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## Seasonal Epidemiological Surveillance on Bacterial and Fungal Pathogens in Broiler Farms in Egypt

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**Abstract:** A total of 1664 environmental samples (litter, water, swabs and air) were collected from commercial broiler farms located in Ismailia and Zagazig Governorates, Egypt. The bacterial and Fungal isolates that were identified included: *Citrobacter Sp*, *E. coli*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Pseudomonas aureuginosa*, *Salmonella Sp*, *Shigella Sp*, *Staphylococcus aureus*, *Streptococcus fecalis* and *Streptococcus pneumonie*, *Yeast Sp*, *Pencillium Sp*, *Aspergillus niger*, *Aspegillus flavus*, *Aspergillus nidulans*, *Mucour* and *Candida albicans*. Prevalence and frequencies of the microorganisms were calculated to detect the most predominant microorganisms. Litter samples showed that *Pseudomonas areuginosa* (24%) and *Yeast Sp* (37.5%) predominated in closed houses; *Klebsiella oxytoca* (12%) and *Penicillium Sp* (28.57%) predominated in open houses in winter, *Klebsiella oxytoca* (33.33%) and *Aspergillus nidulans* (22.73%) predominated in closed houses; *E. coli* (50%) and *Penicillium Sp* (21.40%) in open houses in spring, *Shigella Sp* (34.5%); (47.62%) and *Aspergillus niger* (26.92%); (8.69%) predominated in closed and open houses respectively in summer, *Klebsiella oxytoca* (55.56%) and *Aspergillus niger* (12%) predominated in closed houses; *Klebsiella oxytoca* (41.67%) and *Candida albicans* (59.1%) predominated in open houses in autumn. Water samples showed that *E. coli* (39.47%) and *Candida albicans* (50%) predominated in closed houses; *E. coli* (60.97%) and *Penicillium Sp* (60%) predominated in open houses in winter, *E. coli* (67.57%) and *Penicillium Sp* (37.5%) predominated in closed houses; *E. coli* (89.29%) and *Aspergillus nidulans* (15.83%) predominated in open houses in spring, *Shigella Sp* (42.55%) and *Penicillium Sp* (37.5%) predominated in closed houses; *Shigella Sp* (36.67%) and *Yeast Sp* (66.6%) predominated in open houses in summer, *Klebsiella oxytoca* (36.59%) and *Candida albicans* (33.3%) predominated in closed houses; *Klebsiella oxytoca* (47.22%) and *Candida albicans* (47.62%) predominated in open houses in autumn. Swab samples showed that *Pseudomonas areuginosa* (62.5%) and *Penicillium Sp* (29.41%) predominated in closed houses; *Pseudomonas aureuginosa* (47.06%) and *Candida albicans* (17.64%) predominated in open houses in winter, *Klebsiella oxytoca* (36.49%) and *Aspergillus flavus* (43.48%) predominated in closed samples; *E. coli* (43.48%) and *Aspergillus flavus* (46.51%) predominated in open houses in spring, *Klebsiella oxytoca* (28.98%) and *Penicillium Sp* (34.48%) predominated in closed houses; *E. coli* (35.17%) and *Aspergillus niger* (35.14%) predominated in open houses in summer, *Pseudomonas areuginosa* (31.75%); (42.31%) and *Canidada albicans* (40.32%); (61.4%) predominated in closed and open houses respectively in autumn. Air samples showed that *staphylococcus aureus* (51.72%-45.45%, 52%-56.17%, 59.52-69.44 and 48.78-75%) was predominating in closed and open houses respectively in winter, spring, summer and autumn respectively, while the fungal growth showed that *Aspergillus niger* (66.6%) predominated in closed houses in winter, *Aspergillus niger* (100%) predominated in closed houses; *Aspergillus favlus* (100%) predominated in open houses in spring, *Aspergillus niger* (100%) predominated in closed houses in summer, *Aspergillus niger* (100%) predominated in closed and open houses in autumn.

