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Isolation and Identification of *Aspergillus fumigatus* From Fresh Cow Milk Within Sokoto Metropolis

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ABSTRACT

Fresh cow milk serves as an important source of nutrients and is widely consumed within Sokoto metropolis, yet it is highly susceptible to microbial contamination, including fungal organisms. This study was conducted to isolate and identify *Aspergillus fumigatus* from fresh cow milk and to assess the prevalence of fungal organisms in clinically non-mastitis milk from bovines within Sokoto metropolis. A total of eighty fresh cow milk samples were collected from different associated farms and selected households across the study area. The samples were collected aseptically and transported to the laboratory for mycological examination using standard cultural and morphological identification methods. Each sample was cultured on appropriate fungal media and incubated under suitable conditions to allow fungal growth and identification. Out of the eighty milk samples processed, fifteen samples were found positive for fungal isolates, indicating the presence of fungal contamination in fresh cow milk within Sokoto metropolis. The most common fungal isolates identified included *Rhizopus* species, *Candida* species, and *Aspergillus fumigatus*. The prevalence rates of these isolates were 18.5%, 5%, and 3.75% respectively, with *Rhizopus* species being the most predominant. The presence of *Aspergillus fumigatus* in fresh cow milk is of particular concern due to its potential to cause opportunistic infections and allergic reactions, especially among immunocompromised individuals. These findings suggest that fungal organisms play a significant role in bovine milk contamination within the study area. The study highlights the importance of proper hygiene and sanitation practices during milking and milk handling. Improved farm management and judicious use of antibiotics may help reduce the incidence of fungal contamination. Regular monitoring and quality control measures are recommended to ensure safe milk consumption.

Keywords: *Aspergillus fumigatus,* Fungal contamination, Fresh cow milk, Mycotic mastitis, Sokoto metropolis.

1.0 INTRODUCTION

The microbiological quality of fresh cow milk is an important public health concern, particularly in developing regions where milk is often consumed raw or processed under unhygienic conditions (Ntuli *et al.,* 2023). Fresh cow milk is highly nutritious, containing proteins, fats, vitamins, and minerals that support microbial growth when contamination occurs. Among the various microorganisms that may contaminate milk, fungi have received increasing attention due to their ability to produce harmful mycotoxins and cause opportunistic infections (Dubey *et al.,* 2022). One of the most significant fungal contaminants is *Aspergillus fumigatus*, a ubiquitous mold commonly found in soil, air, feed, and agricultural environments. Its presence in milk may occur through environmental contamination during milking, transportation, storage, or handling (Ntuli *et al.,* 2023). The consumption of contaminated milk poses potential health risks, especially to immunocompromised individuals, children, and the elderly. Therefore, investigating the occurrence of *Aspergillus fumigatus* in fresh cow milk is essential for ensuring food safety and protecting public health (Olufemi *et al.,* 2024).

In many parts of Nigeria, including Sokoto metropolis, fresh cow milk is widely consumed and sold in local markets without adequate pasteurization or quality control measures (Muhammad and Tomo, 2023). Traditional milking practices, poor sanitation, and exposure to dust and animal feed create favorable conditions for fungal contamination. Additionally, the hot and dry climatic conditions of Sokoto may promote the proliferation and dissemination of fungal spores in the environment (Tafinta *et al.,* 2023). These spores can easily settle on milk during milking or storage, leading to contamination. *Aspergillus fumigatus* is particularly important because of its thermotolerance and ability to grow at elevated temperatures, which makes it adaptable to harsh environmental conditions (Liu *et al*., 2024). The organism is also known for producing allergens and secondary metabolites that may compromise food safety. Consequently, monitoring its presence in fresh cow milk

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within Sokoto metropolis is of considerable importance (Abubakar *et al.,* 2025).

The isolation and identification of *Aspergillus fumigatus* from fresh cow milk involve microbiological and mycological techniques that allow for accurate detection and characterization of the organism (Onoharigho *et al.,* 2022). Standard laboratory procedures such as culturing on selective media, morphological observation, and microscopic examination are commonly employed. These techniques help differentiate *Aspergillus fumigatus* from other fungal species that may be present in milk samples (Onoharigho *et al.,* 2022). Accurate identification is essential for determining the extent of contamination and assessing potential health risks. Furthermore, understanding the distribution of *Aspergillus fumigatus* in fresh cow milk can provide insight into contamination sources (Khan *et al.,* 2026). Such information is valuable for designing effective control strategies and improving hygienic practices. Therefore, laboratory-based isolation and identification remain crucial steps in ensuring milk safety (Hooda *et al.,* 2025).

