Results

Distribution of conjunctivitis among test groups of patients;

In the present study, 214 patients of conjunctivitis were selected from the ophthalmologic Department Al Zahraa University Hospital, Faculty of Medicine, Al Azhar university, Cairo, Egypt were investigated to detect the different microorganisms (bacteria and fungai), that cause inflammatory disease of the conjunctiva. The investigation was carried out on patients during a period of two –years starting from July 2007 till August 2009.

Table (1) and Fig. (1) illustrate the classification of the cases suffering from conjunctivitis according to age and sex. Out of 214 patients, 122 were females and 92 were males. The highest incidence of the disease was estimated in adult patients with age range of 31-50 years (28.5%), followed by adolescents with age range of (13-20) years (15 %). The lowest incidence of conjunctivitis was recorded in patients with age range of 71-81 years (2.8 %).

Table (1): Distribution of total conjunctivitis patients according to age and sex.

Age groups	Male	Female	Total	%
<1-2	6	3	9	4.2
2-6	14	12	26	12.1
7-12	17	12	29	13.6
13-20	15	17	32	15
21-30	10	14	24	11.2
31-50	15	46	61	28.5
51-70	13	14	27	12.6
71-81	2	4	6	2.8

Total	**92**	**122**	**214**	100
%	**42.9**	**57.1**	**100**	

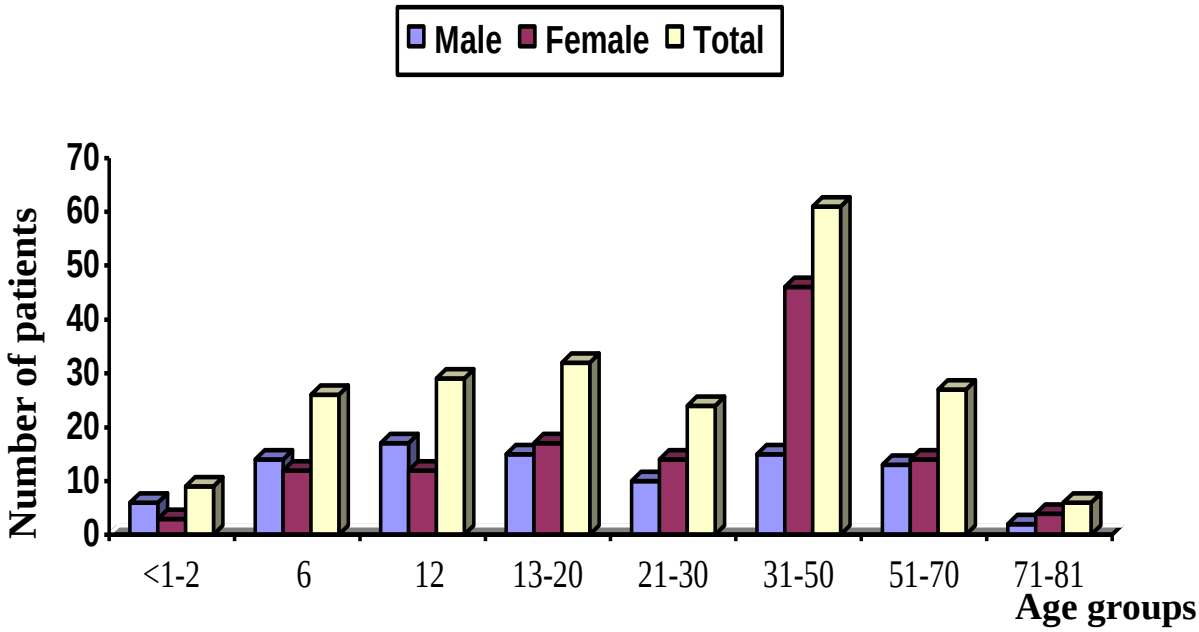


Fig. (1): Distribution of total number of conjunctivitis patients

1 - Distribution of bacterial infection among isolates:-

The number and percentage of patients suffering from bacterial conjunctivitis are presented in (Table 2 and Fig. 2). The data in this table showed that bacterial conjunctivitis were recorded in 143 patients out of 214 patients constituting 66.8%. Patients with age range of (31-50) and (13-20) represented the two groups of patients markedly affected with bacteria was they represented 45.5 %and 38.2 % respectively. Male children, female adolescents and adults were more susceptible to infection than the corresponding sex.

Table (2): Frequencies of bacterial conjunctivitis according to age and sex.

Age groups	Male	Female	Total	%
<1-2	8	4	12	8.4
2-6	12	9	21	14.9
7-12	12	9	21	14.9
13-20	10	14	24	16.8
21-30	7	5	12	8.4
31-50	7	20	27	18.9
51-70	9	8	17	11.9
71-81	3	6	9	6.3
Total	**68**	**75**	**143**	100
%	**47.8**	**52.2**	**100**	

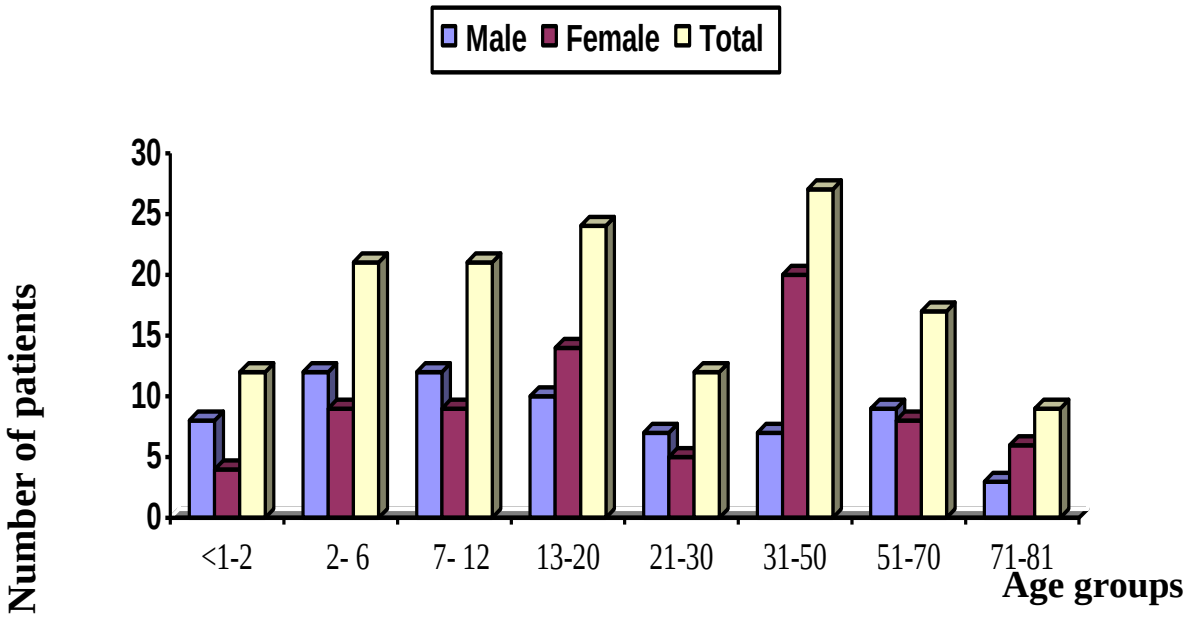


Fig. (2) : Distribution of f bacterial conjunctivitis patients according to age and sex

2 - Distribution of fungal infection among isolates:

Fungal conjunctivitis was diagnosed in 48 patients out of 214 patients matching 22.4% (Table 3 and Fig. 3). The highest incidence of fungal disease observed in males and females lies mainly in age range of 31-50 years (33.3% of total fungal conjunctivitis), where the number of infected females was about two times that of males. The percentage of conjunctivitis was more prevalent in adult cases than in children.

Table (3): Frequencies of fungal conjunctivitis according to age and sex.

Age groups	Male	Female	Total	%
<1-2	0	2	2	4.2
2-6	1	2	3	6.3
7-12	3	3	6	12.5
13-20	5	4	9	18.8
21-30	2	3	5	10.4
31-50	4	12	16	33.3
51-70	3	4	7	14.5
71-81	0	0	0	0
Total	**18**	**30**	**48**	100
%	**37.5**	**62.5**	**100**	

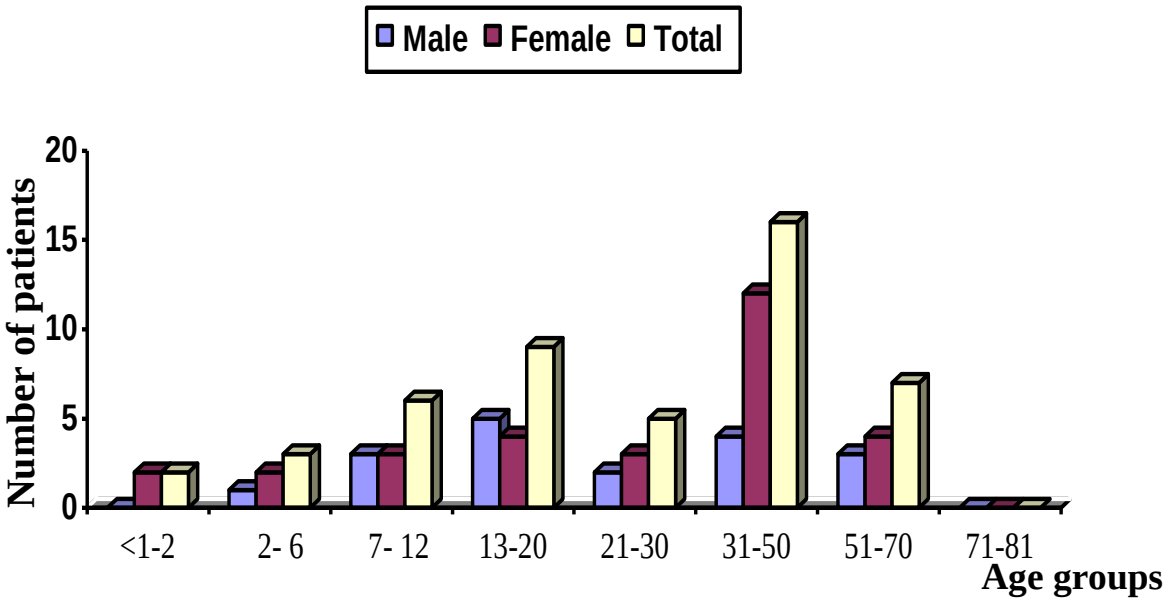


Fig. (3): Distribution of fungal conjunctivitis patients according to age and sex

3- Distribution of mixed infection:

Mixed cultures in conjunctivitis cases were observed, where two different organisms from the same case were isolated (Table 4 and Fig. 4) throughout the 214 patients . studying of conjunctivitis cases recovered 23 patients constituting 10.7% were infected with mixed microorganisms. Most of the mixed cultures were isolated from females. The highest incidence of fungal disease was observed in adult patients with age range of 31-50 years (34.8 %), followed by patients with age range of 21-30 years (26.1 %) and the lowest incidence of fungal disease was observed in patients with age range of 7-12 and 13-20 years.