**Key words:** Broiler, bacterial, fungal, houses system, surveillance

### INTRODUCTION

From the hygienic point of view, Some bacterial pathogens cause serious disorders in animals and poultry farms, such as pathogenic serotypes of *Escherichia coli* which are common in poultry

environment and causes some specific diseases such as coli-septicemia, coli-granuloma, air sac disease (chronic respiratory disease), avian cellulites, peritonitis, salpingitis, swollen head syndrome; Gross (1994). *Staphylococcus aureus* which are known to be

responsible for 30% losses in poultry by causing bacteraemia, endocarditis and yolk sac infection, Calnek *et al.* (1997). Aspergillosis (Brooder pneumonia) caused by *Aspergillus niger* causing high mortality in young chicks in the first three weeks of rearing about 10:50% of the affected chicks dies within 24 h. Also, Aspergillosis have been identified as the real cause of embryo mortality, (Gordon and Jordan, 1982) in addition to aflatoxicosis a condition caused by the toxins of *Aspergillus flavus* which usually occur in young pollets causing mortality which may reach to 50% (Gordon and Jordan, 1982). *Penicillium species* also causing serious damage among poultry farms due to the production of toxins may contaminate food (Marth, 1990). Candidiasis that caused by yeast like fungus *Candida albicans* and the disease which may affect birds from 1-2 weeks of age causing high mortality rate up to 75% (Gordon and Jordan, 1982).

In order to reduce human illness, epidemiological measures have been established because poultry and poultry products often serve as the vehicles for human salmonellosis, (Bean and Griffin, 1990; Persson and Jendteg, 1992), the poultry industry and governmental agencies are focused on eradicating *Salmonella* in live birds and at the processing plant (Hargis *et al.*, 2001). *Salmonella Enterica serovar enteritidis* is the world leading cause of salmonellosis and is often implicated in over 60% of human Salmonellosis in Europe, in the United States it remains the second most common serotype of *Salmonellae*, (Patrick *et al.*, 2004). The current world wide epidemic of *Salmonella enteritidis* started in the middle of 1980s, (Ward *et al.*, 2000), the (USDA) and Food Safety Inspection Services (FSIS) implemented an "in plant" Hazard Analysis Critical Control Point (HACCP) program to reduce the prevalence of the food borne pathogen contamination in meat and egg. Poultry are recognized as important reservoir of *Salmonella*; (Henson, 1997; Lynch *et al.*, 2006), the United States Department of Agriculture implemented to reduce the source(s) of infection. Because food animal are the main reservoir of *Salmonella enteritidis* is mainly poultry often carrying asymptomatic infection, which pass the human pathogen along the food production chain. Especially undercooked or raw eggs and frozen poultry meat represent a high risk for humans.

This study was aiming for conducting an Epidemiological survey to identify the most frequently and persisting pathogenic microorganisms in the broiler's environment. Culture, isolation and identification of pathogens from the environmental samples collected from broiler's environment.

## MATERIALS AND METHODS

**Sampling:** A total of 416 environmental samples were collected from two different systems of bird houses (Natural- artificial) each season (winter, spring, summer,

and autumn). The samples were litter samples (William *et al.*, 1975) Water samples (Lawrence, 1988), Swabs (Collins *et al.*, 1991) that were collected from walls, floors, feed pads and drinkers, Air samples, (Cruickshank *et al.*, 1975).

**Bacterial and fungal culturing:** The processing of the samples was as carried out by pre-enrichment of all litter, water, swabs and air samples in 9 ml of Buffered Peptone Water (Oxoid, Fisher Int.) and incubated at 37°C for 18-24 h. The samples were then inoculated on non-selective quadrate plates of Blood Agar (Oxoid, Fisher Int.), Mac Conkey agar (Lab-M, Fisher Int.), Cystein Lactose Electrolyte Deficient Agar (CLED) (Lab-M, Fisher Sci.) and Sabaroud Dextrose Agar (SDA) (Oxoid, Fisher Sci.), the plates were streaked for isolation using a sterile cotton swab. The plates were then incubated at 37°C for 18-24 h.