The presence of fungal contaminants in fresh cow milk also has economic implications for dairy farmers and milk vendors (Ntuli *et al.,* 2023). Contaminated milk may lead to spoilage, reduced shelf life, and potential rejection by consumers. In addition, fungal contamination can affect the quality of dairy products derived from contaminated milk (Onoharigho *et al.,* 2022). The detection of *Aspergillus fumigatus* may indicate poor handling practices and inadequate sanitation during milk production. Identifying these issues can help stakeholders implement corrective measures to improve milk quality (Fiorillo *et al.,* 2024). Improved hygiene during milking and storage can significantly reduce contamination levels. Thus, studies focusing on fungal contamination contribute not only to public health but also to economic sustainability (Singh and Thakur, 2023).

Given the increasing demand for fresh cow milk in Sokoto metropolis, there is a need to evaluate its microbiological safety, particularly concerning fungal contamination (Akinyemi *et al.,* 2025). Investigating the occurrence of *Aspergillus fumigatus* will help determine the level of exposure among consumers. The findings from such studies can provide baseline data for regulatory authorities and public health agencies (Nwakoby *et al.,* 2025). Additionally, the results may promote awareness among dairy farmers, vendors, and consumers about the importance of hygienic milk handling practices. This research also contributes to the broader understanding of foodborne fungal pathogens in Nigeria (Adediran *et al.,* 2024). Ultimately, the isolation and identification of *Aspergillus fumigatus* from fresh cow milk within Sokoto metropolis will support efforts to improve milk safety. Such efforts are essential for safeguarding public health and enhancing food quality standards (Olufemi *et al.,* 2024). This research was therefore aimed at isolating and identifying agents of fungal mastitis, especially *Aspergillus fumigatus* from milk freshy collected from animals within Sokoto metropolis.

2.0 METHODOLOGY

2.1 Study area

The study was conducted in Sokoto metropolis, the capital of Sokoto State, Nigeria. Geographically, Sokoto State lies between latitude 12°N and 13°58′N and is approximately 308 meters above sea level (Sokoto, 2001). The state is characterized by short grass savannah vegetation in the southern part and thorny vegetation in the northern region. Sokoto State shares boundaries with Zamfara State to the east, Niger Republic to the north, and Kebbi State to the west and southwest (Sokoto, 2001).

Samples were collected from associated farms and selected households within Sokoto metropolis. Sokoto metropolis primarily comprises Sokoto North and Sokoto South Local Government Areas. However, parts of Dange-Shuni, Wamakko, and Kware Local Government Areas also form part of the metropolitan area. These locations were selected due to their high concentration of dairy farming activities and fresh cow milk consumption within the study area (Abdullahi *et al.,* 2024).

2.2 Sample Collection

The cows were physically restrained and prior to sampling, teat ends were swabbed with 70% alcohol. Milk was expressed using hand milking technique. The initial milk stripped from each udder was discarded and the next 10 ml were collected in a sterile container. The milk samples were collected in plain sample bottles. Separate samples were packed in a cooler containing ice block and transported to the Veterinary public health laboratory for further processing and analysis (Orregård, 2013).

2.3 Sterilization of Glass Wares

Petri-dishes, test tubes, conical flasks, beakers, pipettes, etc. was sterilized in hot air oven at 180ºC for two hours (2 h) and stored at 40ºC (Tegegn *et al.,* 2025).

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2.4 Preparation of Culture Media

Sabouraud dextrose agar was weighed at 65.0g which was added in 1000ml of distilled water which was properly dissolved using magnetic stirrer and was autoclaved at 121ºC for 15minutes. The prepared medium was allowed to cool for few minutes then poured into different sterile petri dishes and allowed to solidify within 30minutes on a sterile table. The table was sterilized using 70%alcohol and cotton wool to clean the surface of the table (Kumar *et al.,* 2020).

2.5 Spread Method for Fungal Growth

Exactly 0.1ml of the milk sample was pipetted out from each of the labelled milk sample using a micro titre pipette unto each labelled plate. The plate ware opened quickly to avoid contamination. Using a sterile glass spreader, the inoculum was spread around the surface of the agar until traces of liquid disappeared (Deka *et al.,* 2020). The Petri dishes were completely sealed using masking tape to allow the growth of only fungal organism. The plates were placed upright (lid on top) at room temperature for 24-72 hours for observation of growth (Gautam and Avasthi, 2019).