Table (4): Frequencies of mixed culture microorganisms causing conjunctivitis according to age and sex.

Age groups	Male	Female	Total	%
<1-2	0	0	0	0
2-6	1	3	4	17.4
7-12	1	0	1	4.3
13-20	0	1	1	4.3
21-30	1	5	6	26.1
31-50	1	7	8	34.8
51-70	1	2	3	13.1
71-81	0	0	0	0
Total	**5**	**18**	**23**	100
%	**21.7**	**78.3**	**100**	

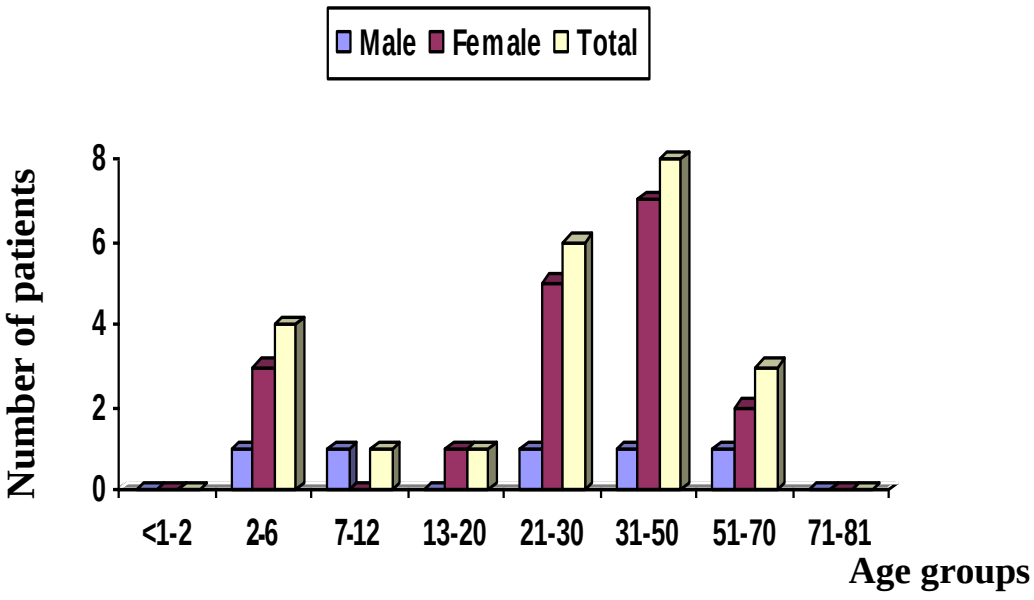


Fig. (4): Distribution of mixed culture microorganisms causing conjunctivitis according to age and sex

2- Identification of isolated Microorganisms:

2.1- Identification of bacterial isolates:

Identification of isolated bacterial strains carried out according to morphological and biochemical characters.

One hundred forty three bacterial isolates were collected from patients as described before the identification process up till species level was preceded according to references by: **Bergey's Manual of Systematic Bacteriology Volume A, B, C and D (2001, 2004, 2008 and 2009).**

The most morphological and biochemical characteristics of bacterial isolates that were required from their identification are summarized in the following results .There after mentioned the description of each isolates is mentioned.

2-1 Morphological and biochemical characteristics for Identification of Staphylococcus aureus:

Cells of *Staphylococcus aureus* were showed spherical 0.5-1.5μm occurring single pairs and irregular clusters, colones are usually yellow to orange, Gram negative stain, non motile, its optimal temperature 30-37°C

The other identification criteria are listed in table (5)

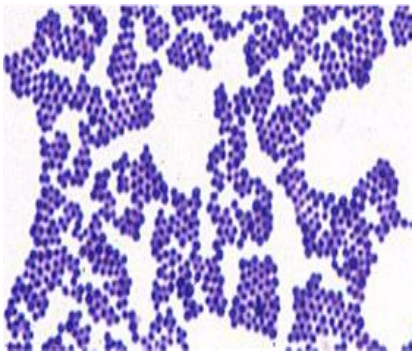


Fig (5):
cells of *Staphylococcus aureus stained by gram stain (x 100)*

Table (5) Some identification criteria of *Staphylococcus aureus:*

Test	*Result*
Pigment	+
Aerobic	+
Anaerobic	+
Growth on Nacl:	
Growth on 10% Nacl	+
Growth on 15% Nacl	±
Growth at 15-45°C	+
Alkaline phosphate	+
Arginine dihydrolase	+
Hemolysis	+
No_3 reduction	+
Oxidase	-
Urease	+
Fermentation:	
Arabinose	-
Celloliose	-
Fructose	+
Galactose	+
Lactose	+
Maltose	+
Mannitol	+
Mannose	+
Melezitose	-
Raffinose	-
Ribose	+
Sucrose	+
Trehalose	+
Xylose	-
Catalas	+
Spore	-

(-) = negative (+) = positive

2-1-2 Most morphological and biochemical characteristics for Identification of Pseudomonas aeruginosa:

Cells of *Pseudomonas aeruginosa* are straight or slightly curved rods, 0.5-1x1.5-5 μm, they showed negative Gram stain, motile, and have no growth at 41°C and 4°C.

The other identification criteria are listed in table (6)

Table (6) Some identification criteria of *Pseudomonas aeruginosa:*

Test	***Result***
Denitrification	+
PHB Poly hydroxy butyric acid	-
Levan from sucrose	-
Arginine dihydrolase	+
Oxidase	+
Gelatin hydrolysis	+
Strach hydrolysis	-
D-xylose	-
Glucose	+
Arginine	+
Lecithinase (egg, yolk)	-
Maltose	-
Catalase	+
Manitol	+
Ethylene glycol	-
Histiden	+

(-) = negative (+) = positive

2-1- Morphological and biochemical characteristics for Identification of Moraxella lacunata and Moraxella catarrhalis :

Cells of *Moraxella lacunata and Moraxella catarrhalis* were showed rods or cocci 1-1.5x2.5 μm occuring in pairs short chain, the rods are often very short & plump approaching a coccus shape, negative Gram stain, non motile, its optimal temperature 33-35°C.

The other identification criteria are listed in table (7)

Table (7) Some identification criteria of *Moraxella lacunata and Moraxella catarrhalis :*

Test	Result	
	Moraxella lacunata	***Moraxella catarrhalis***
Blood heamolysis	-	-
Gelatin hydrolysis	-	-
Growth in mineral Salts + NH_4^+ + acetate	-	-
Growth at 5°C	-	-
Growth in presence of 6% Nacl	-	-
Growth stimulated by bile salts	+	-
Phenylalanine deaminase	-	-
Urease	-	-
Nitrate reduction	-	+
Nitrite reduction	-	+
Sensitive to penicillin	+	+
* catalase	+	+
* acid production from carbohydrate	-	-
* sensetin to penicillin	+	-

(-) = negative (+) = positive

2-1- Morphological and biochemical characteristics for Identification of Streptococcus species:

Cells belonging to *Streptococcus* species are spherical or oval 0.5-2μm in pairs or in chains . The isolated bacteria appear non motile, non spore form, have positive Gram stain, optimal temp. at 37°C, growth at 10°C and 45°C are negative in all species.

The other identification criteria are listed in table (8)

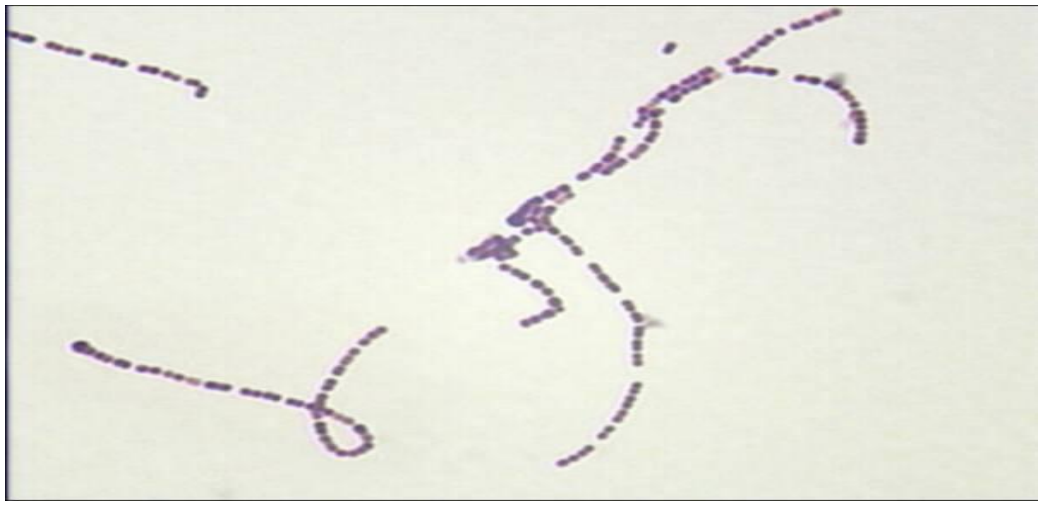


Fig (6):

Cells of *Streptococcus pneumoniae* stained by gram stain *(x 100)*

Table (8) Some identification criteria of *Streptococcus* species *:*

Test	Result		
	Streptococcus pyogenes	***Streptococcus pneumoniae***	***Streptococcus oralis***
Growth in presence 6.5% Nacl	-	-	-
Growth in presence 40% bile salt pH 9.6.	-	-	-
Catalase	-	-	-
arabinose	-	+	-
Erythritol	-	+	-
Fructose	+	+	+
Glucose	+	+	+
Lactose	+	+	+
Maltose	+	-	-
Mannitol	+	-	-
Raffinose	-	+	-
Ribose	-	-	-
Salicin	+	-	-
Sorbitol	+	-	-
Sorbose	-	-	-
Trehalose	+	+	+
Sucrose	+	+	+
Ornithine decarboxylase	-	-	-
urease	-	-	-
VP	-	-	-
Hippurate	-	+	-
Arginine	+	+	-
Esculin	-	-	-
α hemolosis	-	-	+
ß hemolosis	+	-	+
Inulin	-	+	-

(-) = negative (+) = positive

2-1-5 Morphological and biochemical characteristics for Identification of Escherichia coli, Klebsiella pneumoniae and Proteus vulgaris:

Cells belonging to *Escherichia coli, Klebsiella pneumoniae* and *Proteus vulgaris* are –negative Gram stain and have optimal temp. at 37°C. *Escherichia coli.,* are straight rods 1-1.5x2.6μm single or in pairs. *Klebsiella pneumoniae* are straight rods 0.3-1x0.6-6μm in length occurred singly,in pairs in short chain capsule. *Proteus vulgaris* are straight rod 0.4-0.8μm in width x 1-3μm in length.

