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Isolation, Identification and Antimicrobial sensitivity of some fungi causing diarrhea in sheep and goats

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Abstract: The current study identifies the mycotic diseases causing diarrhea in sheep and goats with special focus on isolation and antifungal sensitivity testing on some identified isolates. A total number of 100 fecal swabs from diarrheic animals in small shepherd flocks at Fayoum Governorate, Egypt (70 sheep and 30 goats), 40 samples from workers (buccal samples, skin swabs and nasal samples) and 60 samples from feed stuffs were obtained. The mycological examination declared the isolation of 139 fungal isolates [27 (13.5%) yeasts and 112 mold (56.00%)]. *Rhodotorula spp.*, *C. pseudotropicalis*, *C. tropicalis*, *Torulopsis*, *Geotrichum candidum*, *Saccharomyces*, *A. fiavus*, *A. niger, A. fischeri, A. Carbonarius, Penicillium expansum* and *Fusarium chlamydosporum* were the most predominant fungi from all fecal samples. The examined workers revealed isolation of *C. guilliermondii*, *C. krusei, A. fiavus, A. niger, A. fumigatus* and *A. terreus*. The feed stuffs examinations revealed no yeast but *A. fiavus, A. niger, A.fumigatus*, *A.terreus, A.carbonarius, Penicillium griseofulvum, Penicillium simplicissimum, Rhizopus Spp., Mucor Spp., Eurotium chevalieri* and *Eurotium rubum* were isolated with different rates. There is a strong positive correlation between presence of mold in feed stuff, animal and worker samples. Anti-fungal sensitivity test in diarrheic animals revealed that *C. Krusei*, *C. Pseudotropicdis*, *C. tropicalis* and *Torulopsis* are highly senstive to Voriconazole meanwhile, *Rhodotorula* and *P. expansum* are senstive to Itraconazole. *A. terreus* and *A. flavus* are highly senstive to Clotrimazole and Voriconazole respectively. *Fusarium* is completely resistant to the six used antifungal discs. The fungal human isolates; *C. guilliermondii* and *C. Krusei* are highly sensitive to Voriconazole and Itraconazole resprctively meanwhile *A. flavus* and *A. niger* are highly sensitive to Itraconazole. All fungal isolates from human samples are resistant to Metronidazole. The most fungi still can cause infections to sheep, goat and human.
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1. Introduction

Small ruminants (sheep and goats) play a predominant role in the economy of million people, **(Thornton, 2010).**

Diarrhea in goat and sheep is a very common symptom, it is attributed to many different causes ranging from a sudden change in feed, ingestion of poisonous plants, infection, or in more severe cases, a symptom of a viral, fungal or bacterial disease. Diarrheal disease seems to be one of the major community health hazards both for man and animals in most countries of the world. It is resulted from enteritis, which is the inflammation of the intestinal mucosa, characterized by abdominal pain, loose feces, increase in stool mass and defecation frequency or stool fluidity (dehydration) that contains 70-95% water, the chronic form of diarrhea may last for days or week and may culminate in death **(Radostits *et al.*, 1995)**.

Fungal infections can occur in healthy animals but are more common as opportunistic infections in debilitated and immune-compromised hosts whose normal defense mechanisms are impaired. A fatal outcome is possible in these individuals, as fungal infection may remain undiagnosed **(Randhawa., 2000)**

Aspergillus species are widely distributed in nature; they have been predominantly isolated from soils, decaying vegetation and on a wide variety of organic matter **(Sigler & Verweij, 2003).** *Aspergillus* infections are the most common cause of human opportunistic fungal infections after yeast infections. These infections have increased dramatically in recent years **(Steinbach *et al.*, 2004).** The majority of *Aspergillus* infections are caused by *A. fumigatus, A. flavus, A. niger* and *A. terreus. A. terreus* infections used to be uncommon, but they have become a growing concern in the past few years **(Park & Bakalinsky 2000).**

Yeasts are found on a wide variety of substances such as soil, plants, water, and nectar of flowers, fruits, trees and exudates of animals. They cause diseases in both man and animals such as thrush, disseminated candidosis, cryptococcosis and mastitis **(Asfour *et al.*, 2009).**

So, the objectives of current study are the investigation of different fungi causing diarrhea in sheep and goats, identification of fungal isolates by different conventional methods and application of fungal sensitivity tests on some identified isolates.

2. Materials and methods

1. *Collection of Samples*

A total number of one hundred (100) fecal swabs were collected in sterile tubes, from 70 sheep and 30 goats of different ages and sexes suffering from diarrhea in small shepherd flocks at Fayoum governorate, Egypt. The samples were inoculated onto sterile test tubes having 10 – 15 ml sterile saline. Human samples (buccal samples, skin swabs and nasal samples) were obtained from 40 workers according to **Axéll *et al.*, 1985; Evans & Stevens, 1976 and Polzehl *et al.*, 2005**. A total of 60 feed stuffs samples of the flocks were collected in sterile, clean and dry plastic bags and transferred to the laboratory for mycological examination **(ISO 21527-2, 2008)**. The collected samples were kept in sterile polyethylene bags and preserved in an ice box then transferred to the laboratory in Animal Health Research Institute, Dokki, Giza, Egypt under complete aseptic condition without undue delay to be examined mycologically as shown in table (1).

