RESEARCH ARTICLE

J Res Vet Med. 2024: 43 (2) 113-119
DOI:10.30782/jrvm.1513569

Investigation of Mold and Yeast Contaminations in Cheese Samples

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Received 10-07-2024Accepted 10-12-2024

Abstract

Fungi are common contaminants of cheese. Although they are added during the cheesemaking as starter cultures, they might also contaminate the cheese ripening or storage environments. *Candida*, *Penicillium* and *Aspergillus* species can cause serious systemic mycosis in humans and animals. This study aimed to investigate the diversity of the fungal microbiota in 100 samples of various cheese types and screen the azole resistance of *Aspergillus flavus* isolated from these samples. Twenty aged kashar, 20 fresh kashar, 25 white pickled, 25 curd cheese, and 10 cream cheese were collected from different vendors over six months in Bursa Province. Potato Dextrose Agar (PDA) was used to isolate the fungi. Sabouraud Dextrose Agar (SDA) and PDA were used for conventional identification. After microscopic and macroscopic evaluations, isolates were identified species-wise using the Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) system. Seventy-six mold and 81 yeast isolates were isolated and selected from 94 out of 100 cheese samples. As a result of conventional evaluation, 156 isolates (excluding one yeast isolate) could be identified. Based on the MALDI-TOF MS analysis, 55.6% (79/142) of the isolates were yeasts assigned to 31 species across five genera. In comparison, 44.4% were molds assigned to 28 species across five genera. The predominant fungal genus detected was *Candida* (45.1%, 64/142), followed by *Penicillium* (32.4%, 46/142). The most frequently isolated fungal species, *C. famata* (n=37), was found in all cheese types. One strain of *A. flavus* complex was isolated from one curd cheese sample and was susceptible to azole. This study successfully assesses the fungal microbiota of various cheeses from Bursa, consisting of diverse groups of yeasts and molds. While most of the molds consisted of *Penicillium* spp, detecting azole-sensitive *A. flavus* complex underlines the need for regular monitoring of cheese microflora owing to the risk of resistance development.

Keywords: Azole resistance, cheese microbiology, food hygiene, fungal contamination, public health

Introduction

Fungal infections have been on the rise in recent years. These infections include a variety of diseases caused by molds and yeasts that are considered not to be contagious to animals or humans in the past and therefore not recognized as pathogens. This condition seems to be mainly because of changing trends in patient epidemiology; however, this explanation creates a very narrow view, since in theory every fungus could already cause serious systemic mycotic infections especially in immunocompromised patients (1-3). On the other hand, the "One Health" approach brought a new perspective that diseases are multifactorial conditions. In addition to patient factors, variations in the health systems and advanced treatment procedures, environmental changes, geographic locations, increased contact of humans and animals (increased trade, travel, etc.), changes in agricultural procedures (e.g., increased antifungal usage), and nutritional habits, and similar other global factors have serious places (4,5). The One Health approach recently showed itself with a reduce the severe rates of azole-resistant invasive *Aspergillus* infections related to the wide usage of antifungal agents for agricultural purposes (5).

Cheese has a structure appropriate for developing molds and yeasts under suitable temperature and humidity conditions. Molds are saprophytic microorganisms responsible for the spoilage of dairy products, especially of unripened cheese. Different species can grow on various cheese types. *Aspergillus* and *Penicillium* species can grow from 4 °C to 10 °C at low temperatures. Their growth may result

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in a musty off-flavor, and their appearance may be commercially undesirable, resulting in the downgrading of the cheese. There is also significant potential for mycotoxin production (6). Molds may pioneer severe clinical infections, especially in immunocompromised patients such as elders with comorbidities (1). Conidial spread from a microbiologic culture-like area (cheese) will be intense and exposure may lead to infections (1,6). Yeast species are common in soil, hospital environments, and contaminated foodstuffs (7). These species, known as food spoilage microorganisms and opportunistic pathogens, are part of the intestinal microbiota but can cause systemic mycosis in some cases (7,8). Although the most important species causing human disease is *Candida albicans*, other species such as *C. parapsilosis*, *C. famata*, *C. kefir*, and *C. inconspicua* are also among the species frequently isolated in invasive candidiasis cases in recent years (7).