The other identification criteria are listed in table (9).

Table (9) Some identification criteria of *Escherichia coli, Klebsiella pneumoniae* and *Proteus vulgaris*:

Test	Result		
	Escherichia coli.	***Klebsiella pneumoniae***	***Proteus vulgaris***
Glucose	+	+	+
Arabinose	+	+	-
Cellulose	+	+	+
Glycerol	+	+	-
Inositol	-	-	-
Lactose	+	-	-
Maltose	+	+	+
Mannitol	+	+	-
Mannose	+	+	-
Melibiose	+	+	-
Raffinose	-	+	-
Rhamnose	+	-	-
Cellibiose	-	+	-
Salicin	-	+	-
Sucrose	+	-	+
Trehalose	+	+	+
D-xylose	+	-	+
L-arabinose	+	+	-
Gelatin hydrolysis	-	-	+
Esculin hydrolysis	-	+	+
Urease	-	+	+
Lysine decarboxylase	-	-	-
Arginein dehydrolase	-	-	-
Oxidase	+	+	-
Indole production	+	-	+
MR	+	+	+
VP	-	+	-
Citrate utilization	-	+	-
H_2S	-	-	+
Phenylalanine deaminat	-	-	+
Orithinine decarboxylase	+	-	-
Nitrate reduction	+	+	+
Lipase reduction	+	-	+
Catalase reduction	+	+	+
growth KCN	-	+	+
Malonate	-	-	-
Capsule	+	+	+
Acetate	+	-	-
sorbitol	+	+	-

(-) = negative (+) = positive

2-1-6 Morphological and biochemical characteristics for Identification of Corynebacterium xerosis:

Cells of *Corynebacterium xerosis* are straight cylinder rods, 0.5-0.7x1.0-2.0μm occuring singly or in pairs and sometimes in short chain. The isolates were found to be Gram + positive, motile and its optimal temp at 30°C.

The other identification criteria are listed in table (10):

Table (10) Some identification criteria of *Corynebacterium xerosis*:

Test	Result
Arabinose	-
Xylose	-
Rhamnose	-
Fructose	+
Galactose	+
Mannose	+
Lactose	-
Maltose	-
Sucrose	+
Trehalsoe	-
Raffinose	-
Selicin	+
Dextrin	-
Starch	-
Hydrolysis	
Hippurate	+
Gelatin mufaction	-
Urease	-
Phosphatase	-
Tyrosine	-
Methyl red (MR)	-
Caseinase	-
Nitrate	-
Indole	-
Hameloysis	-
Catalase	-

(-) = negative (+) = positive

2-1-7morphological and biochemical characteristics for Identification of Haemophilus influenzae and Haemophilus aegyptius:

Cells of *Haemophilus* species are spherical oral shaped cells < 1μm in width & variable in length. The isolates exhibited Gram – negative stain for both *Haemophilus influenzae* and *Haemophilus aegyptius*. The isolates were non Motile and optimal temp. was 35-37°C for both species.

The other identification criteria are listed in table (11):

Table (11) Some identification criteria of *Haemophilus* species:

Test	Result	
	Haemophilus influenzae	***Haemophilus aegyptius***
MacCon Key agar, growth	-	-
β-Hemolysis, sheep cells	-	-
MR	+	+
VP	+	+
Arginine dihydrolase	-	-
d-Glucose, gas production	-	-
D-Adonitol	-	-
L-Arabinose	-	-
Cellobiose	-	-
M-Erythritol	-	-
Fructose	-	-
D-Glactose	+	+
D-Glucose	+	+
Glycerol	-	-
M-inositol	-	-
Inulin	-	-
Lactose	-	-
Maltose	+	+
D-Mannitol	-	-
Melezitose	-	-
Melibiose	-	-
Raffinose	-	-
L-Rhamnose	-	-
D-Ribose	+	+
Salicin	-	-
D-sorbitol	-	-
L-sorbose	-	-
Starch	+	-
Sucrose	-	-
Trehalose	-	-
D-xylose	+	+
Catalase	+	+
Oxidase	+	+
Nitrate reduction	+	+
Phosphatase	+	+
Galatinase	+	+
H_2S production	-	-
Ornithine decarboxylase	+	+
Indole production	-	-
Urease	+	+
Erculin hydrolysis	-	-

(-) = negative (+) = positive

Table (12): Some Identification Criteria of *Nessieria catarrhalis*

Nessieria catarrhalis	
Growth characteristics	0.6 – 1 µm in diameter Occur singly, in pairs with adjacent side flattened and some times in fours gram negative.
Gram	-
	Small circular convex, grayish white to dirty white, sometimes erose
Broth:	Turbid, often with a slight pellicle.
optimal temp	37°C
Biochemical reaction	
Test	**Result**
Fermentation	acid not produced from any carbon source.
Gelatin liquifaction	-
Indole	-
Nitrate	-
Acid production by fermentation	-
Glucose	+
Lactose	+
Maltose	+
Mannitol	+
Mannose	+
Melibiose	+
Raffinose	+
Rhamnose	+
Cillibiose	+
Sucrose	+
Trehalose	+
L-avabinose	+
Salicin	+
Glycerol	+
Arabinos	+
Inositol	+
Malonate	+
Acetate	+

(-)= negative (+) = positive

2.2- Identification of yeast isolates:

The most important biochemical and growth characteristics of the isolated pathogenic yeast that were required for their identification are summarized in tables (13 and 14) according to **Larone, (2002)**

Table (13) Surface growth of candida albicans on nutrient media :

Organism	Microscopic morphology on corn meal-Tween 80 agar at 25°C	Growth			Germ tubes
		In Sabouraud broth	With cyclo-heximide at 25°C	On SDA at 37°C	
C. albicans	+	NSG	+	+	+

Abbreviations: SDA, Sabouraud dextrose agar, +, positive and NSG, no surface growth.

Table (14) Biochemical characteristics of *Candida albicans*

Test	Result
Assimilation of	
Dextrose	-
Maltose	-
Sucrose	-
Lactose	-
Galactose	+
Melibiose	-
Cellobiose	-
Inosit ol	-
Xylose	+
Raffinose	-
Trehalose	-
Dulcitol	-
KNO_3	-
Fermentation of	
Dextrose	+
Maltose	+
Sucrose	-
Lactose	-
Galactose	+
Trehalsoe	+
Cellobiose	-
Raffinose	-
Mellibiose	-

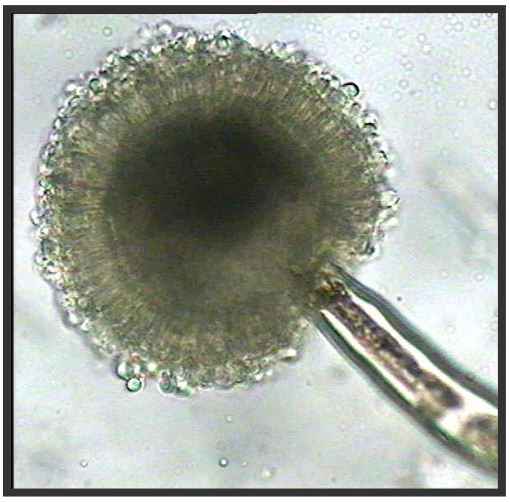
(-) = negative (+) = positive

2.3- Identification of fungal isolates:

The fungal isolates were identified according to their growth characteristics and microscopic examination using the Image Analysis System(light microscop) . The results are summarized as following:

Table (15):Identification criteria of *Aspergillus niger*

Character	Examination
*** Culture Exam.:**	
Growth characteristics	Colonies on Czapek agar growing rapidly attaining a diameter of 5.0 cm in 7 days, white basal mycelium bearing abundant conidial structures with black color and white at the margin.
*** Microscopic Exam.:**	
Conidial heads	500 µm in diameter.
Conidiophore	Conidiophores smooth walled (13.5 µm) in diameter.
Vesicle	Vesicle Globose, 35.0 µm in diameter.
Sterigmata	Strigmata uniseriate (17.2 X5.5 µm).
Conidia	Conidia Globose, 3.5 µm in diameter.



Aspergillus niger

Table (16):Identification criteria of *Aspergillus flavus*:

Character	Examination
*** Culture Exam.:**	
Growth characteristics	Colonies on Czapek agar attaining a diameter of 7.5 cm in 7 days with yellowish green.
*** Microscopic Exam.:**	
Conidiophore	Conidiophores rough walled (7.1 µm) diameter.
Vesicle	Vesicle globose 25.0 µm.
Sterigmata	Strigmata uni or bi seriate, primary (3.9X 2.0) µm. secondary (2.6 X 1.5) µm
Conidia	Conidia subspherical, 3.5 µm.

Aspergillus flavus

Table (17):Identification criteria of *Fusarium oxysporum :*

Character	Examination
*** Culture Exam.:**	
Growth characteristics	Colonies on PDA attaining a diameter of 3.9 cm in 4 days, with pale cream mycelium, the later becoming peach- colored with age. Reverse in yellow pigmentation.
*** Microscopic Exam.:**	
Conidiophores	Conidiophores unbranched at first, later branched with monophialides .
Micro-conidia	0-2 septate, ovoid –ellipsoidal (10 X 2.5) µm.
Macro-conidia	3-5 septate and measure (20 X4.0) µm.
Chlamydospores	Chlamydospores, not produced

Table (18):Identification criteria of *Rhizopus oryzae*:

Character	Examination
*** Culture Exam.:**	
Growth characteristics	Colonies whitish becoming brownish – grey with age; about 10 mm hight, 5.8 cm diameter in 4 days.
*** Microscopic Exam.:**	
Sporangia	Spherical, brownish grey to black 80.0 X 60.0 µm in diameter.
Collumella	Collumella , 35X22 µm .
Sporangiophores	Sporangiophores solitary or in groups (2-4); 9.5 µm in diameter.
Sporangiospores	Subglobose to ellipsoidal; striate, 6.5 X 4.5 µm in diameter.
Chlamydospores	Two type of chlamydospores are observed; globose (30 µm) and cylindrical (24X14 µm).