Table (1): Total number of tested samples from different sources

Total number of all tested sample	Animal		Workers	Feed stuffs				
200	Sheep	Goat	Men	Maize	Hay	Beet		Grasses
						Roots	Leaves	
	70	30	40	28	14	4	6	8

2. *Mycological examination*

A- Isolation of yeast and mould

Mycological examination was conducted according to **Lodder (1970)**. For yeast isolation, swab samples were inoculated into Sabouraud dextrose broth tubes (SDB) for 24-48 hours and subsequently, inoculated into duplicate plates of Sabaroud's dextrose agar (SDA) media with addition of chloramphenicol (50 mg/mL) to the media after autoclaving; to inhibit bacterial growth. and the inoculated plates were incubated at 37 ^{0}C for 48 hours.

Feed and other samples were directly inoculated into Sabouraud dextrose agar plates with chloramphenicol and inoculated plates were incubated at 37 ^{0}C for 48 hours. For mould isolation, the swabs and feed samples were inoculated into Sabouraud dextrose agar plates with chloramphenicol and incubated at 25±2°C for 5-7 days.

Negative plates were not disposed before 2 weeks (**Feingold & Baron, 1986**). All the positive moulds cultures examined for gross and micro morphological characteristics (**Collins & Lyne**, **1984** and **Refai, 1987**).

B. *Identification of yeast isolates*

The isolates were picked up by sterile loop from primary culture and subculture on to Sabouraud dextrose agar slopes and identified by **Lodder (1970)**. Phenotypic methods were done according to the morphological examination of colonies and rapid yeast identification system. The morphological examination was carried by studying the macroscopic and microscopic characters of the isolates **(Finegold & Martin, 1982)**. The Gross appearance of colonies were described through rate and pattern of growth, their size, consistency and surface colour. Slide mount technique was carried by placing small part of the colony on a slide with one drop of lactophenol cotton blue then covered with cover slip and examined under high power magnification for the presence of yeast cells. Smears were prepared from the purified colonies and stained with Gram's stain **(Cruickshank *et al.*, 1975)** then examined microscopically for present of gram positive large spherical yeast cells. Suspected isolates are streaked on CMA (Corn meal agar, Copper and Silvo-Hunter, 1985) plates in a form of four lines and half of all lines were covered with sterile cover slips. The plates were incubated at 25ºC for 24 - 48 hours. The cultures were examined for various morphological features such as pseudohyphae, blastospores, arthrospores and chlamydospores.

C. Identification of mould isolates: **(Pitt & Hocking, 2009)**

Studying the morphological characters of colonies included growth appearance of the cultures, rate of growth, texture and color of the surface and reverse side colonies according to **Al-Dorry (1980)** and **Refai (1987).**The isolates were subcultured on Malt extract agar and Czapek yeast agar by three-point technique method, the plates were incubated at 25^{0}C for 5-7 days. The identification of the colonies was carried out by careful observation and measurements of the macroscopical and microscopical characteristics of the mould colonies. The macroscopical examination of mold colonies included the rate and pattern of growth, as colour, texture, basal and

surface mycelia, reverse of the colony and the rate of colony growth and diameter. The examination was carried out by using a magnifying hand lens. The microscopical examinations were performed through wet mount slide technique **(Arx, 1967)** and/or staining with lactophenol cotton blue **(Dugan, 2006).** The prepared slides were examined under low power and oil immersion lenses to characterize the morphological structures of the mould growth concerning the conidial stage, head, vesicle, sterigmata, conidiophore, and conidia. Also, the Hulle cells or other hyphal peculiarities, sclerotia and ascosporic stage (cleistothecia and asci) were observed and recorded.

3. ***Antifungal Sensitivity testing for isolated moulds***

Two main groups of antifungals are used in the clinical setting to treat fungal infections: polyenes represented by (Amphotericin B, Nystatin,) and azoles with several derivatives such as itraconazole, voriconazole, clotrimazole, fluconazole (Humid); purchased from **HIMEDIA (Ref SD060-IPK)** using disk diffusion assay. The in vitro sensitivity of the isolate to antimicrobials was determined according to standards of National Committee for Clinical Laboratory **(NCCLS, 2002)**. The isolated fungi was subcultured on Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 2 day (yeast) and 25^{0}C for 5 days (mould). Loopfull from pure culture from each isolate was mixed well with 9 ml of sodium chloride solution then spreading over the surface of SDA plate then suction the excess fluid. The six antifungal discs were spread on the surface of inoculated plate. Plates were incubated at 37 ^{0}C for 24 hrs (yeast) and 25^{0}C for 5 days (mould). The diameter of inhibition zone of each disc was measured (mm) and judged.

4. ***Statistical analysis***

Correlation between workers, animal and feed stuffs as well as variance were statistically analyzed using excel 2010 statistics package.

3. Results and Discussion

Fungi causing diarrhea constitute one of the most important problems causing implications in sheep and goats, they may remain undiscovered causing economic losses and may have zoonotic potentials. Few studies were conducted on sheep and goats fungal pathogens in spite of many complications caused by them.

Mycological examination

In the current study, mycological examination revealed the isolation of 27 fungal isolates out of 200 were positive for yeast in a total percentage of (13.5%) and 112 fungal isolates out of 200 were positive for mould at a percentage of (56.00%) as demonstrated in Table (2). The mould were the predominat fungal infections (56%) among all examined samples (animals, workers and feed stuffs) than yeast infecions (13.5%). This may be due to that the examined feed stuffs as well as the nasal and hand samples of the workers were completely free from yeast infections. The high recurrence of mould isolates concurs with **Refai *et al.* (2010)** and **Bassiouny *et al.* (2019)**.