The main goal of this study was to contribute to the "One Health" point of view by investigating the possible fungal exposure originating from cheese, an essential part of everyday nutrition. The study aimed to identify yeast and mold species at genus and species level in yeast-mold populations in cheese samples and also to detect azole resistance in *A. flavus*, the causative agent of mycosis. These fungal species' prevalence and resistance pattern were assessed to provide helpful insights into the food safety risks associated with fungal contamination in cheese. The expected outcome of this work will also contribute to the public health strategy for reducing exposure to fungi and its resultant health effects.

Materials and Methods

Sampling of Cheese Samples

In this study, 100 cheese samples were collected from bazaars in Bursa Province, Türkiye. The samples included aged kashar (n=20), fresh kashar (n=20), white pickled cheese (n=25), curd (n=25), and cream cheese (n=10), with each sample weighing at least 100 grams. All samples were transported under aseptic conditions to the laboratory and stored at 4 °C until analysis.

Isolation and Enumeration of Fungal Species

Samples (10 g) were homogenized in 90 mL of sterile 1% peptone water using a Stomacher 400 (Seward Laboratory Systems, London, UK) for two min at average speed. Samples were serially diluted and spread onto Potato Dextrose Agar (PDA) (Oxoid Ltd., Thermo Fisher Scientific, Massachusetts, USA) (adjusted the pH to 3.5 ± 0.1 with 10% sterile tartaric acid) and incubated for 5-7 days at 25 °C (9). After incubation, mold and yeast colonies were enumerated and selected. The viable colonies were enumerated as log10 CFU g^{-1}. One mold and/or yeast colony was selected from each positive sample and stored at -80 °C for further analysis.

Identification of Fungal Species

The individual colonies were selected according to their color and morphology. Yeast and mold colonies on the plates were examined by direct microscopy for preliminary confirmation and then onto Sabouraud Dextrose Agar (SDA) (RTA Laboratories, Istanbul, Turkey) and PDA, which were then incubated at both 25 °C and 35 °C in ambient atmosphere. After at least 48-h incubation, all new colonies were identified by conventional methods including morphology, germ tube testing, thermotolerance, cornmeal agar, urease testing, pellicle formation in broth, Indian ink capsule observations, cycloheximide resistance, and acid-fast staining for ascospore presence (10-12).

After enough sporulations, all mold colonies were macroscopically examined (growth pattern, speed of growth, pigmentation) (13,14), were tested for thermotolerance, and were microscopically evaluated by both physiologic serum and lactophenol cotton blue staining (Mediko Kimya, Istanbul, Turkey) for special features such as hyphae, septa, vesicles, phialides, conidia, and sexual spores (15). All organisms were stored at -20 °C with 15%-glycerol tryptic soy broth (GBL Laboratories, Istanbul, Turkey) to use in case of further evaluations.

Matrix-assisted laser desorption/Ionization time of flight mass spectrometry (MALDI-TOF-MS) Biotyper instrument equipped with MALDI Biotyper RealTime classification software and FlexControl software (version 3.3) (Bruker Daltonics, Bremen, Germany) was used to identify yeast isolates. A single fresh colony of each yeast isolate was taken from SDA plates, and a thin colony layer was spotted on a 96-spot polished steel MALDI target plate, which was allowed to air dry at room temperature for 30 min. Then, each spot was overlaid by 1 µL of a daily prepared solution of matrix solution (HCCA, α-cyano-4-hydroxycinnamic acid) (16). Afterwards, the sample prepared on the target plate was inserted into the device and analyzed using software associated with the respective system. A score above 2, the threshold value recommended by the manufacturer, was accepted for identification.

To identify mold species by MALDI-TOF MS analysis, subcultures of mold isolates were grown on SDA by incubation at 22-25 °C for 3-5 days. Pre-treatment followed the