Table (19):Identification criteria of *Aspergillus fumigatus*:

Character	Examination
*** <u>Culture Exam.:</u>**	
Growth characteristics	Colonies on Czapek agar at 25 °C attaining a diameter of 4.2 cm within 7 days, with grayish green shades.
<u>* Microscopic Exam.:</u>	
Vesicle diam.	28 µm in diameter.
Sterigmata	6.8 x 2.6 µm.
Conidiophore diameter	20.2 µm in diameter.
Conidia	Globose, rought, 2.9µm in diameter.

Aspergillus fumigatus

Table (20): Identification criteria of *Penicillium albidum:*

Character	Examination
*** <u>Culture Exam.:</u>**	
Growth characteristics	Colonies 4.0 cm diameter on CYA, mycelium white to yellowish becoming dark green, with yellowish brown reverse. Colonies on MEA reached 2.5 – 4.0 cm green with reddish brown reverse. Micro-colonies formed on CYA at 5 °C. No growth on CYA at 37 °C.
<u>* Microscopic Exam.:</u>	
Penicillus type	Biverticillate and terverticillate .
Rami	20.0 X 4.6µm.
Metulae	11.3 X 3.4 µm.
Phialides	8.7 X 2.4µm.
Conidia	Conidia ellipsoidal to sub-spherical 3.7 µm .

Identification criteria of *Alternaria alternata:*

- ***Alternaria alternata*** colonies reaching 6-7cm diameter in seven days at 25-30 on Malt agar media. Conidiophore, Straight or flexuous, Pale brown to olive25-60 x 3-3.5 µm. Conidia Pale brown to light brown Pale brown to light brown 20-63 x 9-18 µm in size.

3-Distributon % of bacterial isolates among different patients groups;

3-1 child age:

Staphylococcus aureus was the most predominant bacterial species which was isolated from 12 patients out of 52 children patients (23%), followed by *Staphylococcus albus* which was found in 10 patients (19.2%). *Moraxella lacunata* came next and was isolated from 8 patients and constituted (15.4%) of the total bacterial isolated from children (Table 21 and Fig. 7). *Haemophilus influenzae and Staphylococcus albus* were recovered in female as mixed cultures and constituted (1.9%), while *Staphylococcus aureus and Streptococcus oralis* were recovered in the male patients as mixed cultures and constituted (1.9%).

Table (21): Distribution% of total bacterial conjunctivitis patients in different number of patients groups ;

Total Species isolates	Child				Adolescent				Adult				Total			
1- Single culture	**Male**	**Female**	**Total**	**%**	**Male**	**Female**	**Total**	**%**	**Male**	**Female**	**Total**	**%**	**Male**	**Female**	**Total**	**%**
Corynebacterium xerosis	1	2	3	5.8	2	2	4	16	2	3	5	7.6	5	7	12	8.4
Escherichia coli	2	2	4	7.7	0	1	1	4	1	2	3	4.5	3	5	8	5.6
Haemophilus aegyptius	0	0	0	0	0	0	0	0	2	4	6	9.1	2	4	6	4.2
Haemophilus influenzae	3	1	4	7.7	1	1	2	8	2	4	6	9.1	6	6	12	8.4
Klebsiella pneumoniae	0	0	0	0	0	0	0	0	1	2	3	4.5	1	2	3	2.1
Moraxella lacunata	6	2	8	15.4	0	1	1	4	2	0	2	3	8	3	11	7.7
Neisseria catarrhalis	4	2	6	11.5	1	2	3	12	3	1	4	6.1	8	5	13	9.1
Proteus vulgaris	0	0	0	0	1	0	1	4	1	1	2	3	2	1	3	2.1
Pseudomonas aeruginosa	0	0	0	0	1	2	3	12	4	2	6	9.1	5	4	9	6.3
Staphylococcus albus	6	4	10	19.2	1	1	2	8	2	8	10	15.2	9	13	22	15.3
Staphylococcus aureus	6	6	12	23	0	1	1	4	1	1	2	3	7	8	15	10.5
Streptococcus pneumoniae	0	0	0	0	2	1	3	12	1	3	4	6.1	3	4	7	4.9
Streptococcus pyogenes	2	1	3	5.8	0	0	0	0	2	5	7	10.6	4	6	10	6.9
Streptococcus oralis	0	0	0	0	1	2	3	12	0	0	0	0	1	2	3	2.1
2- Mixed culture																
H.influenzae + Staph albus	0	1	1	1.9	0	0	0	0	0	0	0	0	1	0	1	0.7
Staph.aureus+ Strept. oralis	1	0	1	1.9	0	1	1	4	0	0	0	0	1	1	2	1.4
Staph. aureus + C.xerosis	0	0	0	0	0	0	0	0	0	2	2	3	0	2	2	1.4
Strept. pneumonia + M.lacunata	0	0	0	0	0	0	0	0	1	1	2	3	1	1	2	1.4
Strept. Pneumonia+C.xerosis	0	0	0	0	0	0	0	0	0	2	2	3	0	2	2	1.4
Total	**31**	**21**	**52**	**100**	**10**	**15**	**25**	**100**	**25**	**41**	**66**	**100**	**67**	**76**	**143**	**100**
%	**59.6**	**40.4**	**100**		**40**	**60**	**100**		**37.9**	**62.1**	**100**		**46.9**	**53.1**	**100**	
Percentage for the total cases	**21.7**	**14.7%**	**36.4**		**7**	**10.5**	**17.5**		**17.5**	**28.7**	**46.2**				**66.8**	

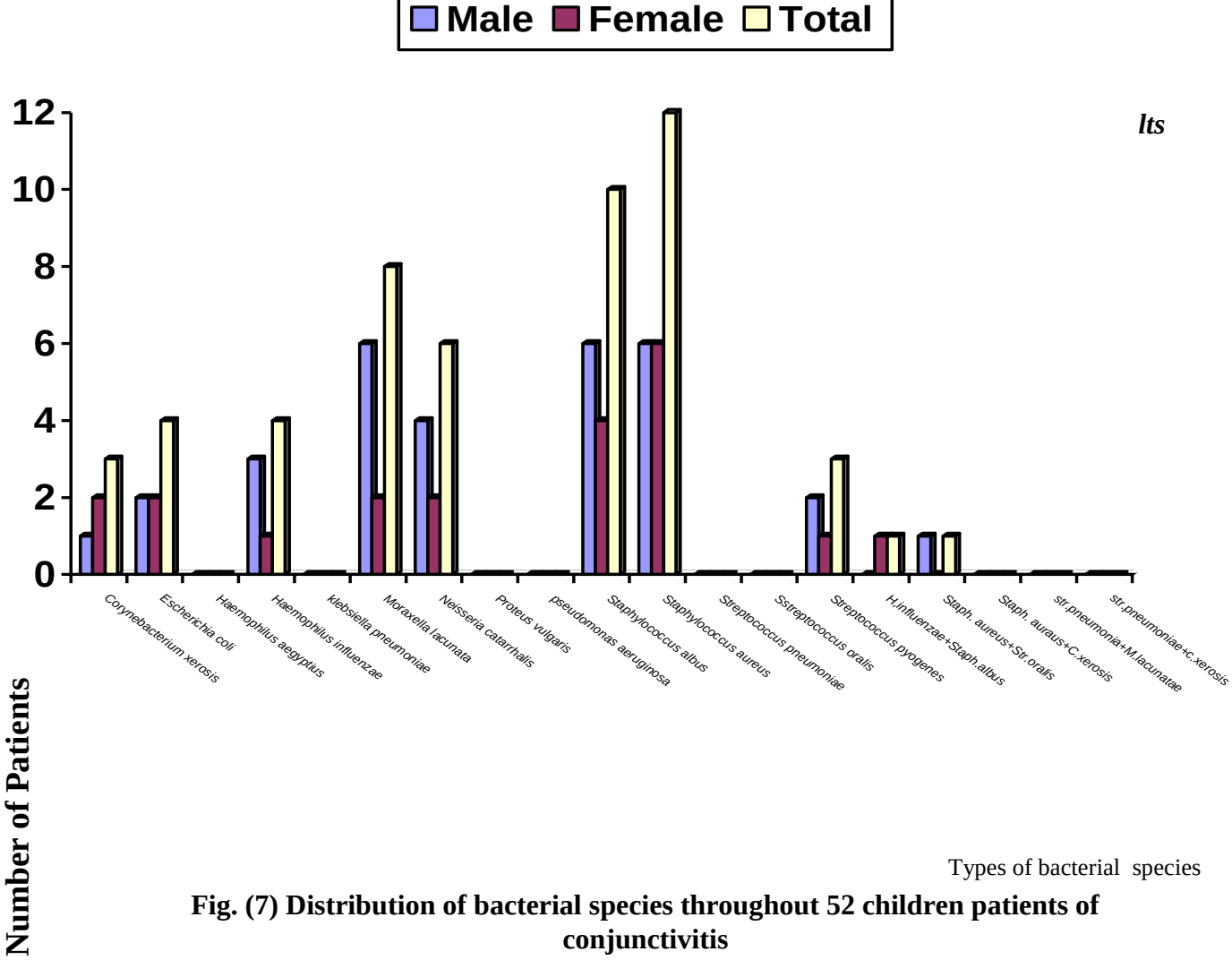


Fig. (7) Distribution of bacterial species throughout 52 children patients of conjunctivitis

3-2 Adolescent age:

Corynebacterium xerosis was the most common species isolated from adolescent patients with age range of (13-20) years, which constituted 16% of the total bacterial isolates, this species was recovered 4 times out of 25 patients Table 21 and Fig. 8. *Pseudomonas aeruginosa*, *Neisseria catarrhalis*, *Streptococcus pneumonia**e*** and *Streptococcus oralis* each was recovered 3 times constituting (12.0%) of the total bacterial isolates. *Escherichia coli*, *Moraxella lacunata*, *Proteus vulgaris* and *Staphylococcus aureus* were of lowest occurrence among the adolescent patients suffering from conjunctivitis and they were isolated once. *Haemophilus influenzae* and *staphylococcus albus* were recovered in one female patient of total isolates (8.0%). Mixed culture of *Streptococcus oralis* and *Staphylococcus*

aureus was isolated ones from a female patient and constituting (4%) of the total bacterial isolates.