Table (2): Incidence of fungi isolated from all examined samples.

Type of sample	Animals						Workers			Feed stuffs					Total number in this study
	Sheep			Goats								Beet			
	ewe	ram	lamb	She	Male	kids	Buccal	Nasal	Hand	Maize	Hay	Roots	Leaves	Grasses	
Number of samples	40	20	10	16	9	5	16	12	12	28	14	4	6	8	200
yeast isolates	4	1	8	5	3	3	3	0	0	0	0	0	0	0	27
Total yeast isolates	13			11			3			0					13.5 %
Mould isolates	28	19	7	9	5	2	6	6	2	14	6	2	4	2	112
Total mould isolates	54			16			14			28					56 %

Incidence rate of isolated yeast and moulds

As summarized in **Table 3**, *Rhodotorula sp.* was the predominated isolated yeast from all fecal samples of diarrheic animals (10.00%) followed by *C. pseudotropicalis* at a percentage of (4.00 %), while *C. tropicalis* and *Torulopsis* were the least isolated yeast (2.00 % for each). *Geotrichum candidum* and *Saccharomyces* isolated at the same rate (3.00% for each) from all fecal samples. However, *A. flavus* was the most predominated mould isolated from the same fecal samples at a percentage of (26.00%) followed by *A. niger* at (17.00%), while the least isolates were *A. fischeri, A. Carbonarius, Penicillium expansum* and *Fusarium chlamydosporum* at equal percentage (1.00% for each) as shown in **Table 4**.

These results is nearly similar to **Jensen *et al.*, (1992)** and **Sarfati *et al.*, (1996)** who declared that the most common mycotic infections in ruminants were aspergillosis, candidosis and zygomycoses. **Jensen *et al.*, (1992)** reported that the respiratory and GI tracts are the portals for mycotic infections and the main organ for infection is the omasum. **Refai (1998)** mentioned that

Candida sp. may cause gastro- intestinal candidiasis in animals. **Hassan *et al.*, (2010)** noticed that, the yeasts isolated from the diarrheic cases of sheep and goats were at higher rates than that from apparently healthy animals. **Radostits *et al.*, (2000)** declared that diarrhea in adult ruminants were mostly associated with mycotic omasitis, rumenitis and enteritis. **Donskey (2004)** managed that the yeasts can infect the digestive tract of animals through setting animals on contaminated soils with yeast pathogens and licking the perianal region of animals infected with anal candidiasis by other healthy animals can transfer infection to the healthy animals causing oral candidiasis and subsequently gastrointestinal tract candidiasis. **Tell (2005)** mentioned that *Asperigillus sp.* is a cause of gastroenteritis in ruminants. **Abou-Elmagd *et al.* (2011)** declared that the fecal samples of sheep and other ruminants contain the higher amount of yeast than milk and rectal swabs suggesting that the GIT provides an important reservoir for *Candida* species. Fungi are opportunistic pathogens having several virulence factors that permit their development and grant infection foundation within the host. Pathogenic fungi secrete proteases **(Hube, 1998)** and phospholipases **(Ibrahim *et al.*, 1995)** which corrupt tissues contain protein and lipids, and this could explain the diarrhea of infected animals with pathogenic fungi.

Table (3): Incidence of yeasts isolated from diarrheic animals

	Animals												Total	
	Sheep						goats							
	Ewe		Rams		lambs		She goat		Male goat		kids			
Samples	**40**		**20**		**10**		**16**		**9**		**5**		**100**	
Rhodotorula sp.	2	5 %	-	0 %	1	100 %	3	18.75 %	2	22.22%	2	40 %	10	10 %
C. pseudotropicalis	-	0 %	1	5 %	2	200 %	1	6.25 %	-	0 %	-	0 %	4	4 %
C. tropicalis	1	2.5 %	-	0 %	-	0 %	-	0 %	1	11.11 %	-	0 %	2	2 %
Torulopsis	-	0 %	-	0 %	2	200 %	-	0 %	-	0 %	-	0 %	2	2 %
Geotrichum candidum	-	0 %	-	0 %	2	200 %	-	0 %	-	0 %	1	20 %	3	3 %
Saccharomyces	1	2.5 %	-	0 %	1	100 %	1	6.25 %	-	0 %	-	0 %	3	3 %

On the other hand; *C. guilliermondii* and *C. krusei* yeasts were isolated from human (workers) buccal samples at rate of (12.50%) and (6.25%), respectively while no *Yeast sp.* was isolated from nasal discharge or hand samples (**Table 5**). Moreover, *A. flavus, A. niger, A. fumigatus* and *A. terreus* moulds were isolated at rate of (12.50 %), (10.00 %), (7.50 %) and (5.00 %), respectively. *A. flavus* was the most predominant in buccal swab, while *A. niger* was the most predominant in nasal ones (**Table 6**).