**Identification of bacterial isolates:** The growing bacterial colonies were first used for gram staining for identification as Gram Negative Bacilli, (Edward and Ewing, 1982), Gram Positive Cocci, (Cherry *et al.*, 1972; Cruickshank *et al.*, 1975).

The colonies were then identified biochemically by inoculation onto TSI (Bialy and Scott, 1978; Edward and Ewing, 1982; Fingegold and Martin, 1982) SIM Citrate; Cruickshank *et al.* (1980), Indol test (Cruickshank *et al.*, 1980), Urease Utilization test (Cruickshank *et al.*, 1980), MR/VP test (Fingegold and Martin, 1982) Gelatin Liquefaction test (Cruickshank *et al.*, 1980), Potassium Cyanide (Cruickshank *et al.*, 1980) for the gram negative bacteria. Meanwhile the gram positive tested against Catalase test (Cruickshank *et al.*, 1980), Coagulase test and Deoxyribonuclease test (Blair *et al.*, 1967).

**Confirmation for the bacterial isolates:** The colonies suspected to be *E. coli* were inoculated into 9 ml of Mac-Conkey broth, incubated at 44°C for 18-20 h. The tubes that revealed growth (gas and acid formation) were streaked again on Eosine Methylene Blue Agar (EMBA) and incubated at 37°C for 18-24 h. The positive colonies were these that have metallic green appearance. Biochemical identification was carried to insure the strain.

The colonies suspected to be *Staphylococcus aureus*: the samples were inoculated into 9 ml Phosphate Buffered Saline (PBS) and incubated for 18-24 h. The samples were then streaked onto Baird Parker Agar and the plates were incubated at 37°C for 20-24 h. The positive colonies showed black colonies on the agar.

The colonies suspected to be *Salmonella Sp*: Three to five colonies were inoculated into 9 ml of Selenite F broth. The tubes then incubated at 37 degree C for 18-24 h. A loopful was streaked onto S-S agar and the plates were incubated at 37°C for 18-24 h. The positive result for *Salmonella* appeared as black colonies with hallow zone.

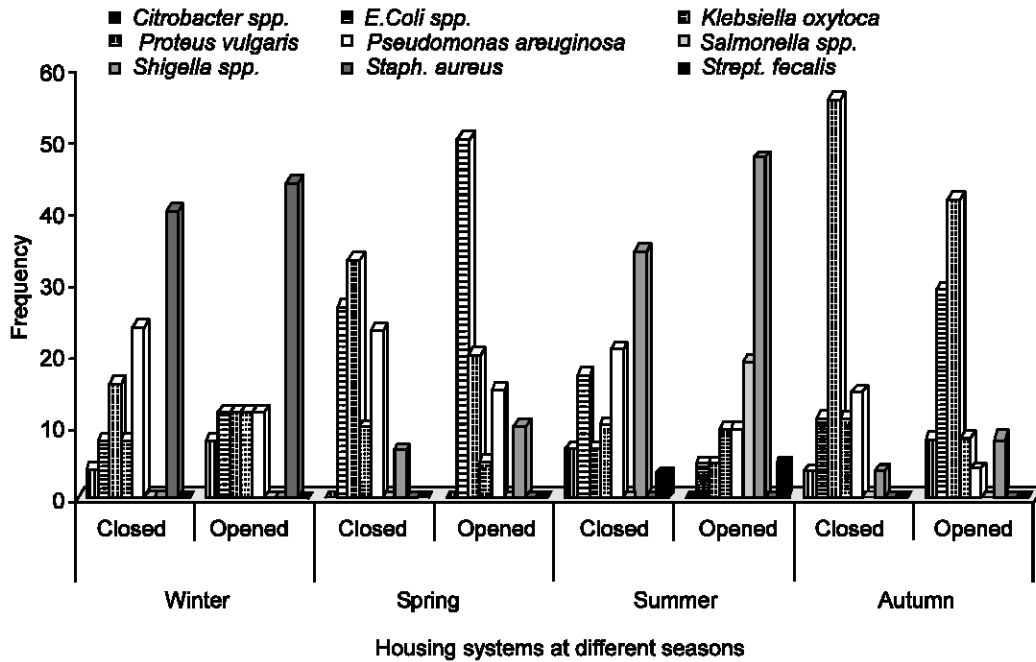


Fig. 1: Frequency distribution of the bacterial isolates in the examined litter samples at different seasons