procedure with some modifications, as described previously by Becker et al. (2015) (17). A 5 mm diameter mold (including the hyphal bed) was removed from the PDA plates (Oxoid Ltd., Thermo Fisher Scientific) and placed into 5 mL of tubes containing SDB (GBL Laboratories) using a swab before starting sporulation (at an early stage). Tubes were kept in an ultrasonic bath (Elma-Transsonic 460H; Techspan Group, Auckland, New Zealand) for 15 sec and then rotated (Stuart rotator SB3; Novatek Analytical Systems Inc., Istanbul, Turkey) at 18 rpm for 18 h. Then tiny colonies were transferred to the microcentrifuge tube (1.5 mL; Eppendorf AG, Hamburg, Germany) by Pasteur pipettes in the amount of 1.5 mL and centrifuged (Electro-mag M4800M; Elektro-Mag, Istanbul, Turkey) for 2 min at 13,000 rpm. SDB (GBL Laboratories) was removed with a micropipette. The colonies were washed with the water of LC-MS grade (Merck Chromatography-LC-MS grade, 1.15333.2500; Merck KGaA, Darmstadt, Germany), and 1,000 µL was left in each tube. The centrifugation and washing processes were repeated twice and finally the remaining liquid was removed. Then, a volume of 300 µL of deionized water (high-pressure liquid chromatography [HPLC] quality; Merck KGaA) was taken into a new microcentrifuge tube and the visibly prepared colony (5-10 mg) was transferred into this tube. It was mixed with the pipette and vortexed (NUVE NM110; Nüve Laboratory & Sterilization Technology, Ankara, Turkey) for 15 min, followed by centrifugation for 2 min at 13,000 rpm. A volume of 900 µL of ethanol (EtOH; Merck KGaA) was added, and the tubes were again vortexed for 5 min, followed by centrifugation at 13,000 rpm for 2 min. The upper phase (EtOH) was removed without damaging the pellets remaining at the bottom and the centrifugation was repeated. The remaining EtOH was removed with a pipette. Pellets were dried at room temperature for 2-3 min and mixed thoroughly with 1-80 µL of 70% (v/v) formic acid (FA; Merck KGaA) and 1-80 µL of acetonitrile (ACN; Merck KGaA) followed by a centrifugation step for 2 min at 13,000 rpm. A 1 µL clear supernatant was placed onto a ground steel MALDI target plate (Bruker Daltonics) and allowed to dry at room temperature. Subsequently, each sample was overlaid with 1 µL of matrix solution (HCCA, α-cyano-4-hydroxycinnamic acid) and dried at room temperature. Then, placed into the Bruker MicroFlex LT device (Bruker Daltonics), the results were evaluated with MALDI Biotyper RealTime Classification software and FlexControl software (version 3.3) programs.

Screening for Azole Resistance of *Aspergillus flavus* Complex

The test was performed according to Guinea et al. (2018) (18). Mold colony identified as *A. flavus* complex with Biotyper software, version 3.3 was next screened for resistance using the agar-based screening for azole resistance test, which consists of a 4-well plate containing agar supplemented with three azole drugs (itraconazole [ITC] at 4 mg/L, voriconazole [VRC] at 2 mg/L, and posaconazole [POS] at 0.5 mg/L) (Spectrum Chemical MFG Corp., NJ, USA) and a growth control without any antifungal drug. A wet sterile swab was used to collect conidia from an *A. flavus* colony to visually make a 0.5 McFarland suspension in 1 mL of sterile water. A disposable pipette was used to add one drop of the suspension (25 µL) to each of the four wells. The plates were incubated at 35-37 °C for 24-48 h. *A. flavus* ATCC 204304 was used as a control strain.

Results

As seen in Table 1, the yeast count varied between 2.30 and 8.23 log10 CFU g^{-1}, while the mold count varied between 2 and 6.85 log10 CFU g^{-1} in all cheese samples. Aged kashar cheese showed the highest incidence of fungal contamination, with 95% of samples testing positive for yeast at concentrations between 4.78 and 8.23 log10 CFU g^{-1}, with an average of 6.73 log10 CFU g^{-1}. Similarly, mold contamination was common in aged kashar cheese samples, showing the highest prevalence (90%) with an average count of 5.07 log10 CFU g^{-1}. This was followed by curd cheese, which had an 84% prevalence rate, showing a relatively higher risk of yeast contamination. The lowest rate of yeast contamination was found in white pickled cheese, with contamination detected in only 56% of samples. In relation to mold contamination, aged kashar had also the highest prevalence with 90% of the samples. In cream cheese, although the prevalence of yeast was lower, the mold contamination rate was relatively high at 70%.