Table (4): Incidence of mould isolated from diarrheic animals

	Animals												Total	
	Sheep						Goats							
	Ewe		Rams		Lambs		She goat		Male goat		kids		Total	
	40		**20**		**10**		**16**		**9**		**5**		**100**	
A. flavus	11	27.5 %	4	20 %	4	40 %	4	25%	2	22.22%	1	20%	26	26.00%
A. niger	6	15 %	7	35 %	-	-	3	18.75 %	1	11.11%	-	-	17	17.00
A. fischeri	1	2.5 %	-	-	-	-	-	-	-	-	-	-	1	1 %
A.carbonarius	-	-	1	5 %	-	-	-	-	-	-	-	-	1	1 %
Penicillium expansum	-	-	-	-	1	10.00 %	-		-	-	-	-	1	1 %
Fusarium chlamydosporum	-	-	1	5	-	-	-		-	-	-	-	1	1 %
A. terrus	1	2.5 %	3	15 %	1	10 %	-	-	1	11.11%	-	-	6	6 %
Rhizopus sp.	4	10 %	2	10 %	1	10 %	1	6.25 %	1	11.11%	-	-	9	9 %
Mucor sp.	5	12.5%	1	5 %	-	-	1	6.25 %	-	-	1	20%	8	8 %

These results are in accordance with that reported by **Marasas & Nelson (1987)** who mentioned that ingestion of contaminated food with *Asperigillus* and *Penicillium sp.* may lead to intoxication in both humans and animals.

In addition, it was observable that no yeast was isolated from feed stuffs. *A. flavus, A. niger, A. fumigatus* and *A.terreus* were the most predominant isolates at incidence rate of (16.66%),(11.66%), (3.33%) and (3.33%), respectively. While; *A.carbonarius, Penicillium*

griseofulvum, Penicillium simplicissimum, Rhizopus sp., Mucor sp., Eurotium chevalieri and *Eurotium rubum* were isolated at incidence rate of (1.66%) each **(Table 7)**. The current results go in accordance with **Radad & Khalil (2011)** who declared that mouldy hay, straw or moist feed may lead to systemic mycosis in ruminants**. Kedar Karki (2015)** reported that feeding on leaves infested with Penicillium may cause outbreaks of systemic mycosis in goats. **Coulombe (1993)** found that faulty storage of feed stuffs and forages may lead to *Asperigillus*, *Fusarium, and Penicillium* growth. **Chenaoui *et al.*, (2017)** isolated *Alternaria; Fusarium; Pythium; Rhizopus; Botrytis; Aspergillus; Rhizoctonia and Penicillium* from sugar beet**.**

Table (5): Incidence of yeasts isolated from workers.

	Workers						Total
	Buccal		Nasal		Hand		
	16		12		12		40
C. guilliermondii	2	12.50 %	-	0.00 %	-	0.00 %	5.0 %
C. krusei	1	6.25 %	-	0.00 %	-	0.00 %	2.5 %

Table (6): Incidence of mould isolated from workers

	Workers						Total	
	Buccal		Nasal		Hand			
	16		12		12		40	
A. flavus	4	25 %	1	8.33 %	-	-	5	12.50 %
A. niger	1	6.25 %	3	25 %	-	-	4	10.00 %
A. fumigatus	1	6.25 %	1	8.33 %	1	8.33	3	7.50 %
A. terreus	-	-	1	8.33 %	1	8.33	2	5.00 %

Table (7): Incidence of moulds isolated from feed stuffs

	Feed stuffs										Total	
	Maize		Hay		Beet-root		Beet-leaves		Grasses			
	28		14		4		6		8		60	
A. flavus	4	17.85%	2	14.28%	1	2.50%	2	33.33	1	12.5%	10	16.66%
A. niger	4	14.28%	1	7.14%	-	-	1	16.66	1	12.5%	7	11.66%
fumigatus	2	7.14%	-	-	-	-	-	-	-	-	2	3.33%
terreus	2	7.14%	-	-	-	-	-	-	-	-	2	3.33%
A.carbonarius	-	-	-	-	1	2.50%	-	-	-	-	1	1.66%
Penicillium griseofulvum	1	3.57%	-	-	-	-	-	-	-	-	1	1.66%
Penicillium simplicissimum	-	-	-	-	-	-	1	16.66	-	-	1	1.66%
Rhizopus Spp.	1	3.57%	-	-	-	-	-	-	-	-	1	1.66%
Mucor Spp.	-	-	1	7.14%	-	-	-	-	-	-	1	1.66%
Eurotium chevalieri	-	-	1	7.14%	-	-	-	-	-	-	1	1.66%
Eurotium rubum	-	-	1	7.14%	-	-	-	-	-	-	1	1.66%

Morphological identification of yeast isolates

Rhodotorula sp. was distinguished on SDA by development of carotenoid pigments; that differ from orange to red (light pink flat colonies); nonetheless, microscopically, showed budding of round, oval enormous cells when stained with Gram's stain **(Fig. 1).** This result is in accordance with some authors **(El-Gamal, 2000; Ali, 2007; Refai *et al.*, 2010; Abbas *et al.*, 2016 and Tartor *et al.*, 2018).**

Candida sp. colonies on SDA seemed smooth hued pale colonies within 48-72hrs. Microscopically, presence of oval; round and extended yeast cells **(Fig. 2).**Nearly similar results were recorded by **(Abbas *et al.*, 2016; Bassiouny *et al.*, 2019 and Abd El-Tawab *et al.*, 2020)** but disagree with **Tartor *et al.*, (2018)**. The observed colonies of all yeast isolates concur with previous reports **(Abou-Elmagd *et al.*, 2011 and Chenaoui *et al.*, 2017)**.