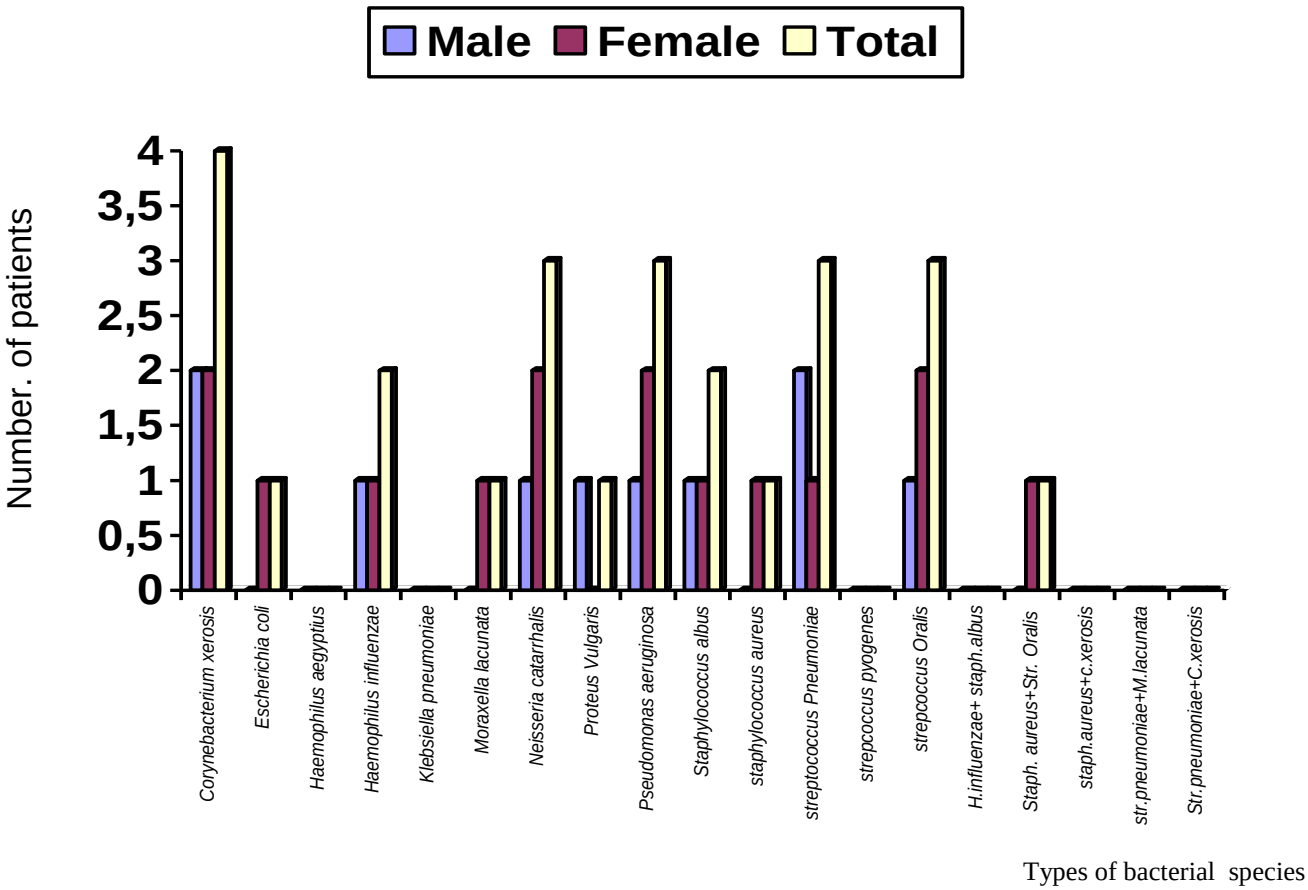


Fig. (8) Distribution of total bacterial species throughout 25 adolescent patients of conjunctivitis

3-3 Adult age;

Fifteen species of pure different bacteria were isolated from 66 patients with age range of (21-81) years. *Staphylococcus albus* and *Streptococcus pyogenes* were the most frequently isolated species from the adults (Table 21 and Fig. 9) they constituted 15.2% and 10.6%, respectively of the total bacterial conjunctivitis isolates. *Haemophilus aegyptius*, *Haemophilus influenaze* and *Pseudomonas aeruginosa* each was recovered 6 times constituting (9.1%) of the total isolates. *Escherichia coli* and *Klebsiella spp*. were recovered 3 times constituting (4.5%) of the total isolates. *Moraxella lacunata*, *Staphylococcus aureus* and *Proteus vulgaris* were of low occurrence and recovered only in 2 cases constituting (3 %).

Mixed cultures were isolated from 6 patients out of 66 tested ones and constituting (9%) of the total bacterial isolates.

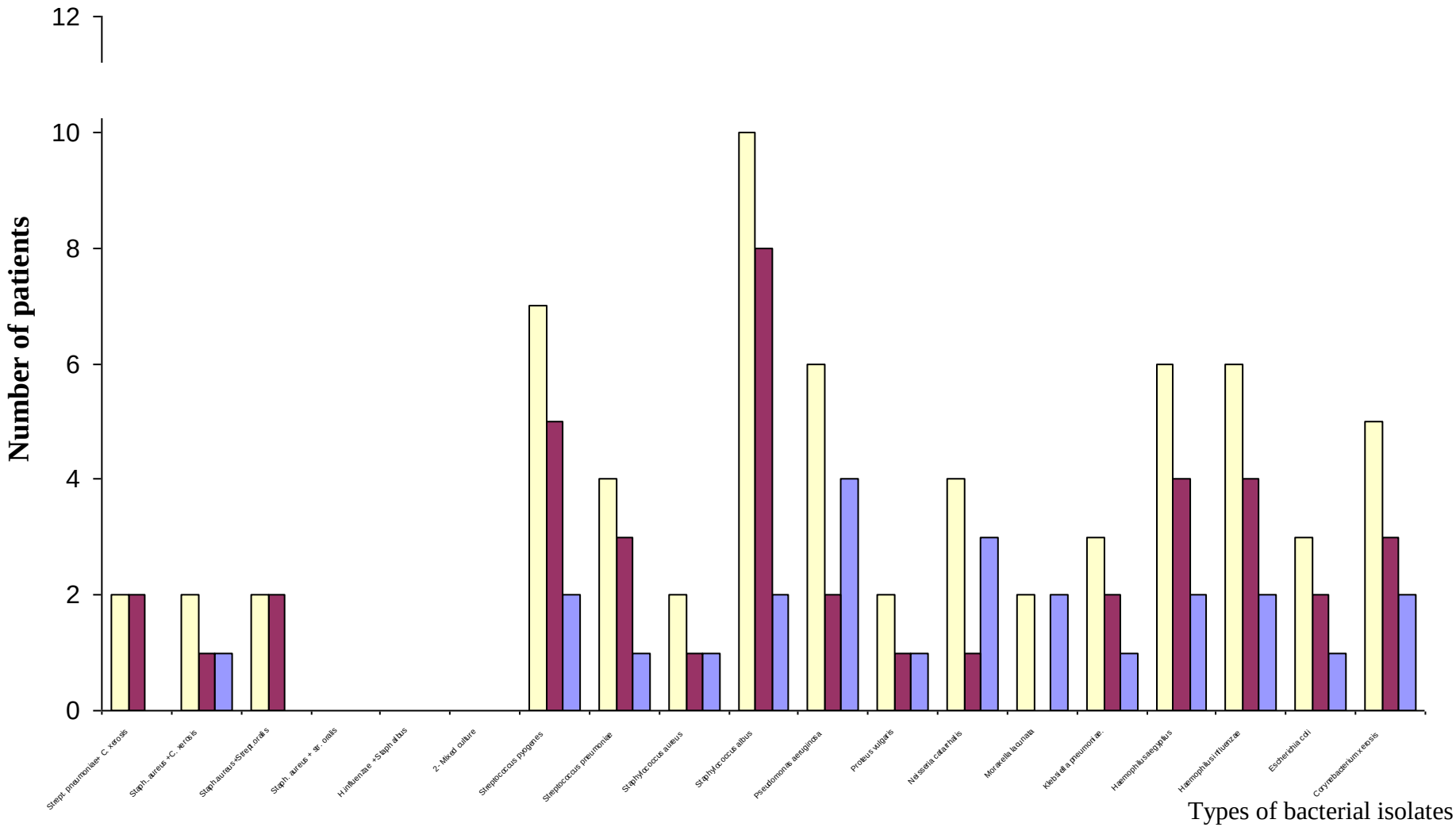


Fig. (9) Distribution of total bacterial species isolated from 66 adult patients of conjunctivitis:

4-Distribution %of fungal isolates among different patients groups;

4-1 Child age.

Nine children cases, 5 females and 4 males were examined and diagnosed as fungal conjunctivitis. Eight species of fungi were recorded in (Table 22 and Fig. 10). In the case of conjunctivitis children incidence was common in females than males (55.6% and 44.4%) respectively. *Penicillium albidum* and *Aspergillus fumigatus* were represented twice-constituting 22.2%.

4-2 Adolescents:

Seven patients of 48 positive fungal infected patients with age range (13-20) years were examined and three patients were females where males constituting four patients (Table 23 and Fig.11). Eight species were isolated. *Aspergillus fumigatus* was isolated twice from males constituting 28.5%.The remaining fungal species were isolated constituting 14.3%.