**Identification of the fungal and mycotic isolates:** The growing fungal colonies were identified using the macroscopical examination of the mould colonies included the rapidity of growth, surface texture of the colony, surface color and reverse color, (Davies, 1976). Further identification for the fungal growth was carried out using the Microscopical examination, Part of the culture was removed with flame sterilized inoculation needle from the agar medium, placed on a slide and mounted into a drop of lacto phenol cotton blue and covered with a cover slip. The coverslip was pressed gently so as to lay the contents on the slide flat. The preparation were viewed under a low power microscope to reveal the desired structure then magnifying it as the conidial stage, head, vesicle, sterigmata, conidiophores and conidia, (Raper and Thom, 1949; Raper and Fennel, 1965; Zycha *et al.*, 1969).

*Candida Albicans* was identified by using the germ tube test (Koneman *et al.*, 1979) which based on the production of filamentous out growth in the form of pseudo-germ or pseudo-mycelium by cells of *Candida albicans* when inoculated into test tube containing 0.5 ml of Rabbit's Serum and incubated at 37°C for at least 3 h. A drop of the incubated yeast serum suspension was placed on a microscope slides, overlaid with a cover slip and examined for the presence of serum tubes known as germ tubes.

**RESULTS AND DISCUSSION**

Figure 1, the frequency distribution of bacterial isolates in the examined litter samples in different seasons showed that *Staphylococcus aureus* (40%) and

*Pseudomonas areuginosa* (24%) predominated in closed houses, *Staph. aureus* (44%) *Klebsiella oxytoca* (12%), *E. coli* (12%), *Pseudomonas areuginosa* (12%), and *Proteus vulgaris* (12%) predominated in open houses in winter. *Klebsiella oxytoca* (33.33%), *E. coli* (26.67%) and *Pseudomonas areuginosa* (23.33%) predominated in closed houses, *E. coli* (50%), *Klebsiella oxytoca* (20%) and *Pseudomonas areuginosa* (15%) predominated in the open houses in spring. *Shigella Sp* (34.5%) and *Pseudomonas areuginosa* (20.69%) predominated in the closed houses, *Shigella Sp* (47.62%) and *Salmonella Sp* (19.02%) predominated in open houses in summer. *Klebsiella oxytoca* (55.56%) and *Pseudomonas areuginosa* (14.81%) predominated in closed houses, *Klebsiella oxytoca* (41.67%) and *E. coli* (29.2%) predominated in open houses in autumn.

Figure 2, the frequency distribution of bacterial isolates in the examined water samples in different seasons revealed that *E. coli* (39.47%), *Pseudomonas areuginosa* (26.32%) and *Proteus vulgaris* (21.05%) predominated in closed houses, *E. coli* (60.97%) and *Pseudomonas areuginosa* (29.27%) predominated in open houses in winter. *E. coli* (67.57%); (89.29%) and *Klebsiella oxytoca* (27.03%) (10.71) predominated in closed and open houses respectively in spring. *Shigella Sp* (42.55%), *E. coli* (21.3%) and *Pseudomonas areuginosa* (17.02%) predominated in closed houses, *Shigella Sp* (36.67%) and *E. coli* (30%) predominated in open houses in summer. *Klebsiella oxytoca* (36.59%), *Pseudomonas areuginosa* (24.39%) and *Proteus vulgaris* (19.44%) predominated in closed houses, *Klebsiella oxytoca* (47.22%) and *Proteus vulgaris* (19.44%) predominated in open houses in autumn.