Morphological identification of mould isolates

Interestingly, *Aspergillus species* exhibited some variety inside similar clas. *Aspergillus flavus (A. flavus)* seemed smooth with various aerial developments; the shading changes from yellow to yellowish green by maturing **(Fig. 3)**. Colonies of *Aspergillus niger (A. niger*) had black color with transmitted edges with wooly surface **(Fig. 4).** Colonies of *Aspergillus fumigatus* (*A. fumigatus*) have distinct edge with some shades of green, blue-green, surface has a powder appearance with a white cover was oserved at the edge in the zone of active growth **((Fig. 5);** and that of *Aspergillus terrus (A. terrus*) were smooth velvety collapsed

buff to dark brown **(Fig. 6).** These outcomes concurred for certain reports **(El-Gamal, 2000; Refai *et al.*, 2010; Bassiouny *et al.*, 2019 and Abd El-Tawab *et al.*, 2020).**

Pencillium sp. colonies were white and fluffy then, transformed into greenish blue in colour while microscopically, there were septated hyphe with un branched condiophores having metula with flagon molded (flask- shaped) strigmata and the metula bear un branched chain of conidia; the whole construction form brush appearance **(Fig. 7).**. *Fusarium sp*. colonies were cottony or wooly in texture, snow white, pink-violet or rosy-red in color, with dispersion of colored pigments into the opposite surface of the medium and microscopically, they seemed long, branched and septated hyphae from which short conidiophores rose separately or in gatherings, and sometimes branched **(Fig. 8).**. This result is in accordance with some authors (**El-Gamal, 2000; Refai *et al.*, 2010 and Bassiouny *et al.*, 2019).**

Colonies of *Mucor sp*. showed up quickly developing, white-to-gray cotton candy, became dark with time and fills the petri dish with fluffy mycelium; and microscopically, non-septated wide hyphae. Sporangiophores are long, might be branched and end with bear terminal round sporangia. The sporangia have a thin wall which when mature disintegrates (or is disrupted) to release round or somewhat ellipsoidal sporangiospores (4-8µm diameter). With the spores dispersed. No rhizoids are formed**(Fig. 9).** ***Rhizopus sp.*** colonies were profoundly cottony; white went to gray-brown on surface with maturing. Microscopically**,** expansive hyphae could be noticed. Sporangiophores are unbranched and connect to each other by septated hyphae forming huge sac-like sporangia that contain sporangiospores **(Fig. 10)**. This result is in accordance with some authors (**Ali, 2007; Refai *et al.*, 2010 and Bassiouny *et al.*, 2019).** The observed colonies of all mould isolates recorded in the current study concur with previous reports **(Abou-Elmagd *et al.*, 2011; Chenaoui *et al.*, 2017; Bassiouny *et al.*, 2019** and **Abd El-Tawab *et al.*, 2020**). **Refai *et al.* (2010)** has characterized *Aspergillus sp.*, *Penicillium sp.* and *Rhizopus sp*. as normal mycoflora and these species may be considered as opportunistic pathogens as many of these genera possess virulence factors which enable them to cause disease especially under favorable conditions.

Statistical Analysis:

A. Correlation test:

Correlation between workers, animal and feed stuffs

	human	***animal***
Workers	1	
Animal	0.835889	1
	feed	***human***
Feed	1	
Workers	0.928572	1
	animal	***feed***
animal	1	
feed	0.90824	1

There is strong positive correlation between presence of mold in feed stuff, animal and worker samples

B. variance test:

t-Test: Two-Samples Assuming Unequal Variances						
	Human	***Animal***	**Animal**	**Feed**	***Human***	***Feed***
Mean	1.5	5.2143	5.214286	1.5	1.5	1.5
Variance	2.2692	59.72	59.71978	9.653846	2.269230769	9.653846154
Observations	14	14	14	14	14	14
Hypothesized Mean Difference	0		0		0	
Df	14		17		19	
t Stat	-1.765		1.668561		0	
P(T<=t) one-tail	0.0497		0.056758		0.5	
t Critical one-tail	1.7613		1.739607		1.729132812	
P(T<=t) two-tail	0.0993		0.113516		1	
t Critical two-tail	2.1448		2.109816		2.093024054	

P value > 0.05 thus null hypothesis cannot be rejected and distribution of mold in feed stuff, diarrheic animal and worker samples has an equal means.

Antifungal sensitivity test

To discern antifungal tolerance and determine the best treatment for a specific fungus, antifungal susceptibility testing methods were used. These methods are used in clinical microbiology to determine the best treatment for a fungal infection and to determine the local and global epidemiology of antifungal sensitivity.

Anti-fungal sensitivity test was conducted using the disc diffusion method for the isolated strains. Results of antifungal sensitivity of different fungal isolates from fecal samples of diarrheic animals and human samples are smmerized in **Tables 8** and **9**. In fecal samples of diarrheic animals, *C. krusei*, *C. Pseudotropicdis*, *C. tropicalis* and *Torulopsis* are highly senstive to Voriconazole meanwhile, *Rhodotorula* and *P. expansum* are senstive to Itraconazole. *A. terreus* and *A. flavus* are highly senstive to Clotrimazole and Voriconazole, respectively. *Fusarium* is completely resistant to the six used antifungal discs. However, antifungal sensitivity of different fungal isolates from human samples showed that *C. guilliermondii* and *C. krusei* are highly sensitive to Voriconazole and Itraconazole, respectively meanwhile *A. flavus* and *A. niger* are highly sensitive to Itraconazole. All fungal isolates from human samples are resistant to Metronidazole.