2.6 Isolation of Pure Culture

Using another prepared Sabouraud dextrose agar plates, individual fungal colony from any of the plates representing each colony was collected using a sterile wire loop, as taken from a high diluted plate as it tends to have pure colonies that are well separated. The wire loop was sterilized, then the selected agar plate was quickly opened near the Bunsen burner, the selected colony was gently picked and transferred into another prepared plates. The sterile wire loop with the colony was smeared at the surface of the sterile plate slant. This procedure was repeated for different growth observed macroscopically. The plates were covered and kept at room temperature for 3 to 7days. This was done to obtain pure colony (Gautam and Avasthi, 2019).

2.7 Cultural Characteristics

The growth pattern, pigmentation and size of colonies were recorded to aid in the identification of the organisms (Campbell and Johnson, 2013).

2.8 Staining with Lactophenol Blue

A drop of lacto phenol cotton blue (LP) was added on a clean microscopic glass slide. The fungal isolate was picked using a sterile wire loop and transferred on to the drop of the lacto phenol cotton blue. Sterile cover slips were placed on the slide containing a drop of lactophenol blue and observe microscopically. It was first viewed at ×10 to focus the lens well then ×40 to get a clearer view. This procedure was done to the remaining sample (Shitayeh *et al.,* 1998).

3.0 RESULTS

The results obtained from this study revealed that out of the 80 fresh cow milk samples examined, 15 samples were positive of fungal growth and 3 samples out of the 15 were *Aspergillus spp*. as shown in table 1 and 2. The growth of different genera showed different cultural characteristics on the agar as displayed in table 1. The different colors used for identification of the fungi was also observed (table 1). These fungal isolates belong to the genera of *Candida, Aspergillus, Absidia Corymbifera* and *Rhizopus spp.* as tentatively identified. Although the most common genus isolated was *Rhizophus spp*. some species of the dominant genus were *Candida spp.*

Table 1: Morphological Appearance of Fungal Isolate

Sample S/N	Colour	Shape	Elevation
17A	Grey-Green	Irregular	Elevated
33A	White	Oval	Elevated
34B	Gray-Green	Circular	Flat
37A	Gray-Green	Circular	Flat
37B	White	Oval	Flat
37C	White	Oval	Elevated
37D	Black	Irregular	Elevated
39A	Gray-Green	Circular	Elevated
39B	Gray-Green	Oval	Elevated
62A	Gray-Green	Irregular	Flat
62B	White	Oval	Flat
65B	Black	Oval	Elevated
65C	Black	Irregular	Flat
69A	Brown	Irregular	Elevated
72D	White	Irregular	Flat

Table 2: Number of Positive Samples of *Aspergillus* Growth

S/N	Test Sample	Positive For Fungal Growth	Positive For *Aspergillus*
17	C	+	-
33	A	+	-
34	B	+	+
37	A	+	+
	B	+	-
	C	+	-
	D	+	-
39	A	+	-
	B	+	-
62	A	+	+
	B	+	.

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65	B	+	-
	C	+	-
69	A	+	-
72	D	+	-

Table 3: Showing percentage prevalence

Fungal isolate	Positive Sample	Total Samples Processed	Percentage Prevalence
Aspergillus fumigatus	3	80	3.75
Candida spp.	4	80	5
Rhizopus	7	80	8.75
Absidia corymbifera	1	80	1.25

Prevalence =18.75%

3.1) Macroscopic Appearance of Fungal Isolates

Plate 1: *Rhizopus* spp.