Table 1: Fungi prevalence and counts (log10 CFU gr^{-1}) in cheese samples

Cheese Type	Samples Count	Positive for Mold			Positive for Yeast		
		No	%	Count	No	%	Count
Aged kashar	20	18	90	Min: 3.0 Max: 6.85 Mean= 5.07 SD=1.23	19	95	Min: 4.78 Max: 8.23 Mean= 6.73 SD=0.97
Fresh kashar	20	11	55	Min: 2.30 Max: 4.90 Mean= 4.07 SD=0.72	14	70	Min: 3.0 Max: 7.18 Mean= 5.22 SD=1.16
White pickled	25	17	68	Min: 2.0 Max: 6.30 Mean= 4.13 SD=1.12	14	56	Min: 3.0 Max: 7.68 Mean= 4.91 SD=1.60
Curd	25	14	56	Min: 2.0 Max: 5.32 Mean= 3.82 SD=0.85	21	84	Min: 2.30 Max: 7.98 Mean= 4.90 SD=1.74
Cream	10	7	70	Min: 3.48 Max: 5.30 Mean= 4.48 SD=0.73	6	60	Min: 3.30 Max: 5.70 Mean= 4.75 SD=0.87

Min: *Minimum;* ***Max:*** *Maximum;* ***Mean:*** *Arithmetic mean ;* ***SD:*** *Standart deviation*

There were 157 isolates (81 yeast and 76 mold isolates) selected after incubation at 25 °C for 5-7 days in PDA of pH 3.5 ± 0.1. As presented in Table 2, fungal contamination

was detected in 94 out of 100 cheese samples. While 156 (80 yeast and 76 mold isolates) of the 157 selected isolates were confirmed conventionally, 142 (79 yeast and 63 mold isolates) were identified at genus and species level by MALDI-TOF MS. The predominant fungal genus detected in 68.1% of the samples was *Candida* (45.1%, 64/142), followed by *Penicillium* genus (32.4%, 46/142). *C. famata* (n=37), *C. zeylanoides* (n=15), *C. lambica* (n=5), *C. kefyr* (n=3), *C. valida* (n=1), *C. parapsilosis* (n=1), *C. intermedia* (n=1) and *C. inconspicua* (n=1) were the identified *Candida* species. *C. famata*, found in all cheese types, was mainly isolated from aged kashar samples (n=13), followed by fresh kashar samples (n=9). Among all selected fungal isolates, only one yeast isolate could not be identified by conventional evaluation, and this isolate was identified as *Geotrichum silvicola* by MALDI-TOF MS.

As a result of MALDI-TOF MS analysis, seven distinct mold species were detected across five types of cheese from a total of 94 out of 100 samples. Various *Penicillium* species, including *P. commune*, *P. digitatum*, *P. camemberti*, *P. italicum*, *P. roqueforti*, and *P. singorense*, were also identified in these samples. *P. commune* was the predominant mold species in 19% (18/94) of the samples. *P. digitatum* was present in 12.8% (12/94) of the samples. The *A. flavus*-oryzae group was isolated and identified from only one (curd cheese), and two or more mold species were isolated from five cheese samples. The azole resistance assay result was negative for the sole *A. flavus* isolate from a curd cheese sample.