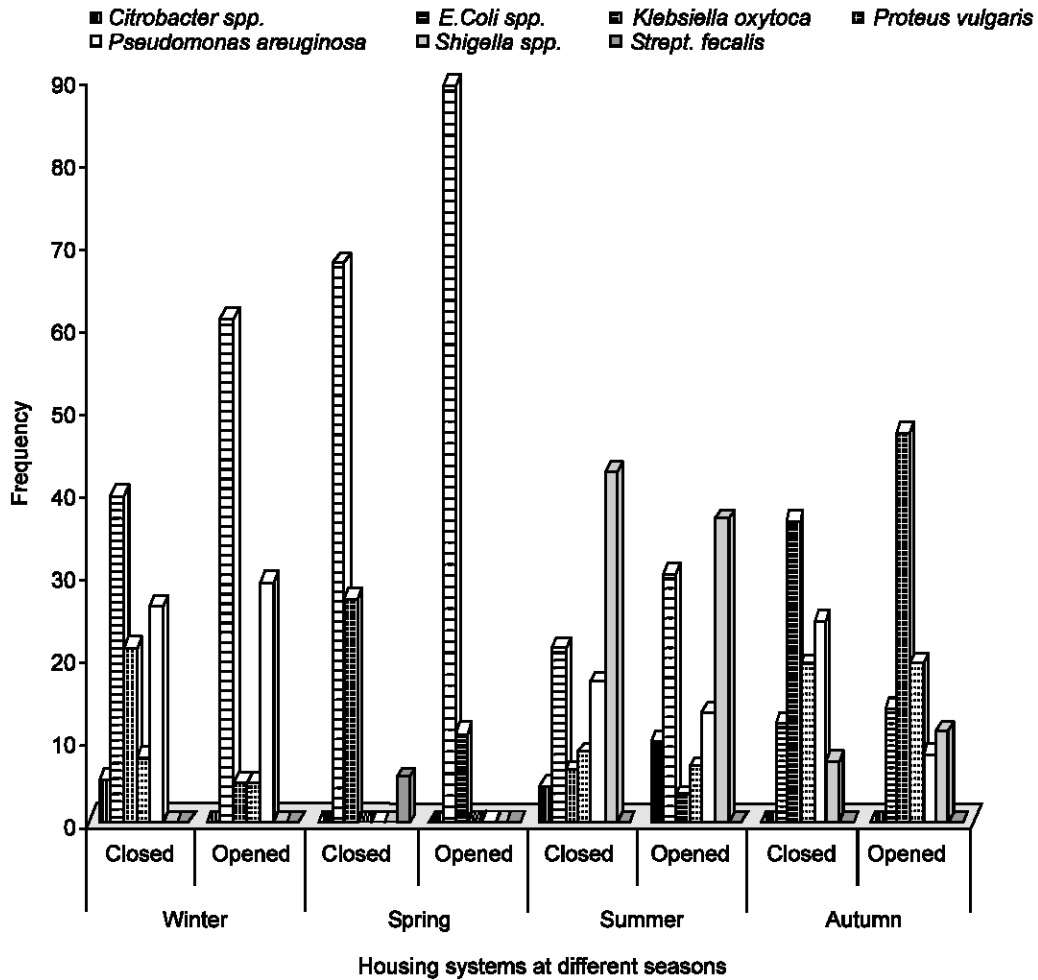


Fig. 2: Frequency distribution of the bacterial isolates in the examined water samples at different seasons

Figure 3, the frequency distribution of bacterial isolates in the examined swab samples in different seasons revealed that *Pseudomonas areuginosa* (62.5%), *Staphylococcus aureus* and *E. coli* (15.63%) predominated in closed houses, *Pseudomonas areuginosa* (47.06%), *E. coli* (29.41%) and *Staphylococcus aureus* (19.61%) predominated in open houses in winter. *Klebsiella oxytoca* (36.49%), *E. coli* (27.03%) and *Pseudomonas areuginosa* (24.32%) predominated in closed houses, *E. coli* (43.48%), *Klebsiella oxytoca* (28.26%) and *Pseudomonas areuginosa* (21.74%) predominated in open houses in spring. *Klebsiella oxytoca* (28.98%), *Staphylococcus aureus* (28.99%) and *Pseudomonas areuginosa* (23.19%) predominated in closed houses, *E. coli* (35.17%), *Staph. aureus* (28.57%) and *Pseudomonas areuginosa* (17.86%) predominated in open houses in summer. *E. coli* (47.62%), (38.46%) and *Pseudomonas areuginosa* (31.75%), (42.31%) predominated in closed and open houses respectively in autumn.