These results concur with previously investigated records. **Ellepolla & Samaranayake (2000)** mentioned that miconazole, clotrimazole, ketoconazole, fluconazole, and itraconazole are effective for the treatment of oral candidiasis**. Kauffman (1996)** recorded that itraconazole is effective for candidiasis. **Kauffman & Carver (1997)** declared that the management of fungal infections has revolutionized by the introduction of the imidazole and azole groups of antifungals. **Hendrickx *et al.*, (2012)** reported that *A.niger* was sensitive to treatment with Voriconazole and Itraconazole. **Tokarzewski *et al.*, (2012)** declared that, *A. niger* exhibited high susceptibility to Voriconazole, low susceptibility to Clotrimazole, Miconazole and Nystatin and resistant to Fluconazole.

Table (8): Results of antifungal sensitivity of different fungal isolates from fecal samples of diarrheic animals

	C. Krusei	***C. Pseudotropicdis***	***C. tropicalis***	***Torulopsis***	***Rhodotorula***	***p. expansum***	***Fusarium***	***A. terreus***	***A. flavus***
NS 100	24 mm	30 mm	13 mm	22 mm	- ve	- ve	- ve	9 mm	20 mm
C C 10	30 mm	25 mm	31 mm	25 mm	8 mm	- ve	- ve	40 mm	- ve
VRC1	40 mm	40 mm	35 mm	25 mm	- ve	- ve	- ve	27 mm	40 mm
IT 10	26 mm	29 mm	33 mm	23 mm	35 mm	10 mm	- ve	30 mm	30 mm
Ap 100	12 mm	19 mm	10 mm	6 mm	- ve	- ve	- ve	9 mm	8 mm
FCA 25	- ve	23 mm	- ve	- ve	- ve	- ve	- ve	- ve	- ve
MTZ5	- ve	20 mm	25 mm	4mm	- ve	- ve	- ve	- ve	- ve

NS 100 = Nystatin**,** C C 10 = Clotrimazole**,** VRC1 = Voriconazole**,** IT 10 = Itraconazole**,** Ap 100 = Amphotericin B**,** FCA 25 = Fluconazole**,** MTZ5 = Metronidazole.

Table (9): Results of antifungal sensitivity of different fungal isolates from human samples

	C. guilliermondii	***C. Krusei***	***A. flavus***	***A. niger***
NS 100	- ve	26 mm	- ve	16 mm
C C 10	30 mm	34 mm	17 mm	20 mm
VRC1	41 mm	30 mm	- ve	- ve
IT 10	- ve	40 mm	18 mm	20 mm
Ap 100	12 mm	15 mm	- ve	10 mm
FCA 25	31 mm	- ve	- ve	- ve
MTZ5	- ve	- ve	- ve	- ve

NS 100 = Nystatin**,** C C 10 = Clotrimazole**,** VRC1 = Voriconazole**,** IT 10 = Itraconazole**,** Ap 100 = Amphotericin B**,** FCA 25 = Fluconazole**,** MTZ5 = Metronidazole**.**

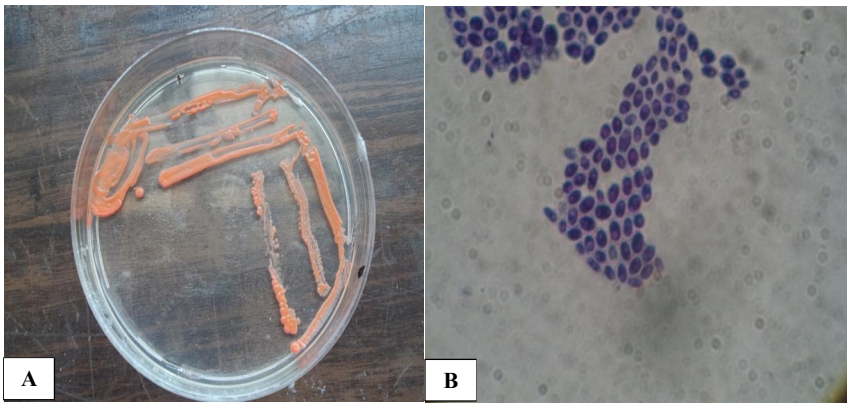

Figure (1): (A): *Rhodotorula* species on SDA produce cartenoid pigment.
(B): *Rhodotorula* species showing round to ovoid cells, single or in clusters, stained by Gram´s stain.

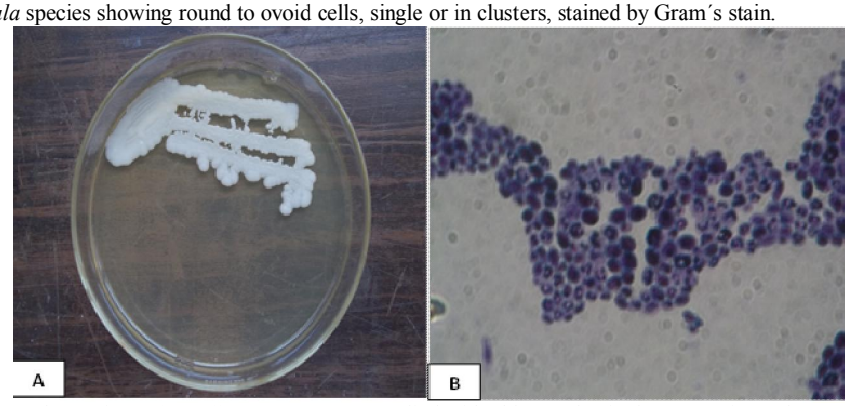

Figure (2): (A): Colony of *Candida albicans* on SDA supplied with chloramphenicol at 37º C for 48 h.
(B): *C. albicans* stained with Gram´s stain.