Table (22): Fungal species isolated from 9 children suffering from conjunctivitis

Type of fungal isolates				
1- Single culture	**Male**	**Female**	**Total**	**%**
Aspergillus flavus	1	0	1	11.11
Candida albicans	1	0	1	11.1
Alternaria alternata	0	1	1	11.1
Aspergillus fumigatus	1	1	2	22.2
Penicillium albidum	0	2	2	22.2
2 Mixed culture	**Male**	**Female**	**Total**	**%**
Candida albicans +A.fumigatus	1	0	1	11.1
A. flavus + Rhizopus oryzae	0	1	1	11.1
Total	**4**	**5**	**9**	**100**
%	**44.4**	**55.6**	**100**	

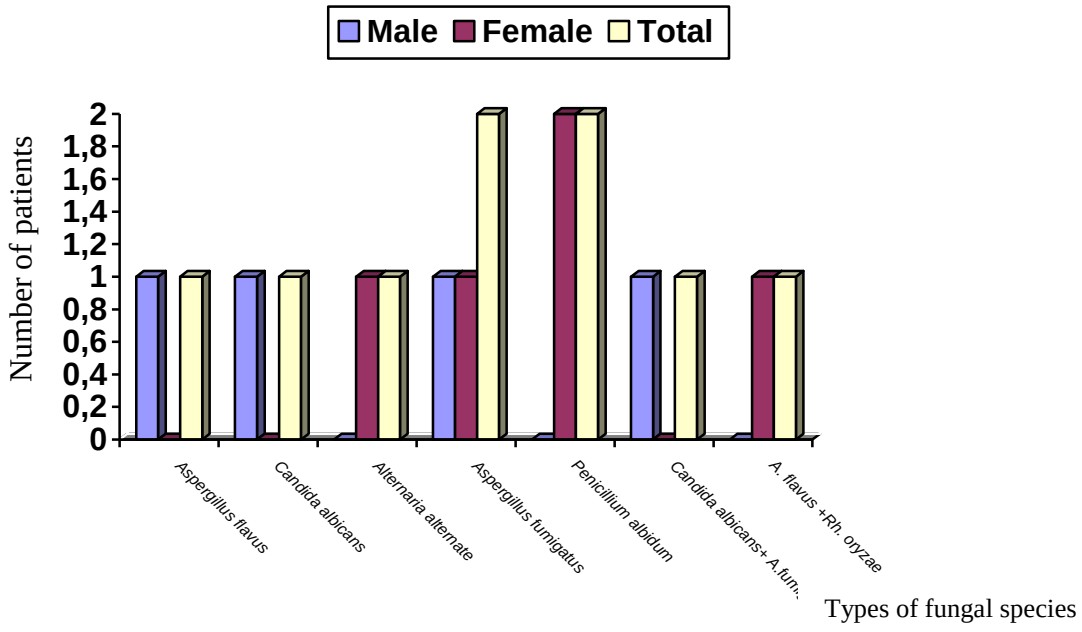


Fig. (10) Distribution of fungal species isolated from 9 children suffering from conjunctivitis

Table (23): Fungal species isolated from 7 adolescents suffering from conjunctivitis

Type of fungal isolates				
1- Single culture	**Male**	**Female**	**Total**	**%**
Alternaria alternata	1	0	1	14.3
Aspergillus flavus	0	1	1	14.3
Aspergillus fumigatus	2	0	2	28.5
Candida albicans	0	1	1	14.3
Rhizopus oryzae	1	0	1	14.3
Penicillium albidum	0	1	1	14.3
Total	**4**	**3**	**7**	**100**
%	**57.1**	**42.9**	**100**	

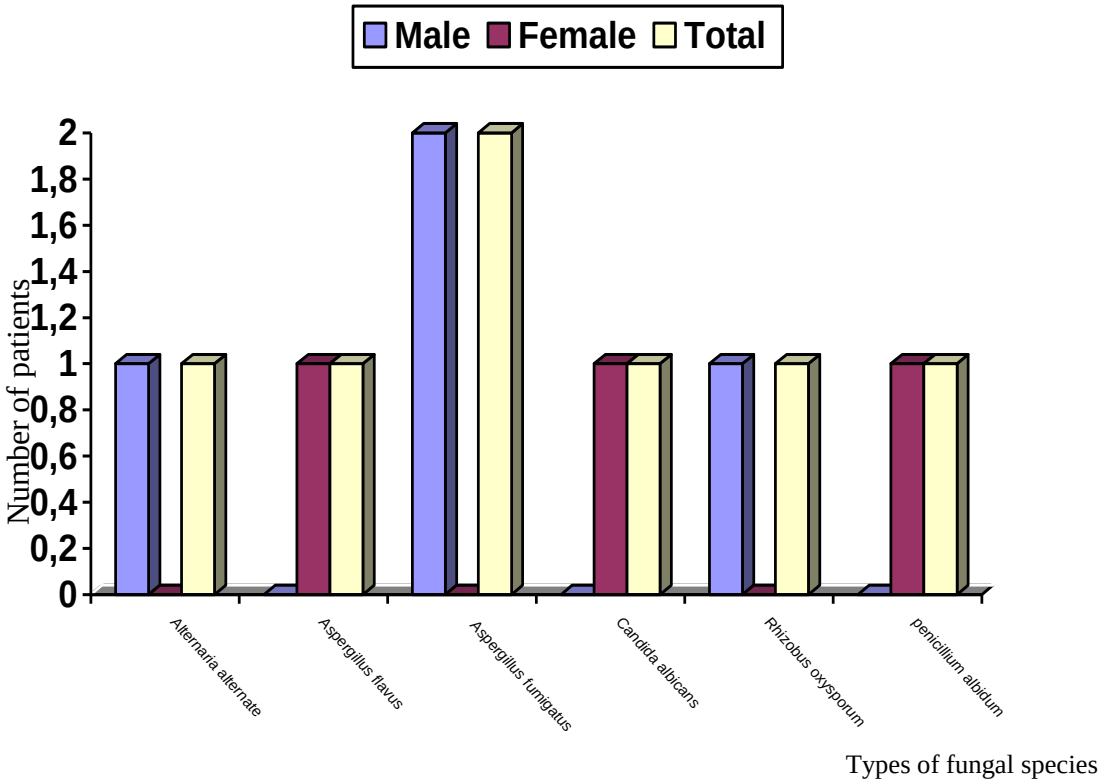


Fig. (11) Distribution of fungal species isolated from 7 adolescent suffering from conjunctivitis

4-3 Adults age;

Fungal conjunctivitis in adult patients was diagnosed in 32 out of 214 cases constituting (15%) of total cases Table 24 and Fig. 12. In this group of patients 32 patients were examined, 24 patients were females and 8 patients were males constituting 75% and 25% respectively. Seven species were isolated, where the highest incidence of fungal conjunctivitis recorded by *Aspergillus flavus* (15.9%), followed by *Penicillium albidum* (15.9%), then *Candida albicans* (12.5%), then *Alternaria alternata* and *Fusarium oxysporum* (9.3%).

On the other hand the highest casual mixed fungal infection were *Alternaria alternata* and *Penicillium albidum* matching 9.3% followed by *Fusarium oxysporum* & *Candida albicans* and *Aspergillus niger* & *Aspergillus fumigatus* as mixed cultures matching 9.3% for each followed by *Aspergillus flavus* and *Aspergillus niger* matching 3.1%.

Table (24): Fungal species isolated from 32 adults suffering from conjunctivitis

Type of fungal isolates				
1- Single culture	**Male**	**Female**	**Total**	**%**
Alternaria alternata	0	3	3	9.3
Aspergillus flavus	2	3	5	15.9
Aspergillus fumigatus	2	1	3	9.3
Aspergillus niger	0	1	1	3.1
Candida albicans	1	3	4	12.5
Fusarium oxysporum	0	3	3	9.3
Penicillium albidum	2	3	5	15.9
2- Mixed culture	**Male**	**Female**	**Total**	**%**
A.alternata +P.albidum	0	3	3	9.3
F.oxysporum + C.albicans	1	1	2	6.3
A.niger+A.fumigatus	0	2	2	6.3
A.flavus+A.niger	0	1	1	3.1
Total	**8**	**24**	**32**	**100**
%	**25**	**75**	**100**	

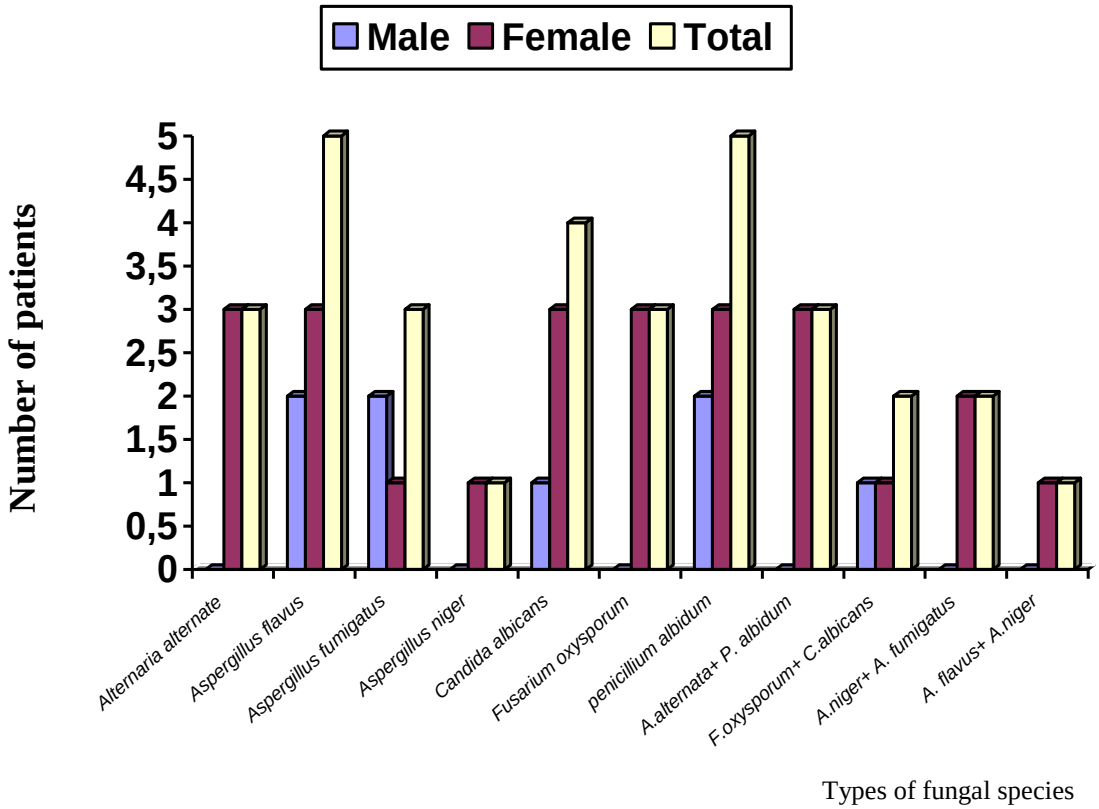


Fig. (12) Distribution of fungal species of 32 adult suffering from conjunctivitis

5-Distribution of microbial conjunctivitis among patients during different seasons;

5-1. Bacterial conjunctivitis;

The highest incidence of bacterial species causing conjunctivitis was recovered in summer season, 71 samples out of 143 total collected samples constituting (49.6%), followed by spring season, 34 samples constituting (23.8%) out of total positive cases, while the rest of the two other seasons were 11.2% for winter and 15.4% for autumn. (Table 25 & Fig. 13)

From different groups of patients studied, the bacteriological analysis of the conjunctivitis specimens revealed the occurrence of 14 species of bacteria were isolated and identified.

In the present investigation, the most common species were found belonging to genus *Staphylococcus* with two species namely *Staphylococcus albus* and *Staphylococcus aureus* with (15.3% and 10.5%) respectively which constituting (25.8%) out of total positive cases.

The highest incidence of *Staphylococcus albus*, were recorded in the summer season, while it has a lowest incidence in other seasons.