Table 2: Identification of fungi isolates from the cheese samples

	Identification of Molds		Identification of Yeasts	
Type and number of cheese samples	**Conventional Evaluation**	**MALDI-TOF MS**	**Conventional Identification**	**MALDI-TOF MS**
Aged kashar cheese (n=20/20)	*Penicillium* spp. (n=19) Order of *Mucorales* (n=2)	*Penicillium commune* (n=11) *Penicillium camamberti* (n=3) *Penicillium digitatum* (n=3) [a]NRI (n=2) *Rhizopus stolonifera* (n=2)	*Candida* spp. (n=17) *Yarrowia lipolytica* (n=2)	*Candida famata* (n=13) *Candida zeylanoides* (n=4) *Yarrowia lipolytica* (n=2)
Fresh kashar cheese (n=18/20)	*Penicillium* spp. (n=8) Order of *Mucorales* (n=3) Dematiaceous fungi (*Cladosporium* sp.) (n=1)	*Penicillium digitatum* (n=3) *Penicillium commune* (n=1) *Penicillium italicum* (n=1) [a]NRI (n=3) *Rhizopus stolonifera* (n=2) *Mucor circinelloides* (n=1) *Cladosporium* sp. (n=1)	*Candida* spp. (n=11) *Candida kefyr* (n=1) *Yarrowia lipolytica* (n=1) *Saccharomyces* spp. (n=1) *Rhodotorula* spp. (n=1)	*Candida famata* (n=9) *Candida valida (Pichia membranifaciens)* (n=1) *Candida parapsilosis* complex (n=1) *Candida kefyr* (n=1) *Yarrowia lipolytica* (n=1) *Torulaspora delbrueckii* (formerly *Saccharomyces delbrueckii*) (n=1) *Rhodotorula mucilaginosa* (n=1)
White pickled cheese (n=22/25)	*Penicillium* spp. (n=15) Order of *Mucorales* (n=2) Dematiaceous fungi (*Cladosporium* sp.? (n=2)	*Penicillium italicum* (n=4) *Penicillium commune* (n=2) *Penicillium roqueforti* (n=2) *Penicillium camamberti* (n=2) *Penicillium singorense* (n=1) [a]NRI (n=4) *Rhizopus stolonifera* (n=2) *Cladosporium* sp. (n=2)	*Candida* spp (n=13) *Saccharomyces* spp. (n=2)	*Candida famata* (n=7) *Candida zeylanoides* (n=4) *Candida lambica* (n= 1) *Candida intermedia* (n=1) *Torulaspora delbrueckii* (formerly *Saccharomyces delbrueckii*) (n=1) *Kluyveromyces lactis (*formerly *Saccharomyces lactis)* (n= 1)
Curd cheese (n=25/25)	*Penicillium* spp. (n=11) Order of *Mucorales* (n=2) Dematiaceous fungi (*Cladosporium* sp.) (n=1) *Paecilomyces* spp. (n=1) *Aspergillus flavus* complex (n=1)	*Penicillium digitatum* (n=5) *Penicillium commune* (n=2) *Penicillium camamberti* (n=2) [a]NRI (n=2) *Rhizopus stolonifer* (n=2) [a]NRI (n=1) *Byssochlamys spectabilis* (*Paecilomyces variotii*) (n=1) *Aspergillus flavus-oryzae* group (n=1)	*Candida* spp. (n=16) *Candida kefyr* (n=2) *Yarrowia lipolytica* (n=2) *Saccharomyces* spp. (n=2) *Rhodotorula* spp. (n=2) [b]NI (n=1)	*Candida famata* (n=6) *Candida zeylanoides* (n=5) *Candida lambica* (n=4) *Candida inconspicua* (n=1) *Candida kefyr* (n=2) *Yarrowia lipolytica* (n=2) *Kluyveromyces lactis (*formerly *Saccharomyces lactis)* (n=1) *Torulaspora delbrueckii* (formerly *Saccharomyces delbrueckii*) (n=1) *Rhodotorula mucilaginosa* (n=1) [a]NRI (n=1) *Geotrichum silvicola* (n=1)
Cream cheese (n=9/10)	*Penicillium* spp. (n=4) Order of *Mucorales* (n=4)	*Penicillium commune* (n=2) *Penicillium digitatum* (n=1) *Penicillium corylophilum* (n=1) *Rhizopus stolonifera* (n=2) *Mucor circinelloides* (n=1) [a]NRI (n=1)	*Candida* spp. (n=5) *Saccharomyces* spp. (n=2)	*Candida famata* (n=2) *Candida zeylanoides* (n=2) [a]NRI (n=1) *Torulaspora delbrueckii* (formerly *Saccharomyces delbrueckii*) (n=1) *Kluyveromyces lactis (*formerly *Saccharomyces lactis)* (n=1)

Discussion

The number of fungal species may increase during ripening, especially in kashar cheeses in direct contact with air. Since one of the dominant flora in aged kashar is *Penicillium spp*, it can be concluded that contamination might have occurred during the ripening stage. *Candida spp*. is the dominant genera in curd cheese; this may be because curd cheese has higher moisture than aged kashar and is a better environment for yeast growth. Lower fungal counts were reported in kashar cheese samples (19-21) and in white cheese samples (22,23) in Türkiye. Godek et al. (2021) (24) found that 74% of the white cheese samples had both yeast and mold, which is relatively higher than what we found. Bouakline et al. (2000) (25) found no fungal contamination

in cream cheese samples. Still, all the soft cheese samples (n=20) had been contaminated with *Geotrichum spp*. and *Candida norvegensis*. While we could identify *Geotrichum silvicola* from one curd cheese sample, *C. norvegensis* was not found in any cheese samples. The samples used in our study were collected from bazaars where they are displayed and sold unpackaged. Exposure to open air, combined with the possible poor hygienic conditions during production, ripening, storage, transportation, and the time spent on market stalls create a potential contamination and breeding environment for fungal species.