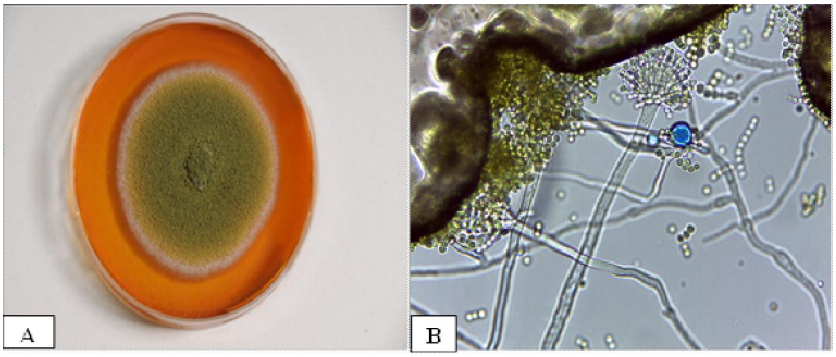

Figure (3): (A): A colony of *A. flavus* on SDA at 25 ºC , one week old.
(B): Typical head of *A. flavus* ,stained by lactophenol cotton stain (40X).

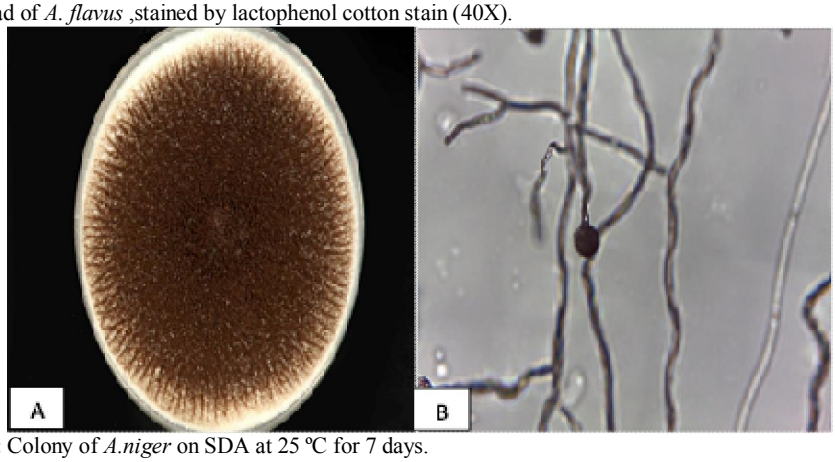

Figure (4): (A): Colony of *A.niger* on SDA at 25 ºC for 7 days.
(B): *A. niger* showing characteristic round head with black conidia, stained by lactophenol cotton blue stain. (40X).

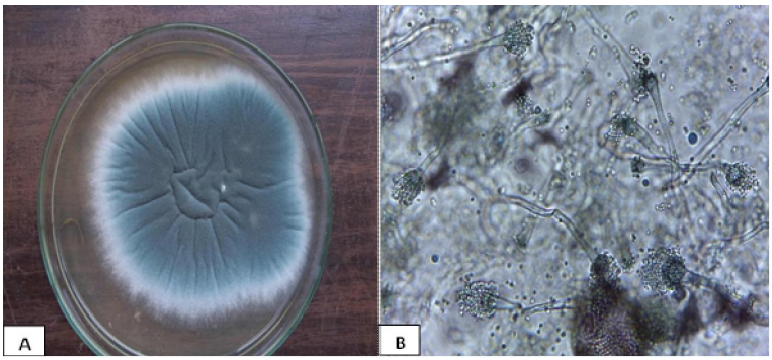

Figure (5): (A): A colony of *A. fumigatus* on SDA at 25º C for 7 days.
(B): *A. fumigatus* with columnar head, stained by lactophenol cotton blue stain (40X).

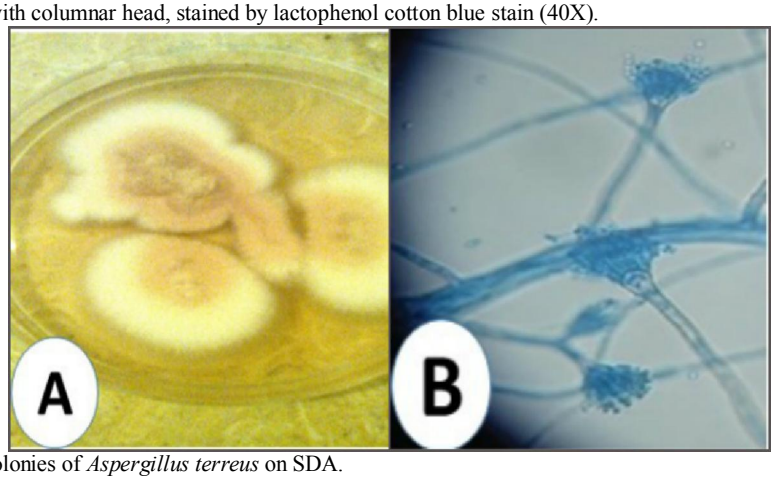

Figure (6): (A): Colonies of *Aspergillus terreus* on SDA.
(B): *A. terreus* showing small hemispherical vesicle.