In case of *Streptococcus* three species also were recovered each of *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Streptococcus oralis* which represented (6.9%, 4.9% and 2.1% respectively). Most of these cases were recorded in summer season and no patients were recorded in winter season.

Infection due to *Haemophilus influenzae* and *Corynebacterium xerosis* come next representing (8.4%), while *Neisseria catarrhalis* was representing 6.3%.

For the total mixed cultures, they were recovered in 9 patients (6.3%) out of 143 cases, which represented the lowest incidence for each one.

Data in Table (21) showed that the total bacterial species causing conjunctivitis of 143 cases constituting (66.8%) out of 214 total positive conjunctivitis cases, where females recovered in 76 patients constituting (53.1%) more than males which recorded as 67 patients constituting (46.9%).

Table (25): Distribution of 143 bacterial conjunctivitis among patients in different seasons throughout the year.

1-Single culture	**Spring**	**Summer**	**Autumn**	**Winter**	**Total**	**Total isolates %**
Corynebacterium xerosis	3	5	2	2	12	8.4
Escherichia coli	2	3	2	1	8	5.6
Haemophilus aegyptius	2	2	1	1	6	4.2
Haemophilus influenzae	3	6	2	1	12	8.4
Klebsiella pneumoniae	1	1	1	0	3	2.1
Moraxella lacunata	3	5	2	1	11	7.7
Proteus vulgaris	0	1	1	1	3	2.1
Pseudomonas aeruginosa	1	5	2	1	9	6.3
Staphylococcus albus	4	11	3	4	22	15.3
Staphylococcus aureus	6	8	1	0	15	10.5
Streptococcus pneumoniae	2	4	1	0	7	4.9
Streptococcus pyogenes	3	6	1	0	10	6.9
Streptococcus oralis	0	3	0	0	3	2.1
Neisseria catarrhalis	3	3	1	2	9	6.3
2- Mixed culture	**Spring**	**Summer**	**Autumn**	**Winter**	**Total**	**Total isolates %**
H.influenzae+Staph.albus	0	1	0	0	1	0.7
Staph. aureus +Strept.oralis	1	1	0	0	2	1.4
Staph.aureus +C.xerosis	0	1	1	0	2	1.4
Strept.pneumoniae+M.lacunata	1	1	0	0	2	1.4
Strept.pneumoniae+C.xerosis	0	0	1	1	2	1.4
Total	**34**	**71**	**22**	**16**	**143**	**100**
%	**23.8**	**49.6**	**15.4**	**11.2**	**100**	

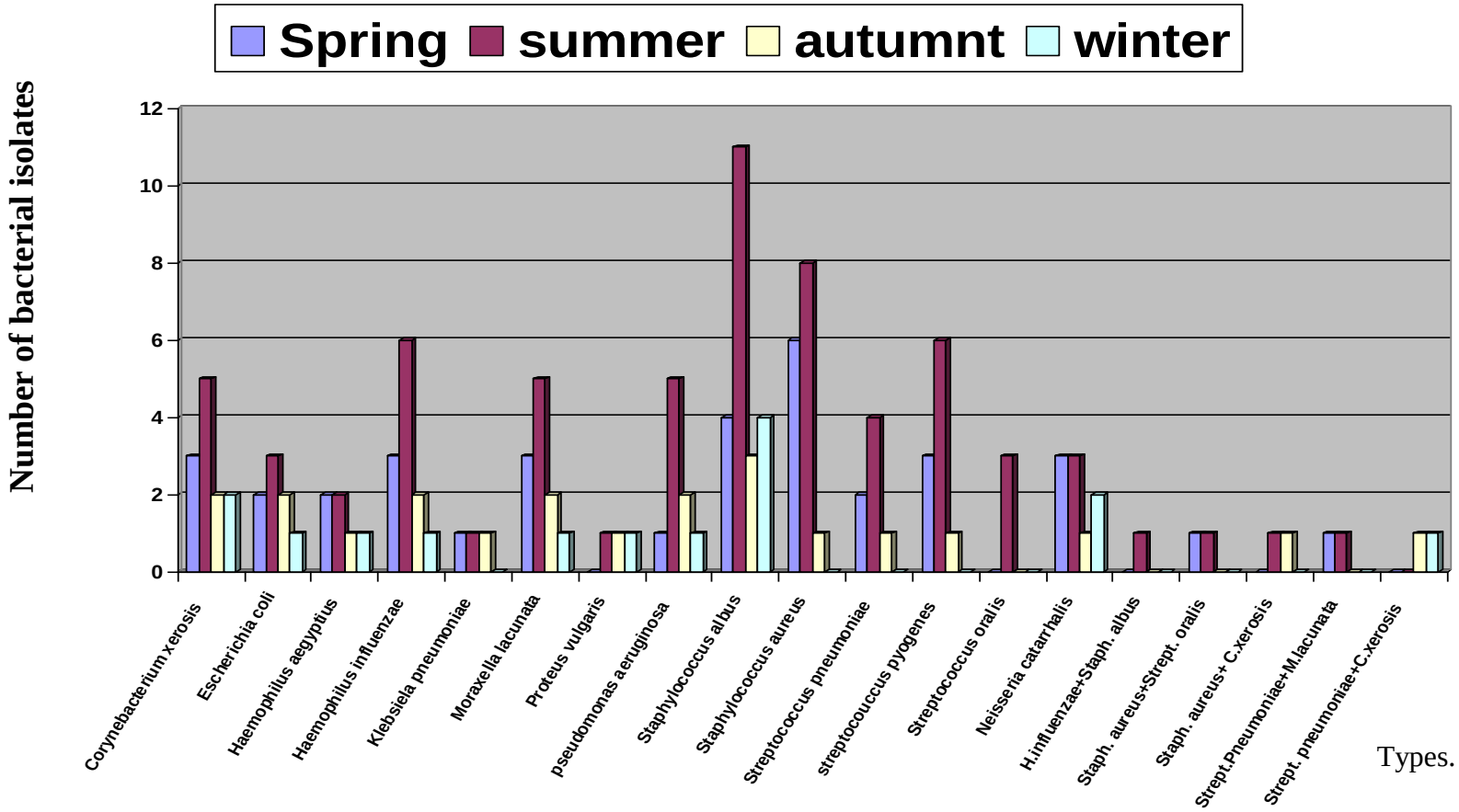


Fig (13): Distribution of 143 bacterial conjunctivitis patients in different seasons throughout the year

5-2 Fungal conjunctivitis;

In this study only 48 patients gave positive fungal growth from the total (214 patients) collected specimens throughout the year which representing 22. 4% (Table 26 Fig. 14). Where 38 out of 48 patients were pure single culture and the least 10 patients which recovered as mixed cultures. The highest number of fungal patients were recovered in spring season (20 patients), where four of them representing the mixed cultures. The lowest incidence number of patients was recorded in winter season with only 4 patients of pure species. In the present investigation, it was revealed that the most common species of these fungi were belonging to genus *Aspergillus*. These species were recovered, namely: representing *Aspergillus flavus, Aspergillus fumigatus and Aspergillus niger*. Incidence of the genus *Aspergillus* was higher in spring season and lowest in the other seasons.

Incidence of yeast spp. comes next. The highest species of these yeast was *Candida albicans*.

The incidence of genus *Penicillium* of the positive cases were *Pencillium albidum*.

Table (26): Distribution of 48 fungal conjunctivitis patients in different seasons

Seasons	Spring		Summer		Autumn		Winter		Total	
	Single	Mixed	Single	Mixed	Single	Mixed	Single	Mixed	Single	Mixed
No. of fungal isolates	16	4	8	4	10	2	4	0	38	10

