CONTENTS

		Page
1.	**Introduction** ……..……...	1
2.	**Review of literature** ……..……...……	4
	2.1. Classification of yeasts……	4
	2.2. *Rhodotorula* infections in cows and buffaloes……	7
	2.2.1. Mastitis……..	7
	2.2.2. Reproductive disorder……	11
	2.2.3. Gastro-intestinal infection in calves……..	13
	2.3. Human infections by *Rhodotorula* species…….	13
	2.3.1. Skin, Hair and Nail infection……	13
	2.3.2. Gastro-intestinal infections……...	17
	2.3.3. Bronchopulmonary mycosis…….	19
	2.3.4. Reproductive disorder……...	21
	2.3.5. Nervous system infections……	22
	2.3.6. Other Rhodotorula infections in human……	23
	2.4. Occurrence of *Rhodotorula* species in environment…...	27
	2.4.1. In air……..	27
	2.4.2. In water…….	29
	2.4.3. In dust and soil……..	33
	2.5. Identification of *Rhodotorula* species…….	35
	2.5.1. Phenotypic methods of identification of *Rhodotorula* spp…….	35
	2.5.2. Genotypic methods of identification of *Rhodotorula* spp…….	38

2.6. Antifungal sensitivity test for *Rhodotorula* species…… 40

3. Material and Methods……..……..... 44

3.1. Materials……..……..…….……..…… 44

3.1.1. Samples…….. 44

3.1.1.1. Animal samples…….. 44

3.1.1.2. Human samples…….. 45

3.1.1.3. Environmental samples…….. 46

3.1.2. Media…….. 48

3.1.3. Material used for identification…….. 49

3.1.3.1. Lactophenol cotton blue……. 49

3.1.3.2. Gram's stain……... 49

3.1.3.3. API 20C Aux……... 49

3.1.3.4. Material used for polymerase chain reaction (PCR)…….. 55

3.1.3.5. Material used for DNA sequencing……... 58

3.1.4. Antifungal sensitivity discs…… 58

3.2. Methods…..…….…… 60

3.2.1. Collection of samples……. 60

3.2.1.1. Collection of animal samples……. 60

3.2.1.2. Collection of human samples……. 60

3.2.1.3. Collection of environmental samples……... 61

3.2.2. Cultivation of samples on SDA with chloramphenicol……. 62

3.2.2.1. Cultivation of animal samples……... 62

3.2.2.2. Cultivation of human samples……... 63

3.2.2.3. Cultivation of environmental samples…….. 63

3.2.3. Identification of isolates……. 64

3.2.3.1. Phenotypic methods of identification of isolates... 64

A- The morphological examination…… 64

B- API 20C Aux system for identification of yeasts…….. 65

3.2.3.2. Genotypic methods of identification of isolates… 68

A- Method of PCR……. 68

B- DNA sequencing…… 72

3.2.4. Antifungal sensitivity test (Disc method)…….. 73

4. Results……..…. 75

4.1. Isolation of genus *Rhodotorula* from samples…….. 75

4.1.1. Isolation of *Rhodotorula* spp. from diseased buffalo samples…… 75

4.1.2. Isolation of *Rhodotorula* spp. from apparently healthy buffalo samples…….. 75

4.1.3. Isolation of *Rhodotorula* spp. from diseased cow samples…… 77

4.1.4. Isolation of *Rhodotorula* spp. from apparently healthy cow samples……... 77

4.1.5. Isolation of *Rhodotorula* spp. from human samples…… 79

4.1.6. Isolation of *Rhodotorula* spp. from surrounding animal environment…… 79

4.1.7. Isolation of *Rhodotorula* spp. from surrounding human environment…… 79

4.2. Identification of isolates…… 81

4.2.1. Phenotypic methods of identification……... 81

A. Morphological identification……. 81

B. Identification by API 20C Aux system…….. 83

4.2.2. Genotypic identification of *Rhodotorula* isolates…. 89

A. Polymerase Chain Reaction (PCR)…… 89

B. DNA sequence polymorphisms…….. 92

4.3. Antifungal sensitivity test (Disc Method)…… 112

5. Discussion……. 115

6. Conclusion……. 125

7. Summary ……. 126

8. References……. 129

9. Vita……...……. 166

Arabic Summary..……

List of abbreviations

Abb.	
5-FC	5-flurocytosin
API 20C	Appareils et proce'de's d'identification
BAL	Bronchial alveolar lavage
C	Candida
CFU	Colony forming units
CSF	Cerebrospinal fluid
DBB	Diazonium blue B salt
DNA	Deoxyribonucleic acid
HIV	Human immunodeficiency virus
ITS	Internal transcribed spacer region
KOH	Potassium hydroxide solution
LPCB	Lactophenol cotton blue
MIC	Minimum inhibitory concentration
MSP-PCR	Mini-micro satellite primed PCR technique
NaCl	Sodium chloride
PCR	Polymerase chain reaction
