminant slaughterhouse, nine isolates of *Geotrichum* spp. were obtained from slaughter ground samples, four isolates from the meat stall, and three isolates from skin/hoof burning site, totalising 4.5% of the isolates in this study [24].

Although *Geotrichum*-related diseases are rare, they may be linked to pulmonary and disseminated infections in immunocompromised individuals [77]. In an Italian study, *Geotrichum candidum*, an opportunistic pathogen linked with high mortality rates among cancer patients [77], was identified in air samples from two poultry slaughterhouses and inhalable dust from one of them [45].

### 3.11. *Scopulariopsis* spp.

*Scopulariopsis* spp. are moulds linked to clinical manifestations most often associated with pulmonary and disseminated infections [78]. *Scopulariopsis*, particularly *Scopulariopsis brevicaulis*, is recognized as a cause of invasive and non-invasive infections, including onychomycosis, keratitis, conjunctivitis, endocarditis, and disseminated infections in both animals and humans [79].

*Scopulariopsis* spp. were identified in less than 1% of the isolates recovered from air samples in the hanging area site in a poultry slaughterhouse in Austria [36]. In contrast, *Scopulariopsis* spp. were isolated in 59.5% of air samples collected from a Portuguese poultry slaughterhouse (n = 950 CFU/m<sup>3</sup>) [13]. Regarding cattle slaughterhouses, *Scopulariopsis brumpti* was detected in 40% of the isolates (n = 40,000 CFU/m<sup>2</sup>) collected from floor samples in a Portuguese study [13] and *Scopulariopsis brevicaulis* was similarly isolated from floor samples in a Serbian study, but representing 2% of the floor samples' isolates [23].

### 3.12. Other Moulds Isolated in Slaughterhouses

Other moulds with occupational hazard potential isolated in slaughterhouses include *Pseudogymnoascus* spp., which represented 2% of the isolates in five Korean swine slaughterhouses [5]; *Trichoderma* spp., obtained in less than 1% of air samples from the hanging area of a poultry slaughterhouse [36]; *Ulocladium chatarum*, which was isolated from air samples in air handling units and sites of evisceration and cutting of turkeys in an Italian slaughterhouse [45]; *Wallemia* spp., detected in 4% of the isolates of the poultry eviscerating area air samples [36]; and *Zygomycetes* spp., isolated from inhalable dust samples collected in another two poultry slaughterhouses [45].

Furthermore, *Acremonium* spp. were isolated in two studies conducted in poultry slaughterhouses [36,45]. *Aureobasidium* spp., were obtained in 11.1% of isolates in air samples from a mixed swine-cattle slaughterhouse in Portugal ( $n = 90$  CFU/m<sup>3</sup>) [13]. *Aureobasidium pullulans* was recovered from air samples collected in the turkey evisceration site of an Italian poultry slaughterhouse [45].

*Microsporium* spp. were isolated in a Saudi Arabian slaughterhouse [38]. Flies (*Musca domestica*) in slaughterhouses were previously reported as carriers of *Nannizzia gypsea* (formerly *Mycrosporium gypseum*) [42,44].

*Monascus ruber* [45], *Chrysonilia* spp., *Cunninghamella* spp. [36], *Emmericella* spp. [36,45], *Englyodontium album* [45], *Epicoccum* spp. [36], *Geomyces* spp. [5,45], and *Phoma* spp. [36,45], *Talaromyces* spp. [45] are also moulds isolated in slaughterhouses.

## 4. Occurrence of Yeasts in the Slaughterhouse

Yeasts are microorganisms found in various environments, including food production facilities such as slaughterhouses [11]. Their presence in these settings may result from contamination of equipment, air, and surfaces throughout the processing chain. While some yeasts are considered harmless, others can act as opportunistic pathogens.

### 4.1. *Candida* spp.

*Candida* species are part of the mucous flora and can cause a broad scope of human infections. The incidence of infections caused by *Candida* genus has increased significantly in the last decades [80]. *Candida* species are responsible for most human infections caused by fungal pathogens [81].