While recent studies (26) identified *Mucor* and *Candida* (especially *Issatchenkia orientalis*) as dominant species in cheese, we found *C. famata* as the most prevalent yeast and *P. commune* as the most common mold. Unlike our study, Hameed (2016) (27) found only three yeast species: *C. albicans*, *C. krusei*, and *Debaryomyces hansenii*; *C. albicans* was dominant among all yeasts with 24%, 30%, and 67% ratios from three different cheese samples. Out of all the mold isolates, *Cladosporium cladosporidis* was the dominant species, followed by the only detected *Penicillium* species, which was *P. corylophilum*, and isolated four *Aspergillus* species. *C. albicans* was not detected in our samples, while *P. corylophilum* was only encountered once. Moreover, the dominant mold species was *P. commune* (one of the fungal species known to cause invasive fungal infections) (28), followed by *P. digitatum*, and one *Aspergillus* species was detected in one of our curd cheese samples. Several studies have reported that the dominant mold in cheeses is *Penicillium*, with the most dominant species being *P.* roqueferti (29,30) and *P. communae* (31), respectively. Our data revealed that the predominant fungus was *Candida*, succeeded by *Penicillium*, suggesting significant variations in the fungal species isolated across previous investigations and the prevailing species. The diversity in fungal communities in cheese is thought to be due to regional, environmental, and production-related factors, including differences in sampling, microbial ecology, and climate.

When considering the technologies of these cheeses, the milk used must be heat processed before the starters are added, and these cheeses also do not have fungi as starter cultures. Heat processing inactivates fungi spores due to their thermo-susceptibility (32). After cheese production, there are different ways that the product might get contaminated. The air is one of the common fungal contamination sources. Air quality plays a considerable part during the production, ripening, and storage stages (33). There also is a brined cheese, in which the brine is a potential contamination source. In addition, different areas of the production plant may have different levels of microorganism concentration, although *P. commune* is still the most common contaminant (34).

Azole compounds are used as both antifungal agents in human and veterinary medicine and as fungicides in agriculture (35). Resistance development in fungi seems much more complicated than in bacteria, which can quickly transfer the resistance genes (36). Some studies showed that azole resistance might develop in fungi isolated from infected patients, considering their extensive use (37,38). The prevalence of azole-resistant *Aspergillus spp* is increasing worldwide and is thought to be related to improper use of azole antifungals in agriculture. In a study conducted in France, 208 *Aspergillus* isolates were obtained from clinical and soil samples and eight isolates resisted at least one azole drug (39). In Japan, another study identified two azole-resistant *A. flavus* isolates among 99 *Aspergillus spp*. obtained from 50/692 food samples containing agricultural products (40). Additionally, a separate investigation reported that 18.6% of *Aspergillus* isolates were azole-resistant (41). All these studies indicate that azole is misused in agricultural crop cultivation and that azole resistance is spreading. Proximity to agricultural crop fields, where fungicides are used intensively, and consumption of food contaminated with fungal species may increase the spread of resistant strains into larger populations.

Our study showed that one sample, which constituted 1% of the samples, contained *A. flavus* which was susceptible to azole. The transfers of genes amongst fungi, let alone inter-species, are a very complex process. Application of azoles-both as medicines and also as fungicides in agriculture-needs to be carefully policed. This finding has proved significant in establishing the fact that in our population, *A. flavus* is still susceptible to treatments based on azoles. Azoles are extensively applied in clinical and agricultural use to inhibit fungal growth. Thus, the future research could be on prevalence and resistance profile of *A. flavus* from various types of cheese and different geographical regions showing potential risks and effective management.

Conclusion

In our study, *Candida* was found to be the dominant fungus genera in five different types of cheese. In addition, while the primary source of fungus contamination was determined to be aged kashar cheese, the absence of azole resistance in *A. flavus* isolated from curd cheese was reassuring regarding the prevalence of antifungal resistance. Consumption of cheeses with high levels of fungus genera such as *Candida*, *Penicillium* and *Aspergillus*, which are

known as causative agents of systemic mycosis, can pose significant health risks, particularly for individuals with compromised immune systems. Improving production, ripening, and storage could greatly reduce contamination rates of fungus species in the food production-consumption chain.

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