Plate 2: *Asprgillus Fumigatus*

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Plate 3: Candida

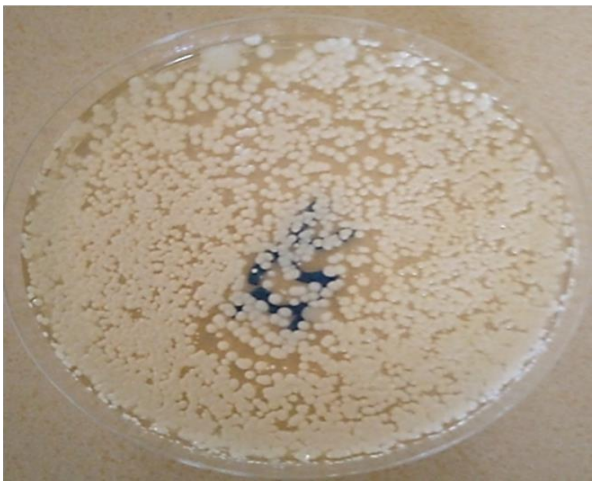

Plate 4: *Absidia corymbifera*

3.2) Microscopic Appearance of Fungal Isolates

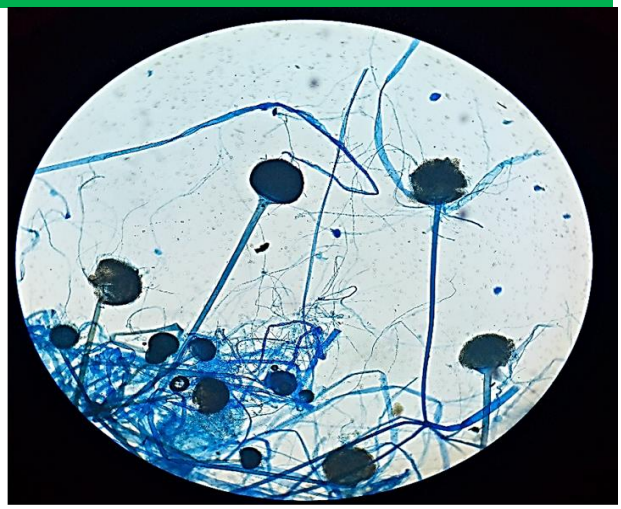

Figure 1: *Rhizopus* spp.

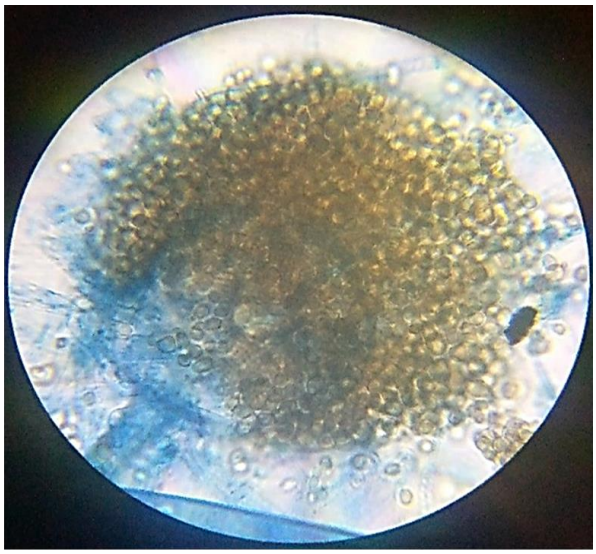

Figure 2: *Aspergillus fumigatus*

4.0 DICUSSION

From this study, the overall prevalence was found to be 18.75% with *Rhizopus* and *Candida spp*. as dominant organism having 8.75% and 5% respectively. Other isolated fungal organisms were *Aspergillus spp.* with prevalence of 3.75% and *Absidia corymbifera* with prevalence of 1.25%. Fungi as an etiological agent of mastitis have also been reported by various workers. In a view of closely similar research, a prevalence of 60% was observed (Pachauri *et al.,* 2013).

According to Ahmed *et al.* (2024), The incidence of fungal mastitis may be due to unhygienic condition of the animals shed supporting the growth of fungal spores and hyphae in the vicinity of lactating animals, thereby

increasing the probability of fungal spores and yeast cells to enter in to the udder parenchyma which provides the best environment for the growth of these fungi. It can also be attributed to geographical location, and variation of test animals that were employed as this research is strictly on clinically non mastitis animal which differs from previous research. There may be seasonal influences like high humidity along with favorable ambient temperature and excessive use of anti-biotic may increase the chances of fungal growth such as candida and *Aspergillus* species.

5.0 CONCLUSION

This study investigated the isolation and identification of Aspergillus fumigatus and other fungal organisms from fresh cow milk within Sokoto metropolis. Out of eighty milk samples examined, fifteen showed fungal growth, giving an overall prevalence of 18.75%. The predominant isolates were *Rhizopus* species, *Candida* species, *Aspergillus fumigatus*, and *Absidia corymbifera*. *Rhizopus* species recorded the highest occurrence, followed by *Candida* species, while *Aspergillus fumigatus* was detected in three samples. The presence of these fungi indicates contamination of milk from environmental sources, poor hygienic milking practices, and inadequate handling during collection and storage. The occurrence of fungal organisms in clinically non mastitis cows suggests potential risk for subclinical infections and reduced milk quality. These findings highlight the importance of regular screening and proper sanitary practices in dairy farms. Improved farm hygiene, careful use of antibiotics, and proper handling during milking are essential to reduce contamination. Continuous monitoring and awareness among dairy farmers are necessary to improve milk safety, protect public health, and enhance dairy productivity within Sokoto metropolis and surrounding areas effectively.

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