In a Nigerian study, 5.6% of the isolates in a ruminant slaughterhouse belonged to *Candida* spp. [24]. These were also detected in a Saudi Arabian study [38].

More specifically, *Candida albicans* was respectively isolated from 23.3% and 16.7% of the isolated yeasts in broiler carcasses and workers' swabs collected in two Egyptian poultry slaughterhouses [82]. Moreover, in two Iraqi sheep slaughterhouses, *Candida albicans* was isolated by 10% in sheep organs ( $n = 10$  isolates), by 8% in equipment swabbed before the slaughter process ( $n = 4$  isolates), and by 12% in equipment swabbed after slaughter ( $n = 6$  isolates) [11]. *Candida albicans* was also one of the fungal species that were previously described to be carried by flies (*Musca domestica*) present in an Iraqi slaughterhouse ( $n = 11$  isolates) [43]. *Candida albicans*, a commensal organism, is part of the microbiota in healthy individuals. However, under certain conditions, it can transition from a commensal to a pathogenic state [83].

Another recognized opportunistic pathogen is *Candida tropicalis*, which is considered the most prevalent pathogenic yeast species within the *Candida* non-albicans group [84]. In humans, *Candida tropicalis* is associated with superficial mycoses, such as onychomycosis, otomycosis, oral and skin candidiasis, keratitis, and genital tract infections [85]. *Candida tropicalis* was reported in swabs from broiler carcasses (16.7%,  $n = 5$  isolates) [82], from sheep organs (1%,  $n = 1$  isolate) [11], and from flies (4.5%,  $n = 7$  isolates) [43].

*Candida parapsilosis*, one of the most prevalent species responsible for candidemia worldwide [86], was isolated in broiler carcasses swabs (13.3%,  $n = 4$  isolates) and in swabs from workers' hands (6.7%,  $n = 2$  isolates) in a study carried out in two poultry slaughterhouses located in Egypt [82]. [11]

have also isolated *Candida parapsilosis* in swabs from sheep organs (4%, n = 4 isolates) and from equipment before (8%, n = 4 isolates) and after slaughter (2%, n = 1 isolate).

Other isolated *Candida* strains consist of *Candida lusitanae*, detected in two poultry slaughterhouses and in two sheep slaughterhouses [11,82]; *Candida famata*, isolated in an Italian poultry slaughterhouse and in two Iraqi sheep slaughterhouses [11,45]; *Candida guilliermondii* and *Candida zeylanoides*, detected in sheep organs and equipment swabs [11]; *Candida rugosa* obtained from equipment swabs in sheep slaughterhouses [11]; *Candida sphaerica*, in sheep organs' samples [11]; and *Candida minuta*, found in air samples of an Italian slaughterhouse [45].

#### 4.2. *Cryptococcus* spp.

Cryptococcosis encompasses infections caused by species within the genus *Cryptococcus*, usually leading to pneumonia and/or meningitis [87]. *Cryptococcus* spp. are ubiquitous, non-motile yeasts found worldwide, affecting both humans and animals [88,89].

*Cryptococcus* spp. were isolated from knife, blade and saw swabs in a Saudi Arabian slaughterhouse [38]. Additionally, *Cryptococcus albidus* was isolated from broiler carcasses (10%, n = 3 isolates) [82] and equipment swabbed before slaughter (12%, n = 6 isolates) [11].

*Cryptococcus albidus* (syn. *Naganishia albida*) rarely causes infections in humans, but it can act as an opportunistic pathogen in immunocompromised patients. This saprophytic yeast exhibits pathogenic potential by enhancing its resistance to host defences [74,90].

#### 4.3. *Rhodotorula* spp.

Human fungal infections by *Rhodotorula* spp. have been increasing in the last decades, and these are considered emerging pathogens that mainly affect immunocompromised individuals [91]. Reports indicate that *Rhodotorula* spp. can cause fungaemia, meningitis, cutaneous infections, peritonitis, keratitis, ventriculitis, and other less common conditions [91].