PFGE	Pulsed field gel electrophoresis
Prolysis-GC-MS	Analytical prolysis-gas chromatography-Mass spectrometry
R	Rhodotorula
RAPD	Random amplification of polymorphic DNA analysis
rDNA	Recombinant- Deoxyribonucleic acid
rpm	Revolution per minute
rRNA	ribosomal Ribonucleic acid
S	Saccharomyces
SBS	Sick building syndrome
SDA	Sabouraud’s dextrose agar medium
SDS-PAGE	Sodium dedecyl sulfate polyacrylamide acyl gel electrophoresis
spp	Species
T	Torulopsis

7. SUMMARY

This study was conducted on 1830 samples which were collected from human (100 samples of infected nails of Onychomycosis), diseased animals (350 buffaloes and 290 cows) and apparently healthy animals (400 buffaloes and 285 cows).

The samples collected from diseased cases included mastitic milk samples (150 buffaloes and 100 cows), rectal swabs from animals suffering from diarrhea (100 buffaloes and 90 cows), nasal swabs from animals showing nasal discharge (50 buffaloes and 50 cows) and vaginal swabs from animals suffering from any reproductive disorders (50 buffaloes and 50 cows).

The normal samples were collected from apparently healthy animals including 100 samples from each type of normal milk, rectal, nasal and vaginal swabs of apparently healthy buffaloes, also 60 samples of normal milk from cows and 75 samples from rectal, nasal and vaginal swabs of apparently healthy cows.

Environmental samples included 225 samples which were collected from environment of animal farms (90 air samples, 60 water samples and 75 soil samples) and 180 samples from different places surrounding human (60 samples from each type, air, water and soil).

All the samples were cultured on SDA with chloramphenicol and incubated at 30°C for 48-72 hours for mycological examination. The obtained results revealed that *Rhodotorula* species were isolated from human samples with an incidence of (5%) and from diseased cases of buffaloes with an incidence of (6.7%, 20%, 10% and 16%) from milk samples, rectal, nasal and vaginal swabs, respectively, while those obtained from apparently health buffaloes were with an incidence of (3%, 10%, 10% and 10%) from milk samples, rectal, nasal and vaginal swabs.

Rhodotorula isolates presented (8%, 22.2%, 24% and 22%) from diseased cases of cows and (10%, 20%, 13.3% and 9.3%) from apparently healthy cases of cows from milk samples, rectal, nasal and vaginal swabs, respectively.

Rhodotorula species were isolated with an incidence of (13.3%, 20% and 20%) from environment surrounding animals and with an incidence of (13.3%, 20% and 13.3%) from environment surrounding human from air, water and soil, respectively.

Yeast colonies which have carotenoid pigment were subjected for further identification by phenotypic and genotypic methods. All isolates which were identified morphologically by macro and microscopically examination revealed carotenoid

pigments colonies on SDA with orange to red surface color, large round, ovoid or elongated cells with lactophenol cotton blue, large gram positive yeast cells with Gram's stain and absence of hyphae and pseudohyphae with large blastospare on rice agar.

The identification of *Rhodotorula* isolates into species was performed using API 20C Aux system according to its substrate assimilation profile. Among 25 selective isolates, 22 isolates were *R. mucilaginosa*, 2 were *R. glutinis* and one was *R. minuta*.

On the other hand, 14 isolates were examined by molecular method polymerase chain reaction (PCR). ITS 2 of PCR products obtained from all isolates identified them as genus *Rhodotorula* at 429 bp.

DNA sequences for 5 isolates from them identified 3 as *R.mucilaginosa*, one as *R. slooffiae* and one was identical to *Rhodotorula* species known in gene bank as strain CBS 8885.

On the other hand, antifungal sensitivity test for *Rhodotorula* isolates revealed high sensitivity for nystatine, followed by ketoconazole and clotrimazole, while resistance was observed with fluconazole and amphotericin B.