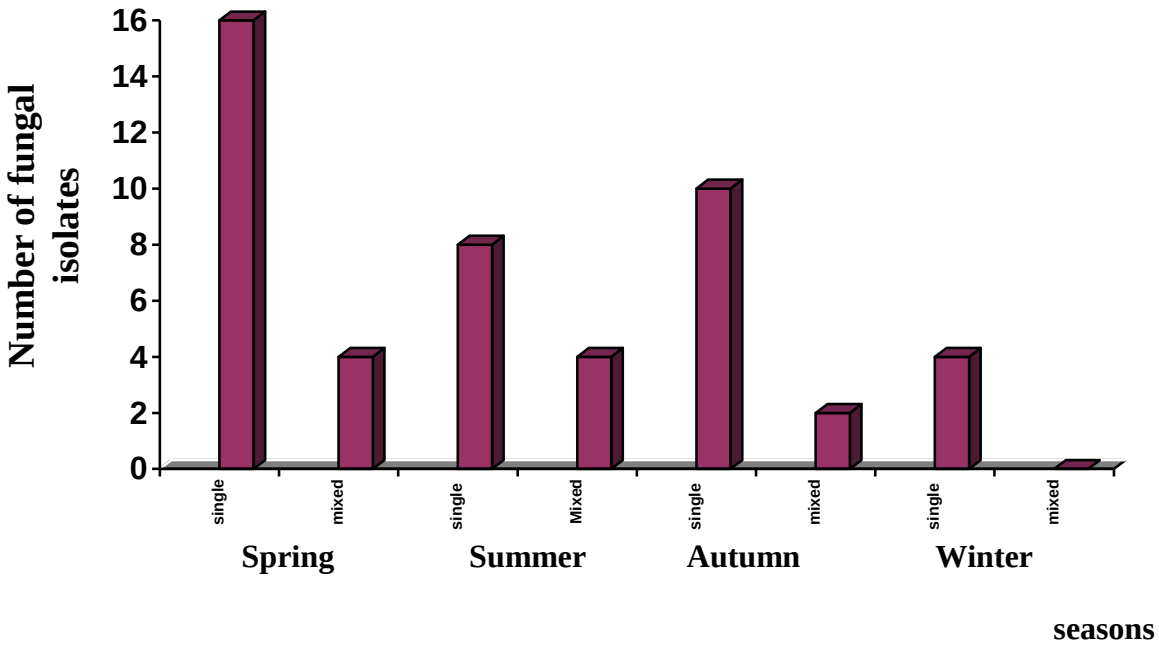


Fig (14): Distribution of 48 fungal conjunctivitis patients in different seasons

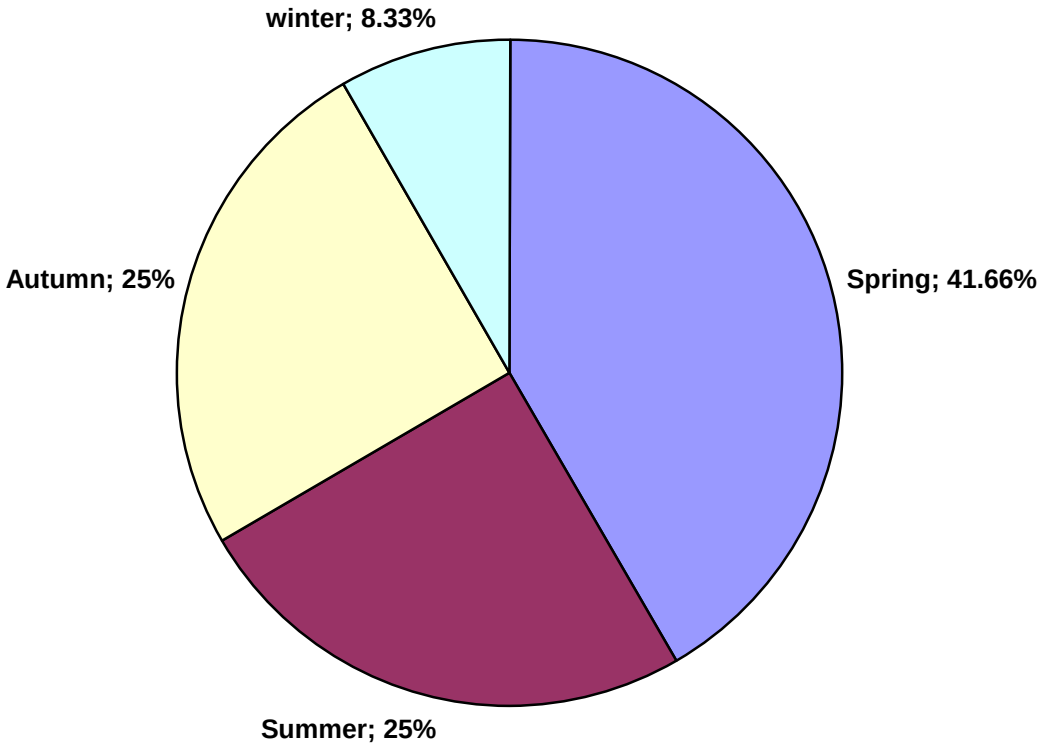


Fig. (15): Distribution and frequencies of total fungal conjunctivitis patients incidence throughout the year

6- Screening of antibacterial activity of ethanolic extracts of propolis (EEP) and bee venom:

Tables(27 and 28) clarified that both ethanolic extracts of propolis (EEP) and bee venom have antibacterial activity against all tested bacteria. Ethanolic extract of propolis showed antibacterial activity against Gram positive bacteria and Gram negative bacteria. The largest inhibition zones were noticed against *Staphylococcus albus* (26mm) and against *Staphylococcus aureus* (23mm), followed by *Streptococcus oralis* (22mm), *Streptococcus pyogenes* (20 mm), *Streptococcus pneumoniae* (19 mm) and *Corynebacterium xerosis*(13 mm).

On the other hand, *Proteus vulgaris* was the most susceptible tested Gram-negative bacteria to propolis with inhibition zone diameter (20 mm), followed by *Haemophilus aegyptius* (18 mm), *Haemophilus influenzae*(16 mm), *Neisseria catarrhalis* (13 mm), *Escherichia coli* and *Moraxella catarrhalis* (8 mm), *Klebsiella pneumoniae* (7 mm), *Pseudomonas aeruginosa* (3 mm). The control (absolute ethanol, v/v) showed no inhibitory zone against any tested bacteria.

Bee venom seemed to have higher antibacterial activity than propolis. *Staphylococcus albus* seemed to be the most sensitive tested bacteria (34mm) followed by *Staphylococcus aureus* (33mm),then *Streptococcus oralis* (32mm), then *Streptococcus pyogenes* (30mm) and then *Streptococcus pneumoniae* (29mm) and *Corynebacterium xerosis* (25mm).

On the other hand, *Proteus vulgaris* was the most susceptible tested Gram-negative bacteria with inhibition zone diameter (24 mm), followed by *Haemophilus aegyptius* (22 mm), *Haemophilus influenzae*(20 mm), *Neisseria catarrhalis* (18 mm), *Klebsiella pneumoniae*(18mm), *Escherichia coli* and *Moraxella catarrhalis*(15 mm),

then *Pseudomonas aeruginosa* seemed to be the least sensitive bacteria (5 mm) (Table 28).

Table (27):- Antibacterial activity of ethanolic extract of propolis (EEP) samples, absolute ethanol and commonly used antibiotics disc against (Gram-positive and Gram-negative bacteria):

SAMPLE / Tested bacteria	absolute Ethanol	EEP	Nitrofurontion (300)	Penicillin (10)
	Inhibition Zone Diameters (mm)			
Gram-Positive Bacteria				
Staphylococcus aureus	NA	23± 0.1	32± 0.9	28± 0.8
Staphylococcus albus	NA	26± 0.2	33± 2.0	31± 0.5
Streptococcus pyogenes	NA	20± 0.6	29± 0.3	25± 0.9
Streptococcus oralis	NA	22± 1.0	30± 0.1	26± 0.2
Streptococcus pneumoniae	NA	19± 0.6	31± 0.5	28± 0.2
Corynebacterium xerosis	NA	13± 0.3	28± 0.1	26± 0.3
Gram-negative Bacteria				
Escherichia coli	NA	8± 0.1	25± 0.7	24± 0.9
Klebsiella pneumoniae	NA	7± 0.1	27± 0.1	25± 0.1
Moraxella lacunata	NA	6± 0.3	28± 0.9	25± 0.7
Moraxella catarrhalis	NA	8± 0.5	29± 0.2	27± 0.1
Proteus vulgaris	NA	20± 0.6	20± 0.4	21± 0.4
Pseudomonas aeruginosa	NA	3± 0.2	23± 0.2	24± 0.3
Haemophilus aegyptius	NA	18± 0.4	26± 0.5	25± 0.1
Haemophilus influenzae	NA	16± 0.5	25± 0.1	23± 0.5
Neisseria catarrhalis	NA	13± 0.4	22± 0.3	20± 0.2

***Data are expressed in the form of mean ± SD**

***NA : No activity**

Table (28):- Antibacterial activity of ethanolic extract of bee venom, and commonly used antibiotics disc against (Gram-positive and Gram-negative bacteria)

SAMPLE / Tested bacteria	Bee venom	Nitrofurontion (300)	Penicillin (10)
	Inhibition Zone Diameters (mm)		
Gram-Positive Bacteria			
Staphylococcus aureus	33± 0.1	32± 0.9	28± 0.8
Staphylococcus albus	34 ± 0.2	33± 2.0	31± 0.5
Streptococcus pyogenes	30± 0.6	29± 0.3	25± 0.9
Streptococcus oralis	32 ± 1.0	30± 0.1	26± 0.2
Streptococcus pneumoniae	29 ± 0.6	31± 0.5	28± 0.2
Corynebacterium xerosis	25 ± 0.9	28± 0.1	26± 0.3
Gram-negative Bacteria			
Escherichia coli	15± 0.1	25± 0.7	24± 0.9
Klebsiella pneumoniae	18± 0.1	27± 0.1	25± 0.1
Moraxella lacunata	12± 0.3	28± 0.9	25± 0.7
Moraxella catarrhalis	15	29± 0.2	27± 0.1
Proteus vulgaris	24± 0.9	20± 0.4	21± 0.4
Pseudomonas aeruginosa	5± 0.1	23± 0.2	24± 0.3
Haemophilus aegyptius	22± 0.2	26± 0.5	25± 0.1
Haemophilus influenzae	20± 0.6	25± 0.1	23± 0.5
Neisseria catarrhalis	18± 0.5	22± 0.3	20± 0.2

***Data are expressed in the form of mean ± SD**

7-**The minimum inhibitory concentration (MIC) of ethanolic extracts of propolis (EEP) and bee venom:**

Table (29) clarified the minimum inhibitory concentration (MIC) of ethanolic extracts of propolis (EEP) and bee venom

propolis exhibited differences in its (MIC) against tested bacteria. Propolis had the lowest MIC against *Staphylococcus albus* (0.175 mg/ml), and *Staphylococcus aureus* (0.35 mg/ml), followed by *Streptococcus oralis* (0.7 mg/ml). Propolis had the same MIC against *Streptococcus pyogenes* and *Streptococcus pneumoniae* (1.4 mg/ml) and *Corynebacterium xerosis* (5.6 mg/ml).

On the other hand propolis had the same MIC against *Proteus vulgaris* and *Neisseria catarrhalis* (5.6 mg/ml), *Haemophilus aegyptius* (1.4 mg/ml), *Haemophilus influenzae*(2.8 mg/ml), *Klebsiella pneumoniae* (22.4 mg/ml), *Escherichia coli* and *Moraxella catarrhalis* (11.2 mg/ml), then *Pseudomonas aeruginosa*(89.6 mg/ml).

Bee venom had the same MIC against *Staphylococcus albus* and *Streptococcus oralis* (0.043 mg/ml), and also the same MIC against *Staphylococcus aureus* and *Streptococcus pyogenes* (0.087 mg/ml), followed by *Streptococcus pneumoniae* (0.175 mg/ml) and *Corynebacterium xerosis* (1.4 mg/ml).

On the other hand Bee venom had MIC against *Proteus vulgaris* (0.35 mg/ml), *Neisseria catarrhalis* (2.8 mg/ml), *Haemophilus aegyptius* (0.7 mg/ml), *Haemophilus influenzae*(0.35 mg/ml), *Klebsiella pneumoniae*, *Escherichia coli* and *Moraxella catarrhalis* (5.6 mg/ml), then *Pseudomonas aeruginosa* (22.4 mg/ml).

Table (29): MIC(mg/ml)of EEPand Bee venom extracts against tested bacteria;

SAMPLE / Tested bacteria	EEP	Bee venom
	Minimum inhibitory concentration (mg/ml)	
Gram-Positive Bacteria		
Staphylococcus aureus	0.35	0.087
Staphylococcus albus	0.175	0.043
Streptococcus pyogenes	1.4	0.087
Streptococcus oralis	0.7	0.043
Streptococcus pneumoniae	1.4	0.175
Corynebacterium xerosis	5.6	1.4
Gram-negative Bacteria		
Escherichia coli	11.2	5.6
Klebsiella pneumoniae	22.4	5.6
Moraxella lacunata	22.4	11.2
Moraxella catarrhalis	11.2	5.6
Proteus vulgaris	5.6	0.35
Pseudomonas aeruginosa	89.6	22.4
Haemophilus aegyptius	1.4	0.7
Haemophilus influenzae	2.8	0.35
Neisseria catarrhalis	5.6	2.8