*Rhodotorula* spp. were previously isolated from air samples in two Italian poultry slaughterhouses [45]. *Rhodotorula* spp. was also recovered from broiler carcass swabs (26.7%, n = 8 isolates) in an Egyptian study and represented 6% of the detectable genera isolated in a study carried out in Korean swine slaughterhouses [5].

*Rhodotorula mucilaginosa* (formerly *Rhodotorula rubra*) was detected in swabs from equipment taken before (18%, n = 9 isolates) and after slaughter (12%, n = 6 isolates) [11]. This strain was also recovered from air samples collected in a cutting site [45] and blade swabs [38].

*Rhodotorula mucilaginosa*, although rarely seen as an opportunistic pathogen, is one of the most common causative species of fungemia and may be associated with endocarditis in chronic kidney disease patients [91,92].

Other *Rhodotorula* isolates include *Rhodotorula aurantiaca* and *Rhodotorula minuta*, both isolated from air samples collected from an Italian poultry slaughterhouse [45].

#### 4.4. Other Yeasts Isolated in Slaughterhouses

Various yeast genera have been isolated in slaughterhouses, with studies from different countries highlighting their presence in poultry and swine slaughterhouses. In a Korean study, *Apiotrichum* spp. (3% of the detected genera), *Naganishia* (2% of the detectable genera), *Cystobasidium* spp. (7% of the detected genera), *Filobasidium* spp. (2% of the detected genera) were isolated [5].

In an Egyptian study, *Torulopsis* spp. were identified in 6.7% of the isolated yeast species in broiler carcasses swabs (n = 2 isolates) [82]. *Saccharomyces* spp. were identified under the same circumstances [82].

## 5. Microbiological Monitoring in Slaughterhouses: the Overlooked Impact of Fungi

*Penicillium* spp. [23,37,40], *Aspergillus flavus* and *Aspergillus niger* [x – arire et al., ; x - Ja'afaru], *Cladosporium* spp. [5,36], *Scopulariopsis* spp. [13], *Candida* spp. [82], and *Rhodotorula mucilaginosa* [11] were the most frequently isolated fungal species from slaughterhouses in the limited research carried out over the last twenty-five years. This could present a risk to both food safety and occupational health. However, there is a notable absence of risk assessments and regulations addressing the potential dangers associated with fungi and their secondary metabolites.

Most of the studies addressing the isolation of moulds and yeasts in the slaughterhouse are outdated and, in addition to being scarce, often describe the isolation of fungal species in slaughterhouses without establishing a statistical correlation with other factors, such as isolation sites, slaughterhouse type (species slaughtered, size), indoor humidity, ventilation, disinfection practices, and more. There is also a lack of studies conducted in slaughterhouses regarding the presence of mycotoxins in meat and the surrounding processing environment.

Toxic residues in animal products mainly arise from contaminated feed. Mycotoxins can be excreted in urine, faeces, and milk, or accumulate in eggs, meat, and internal organs, with aflatoxins predominating in the liver, gizzard, kidney, milk, eggs, and meat [93]. These products are significant sources of human mycotoxin intake [93]. As described in this review, meat contamination may also occur during slaughter. However, the current microbiological monitoring in slaughterhouses does not include fungi or mycotoxins in the protocols.

According to European legislation, point 3.2 in chapter 3 of the Commission Regulation (CE) No. 2073/2005 [94] states that *Salmonella*, *Enterobacteriaceae*, bacterial aerobic colony count, and *Escherichia coli* must be sampled and monitored in the context of slaughterhouses, thus not mentioning fungal species. The Commission Regulation (CE) No. 2023/915 [95], which sets maximum levels for certain contaminants in food, does not set a maximum level of mycotoxin in meat.