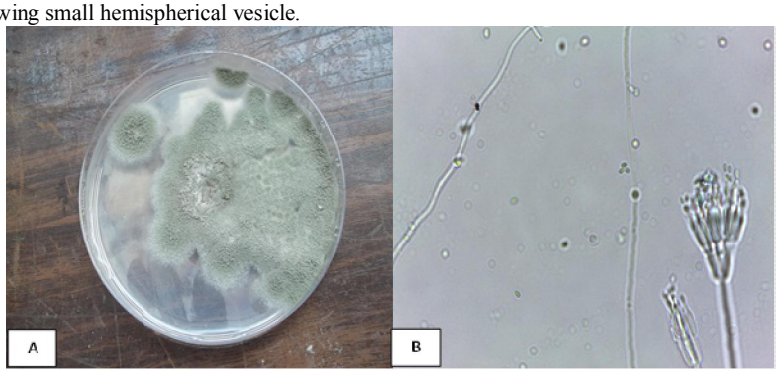

Figure (7): (A): *Penicillium sp.* showing greenish blue colonies in colour on SDA.
(B): *Penicillium sp.* showing brush- like arrangement under microscope

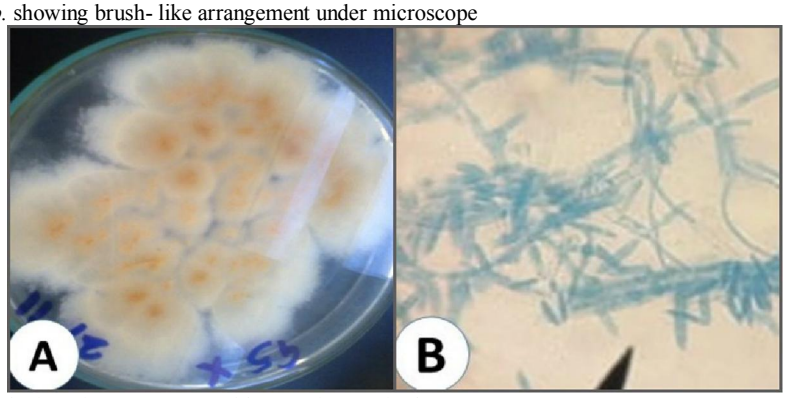

Figure (8): (A): Colonies of *Fusarium sp.* with rose pigment in the center on SDA
(B)*: Fusarium sp.* under light microscope.

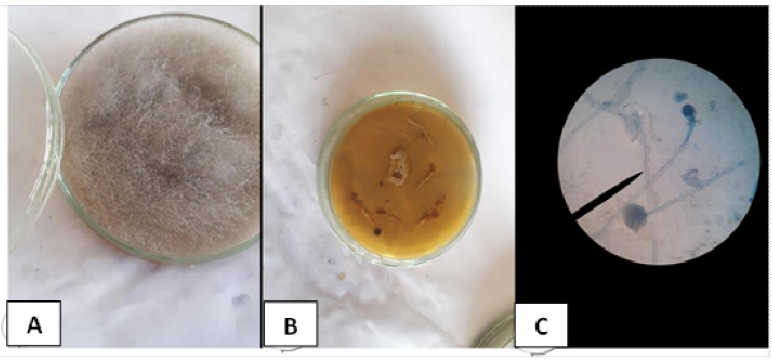

Figure (9): (A): Colonies of *Mucor sp*.; Petri dish…Surface side.
(B)*:* Colonies of *Mucor sp*.; Petri dish… reverse side.
(C): hyphae stained with LCB showing characteristic round sporangia.

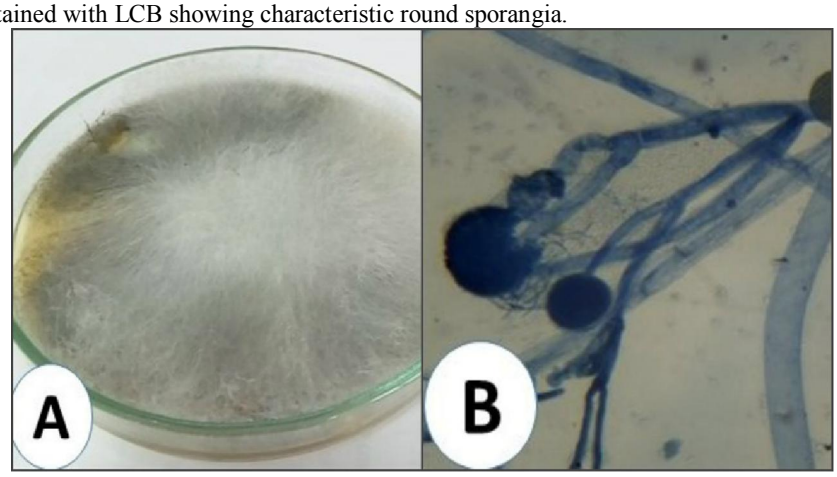

Figure (10): (A): Rhizopus colonies spread over the surface on SDA showing dens deeply cottony mycelium. Sporangia are seen as small black dots.
(B)*: Rhizopus sp.* showing long, branched Sporangiophores and terminate with rhizoids (X200)

Conclusion

In conclusion, different fungal isolates are associated with diarrhea in sheep and goats and can be transmitted to humans. There is a strong positive correlation between the presence of mold in feed stuff, animal and worker samples so proper and sanitary management practices should be adapted to decrease fungal infections. Most the fungal infections are sensitive to Voriconazole, Itraconazole and Clotrimazole. *Fusarium* is completely resistant to the six used antifungal discs. All fungal isolates from human samples are resistant to Metronidazole.

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Conflict of Interest:

The authors declare no conflicts of interest.

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