Monitoring mycotoxigenic fungi could be an added component of the HACCP programs in the meat production chain and mould removal should be incorporated into the Standard Sanitary Operating Procedures (SSOPs) of the slaughterhouses and meat production companies.

Furthermore, based on the data collected in this review, the study of the role of possible vectors in slaughterhouses, such as flies and other insects, should be evaluated.

Regarding occupational exposure, among the fungal species mentioned in this review, only *Aspergillus* spp. (including *Aspergillus flavus* and *Aspergillus fumigatus*), *Candida parapsilosis*, and *Candida tropicalis* are included in the list relating to the protection of workers against risks related to exposure to biological agents at work published in the Commission Directive (EU) 2000/54 [96], emended by Commission Directive (EU) 2019/1833 [97]. These species are classified as Group 2 biological agents, meaning that the agents can cause diseases in humans and pose a danger to workers. Still, the probability of its spreading in the community is low, and generally, there are effective means of prophylaxis or treatment [96 - Commission Directive (EU) 2000/54].

Moreover, although the most recent report published by the European Agency for Safety and Health at Work [26] mentions the possibility of occupational exposure to fungi, such as histoplasmosis and cryptococcosis, it does not provide detailed insight into the exposure of slaughterhouse workers to other fungal species not associated with direct or indirect contact with animals and their fluids.

Currently, no specific regulation in the European Union exclusively addresses mycotoxins as an occupational risk. Previous studies, including those by Viegas et al. (2016) [28] and Viegas et al. (2016) [13], have highlighted the exposure of slaughterhouse workers to various fungal species and the mycotoxin Aflatoxin B1, revealing a significant gap in the comprehensive assessment of the occupational burden caused by fungal exposure in slaughterhouses across Europe. Despite this, there is still a lack of studies focused on assessing the risk of mycotoxin exposure for slaughterhouse workers.

Mycotoxin exposure requires the presence of mould and favourable conditions for toxin production, with humidity being a key factor [98]. However, exposure can occur even without visible mould, as small fungal biomass may produce significant mycotoxins. Assessing occupational risks involves considering airborne mycotoxin concentration, exposure duration, and frequency. Two main scenarios exist: regular low-level exposure or occasional high peaks. Health risk evaluation remains challenging due to the absence of regulatory limits for airborne mycotoxins [98].

The lack of monitoring of these compounds in workplaces makes it challenging to compare exposure levels, highlighting the need for standardized methods. Airborne fungi are often used as indirect indicators of mycotoxins [99]. However, in addition to the unreliability of this approach, as mycotoxins can persist even after fungi are eliminated [99], there is also a lack of standardization in fungal identification in slaughterhouses.

Additionally, insufficient epidemiological studies hinder assessing acute and chronic health effects of mycotoxin exposure, making it crucial to establish occupational exposure limits for individual mycotoxins and their mixtures [99].

Protective measures should be taken to reduce the potential exposure to fungal burden. Preventive measures should be implemented, such as disinfection and wearing personal protection devices, such as filtration masks and gloves [13]. Increased awareness and education on this topic should be provided to both workers and employers.

Ultimately, it is important to highlight that the presence of fungi capable of producing mycotoxins in slaughterhouse wastewater could present an environmental risk [100].

## 6. Conclusion

Fungal contamination in slaughterhouses remains an overlooked issue. This fact may pose implications for meat safety and occupational health. However, the current microbiological monitoring protocols and regulatory frameworks do not address the presence of mycotoxigenic fungi in such an environment. Specific guidelines for assessing and mitigating these risks are still lacking, maybe due to the gap in research and available data. Further research would be essential to establish the limits of mycotoxins in meat and occupational exposure limits and assess the long-term health impacts of mycotoxin exposure in the slaughterhouse.

Furthermore, implementing standardized fungal monitoring programs, integrating mould control and cleaning on SSOPs, and raising the awareness of workers, employers, and regulatory entities about the issue are essential steps to enhance public health.

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