Figure 4, the frequency distribution of bacterial isolates in the examined air samples in different seasons

revealed that *Staphylococcus aureus* (51.72%), (45.45%), *E. coli* (34.48%), (45.45%) and *Streptococcus Pneumonie* (13.79%); (9.09%) predominated in closed and open houses respectively in winter, *Staphylococcus aureus* (52%), (56.17%), *E. coli* (40%) (23.33%) and *Streptococcus Pneumonie* (8%); (20%) respectively in summer. *Staphylococcus aureus* (59.52%) (69.44%), *E. coli* (23.81%), (25%) and *Streptococcus Pneumonie* (16.67%), (5.55%) respectively in summer, *Staphylococcus aureus* (48.78%), (75%), *E. coli* (36.59%), (13.89%) and *Streptococcus Pneumonie* (14.63%) (11.11%) respectively in autumn.

Figure 5, the frequency distribution of fungal and mycotic isolates in the examined litter samples at different seasons revealed that *Yeast* (37.5%), *Pencillium Sp* (31.25%) and *Candida albicans* (18.75%) predominated in closed houses, *Yeast*, *Pencillium Sp* and *Candida albicans* by (28.57%) predominated in open houses in winter. *Aspergillus nidulans* (22.73%), *Pencillium spp.* (22.73%) and *Aspergillus niger* (18.18%) predominated in closed houses, *Yeast*, *Pencillium Sp.* and *Mucour* in a percentage of (21.4%) predominated in open houses

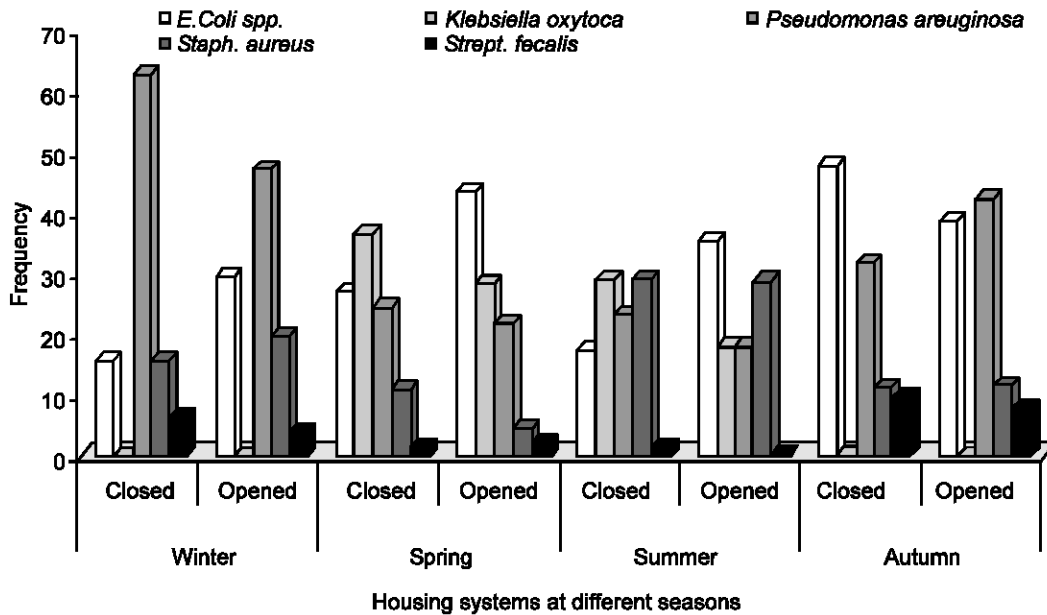


Fig. 3: Frequency distribution of the bacterial isolates in the examined swab samples at different seasons

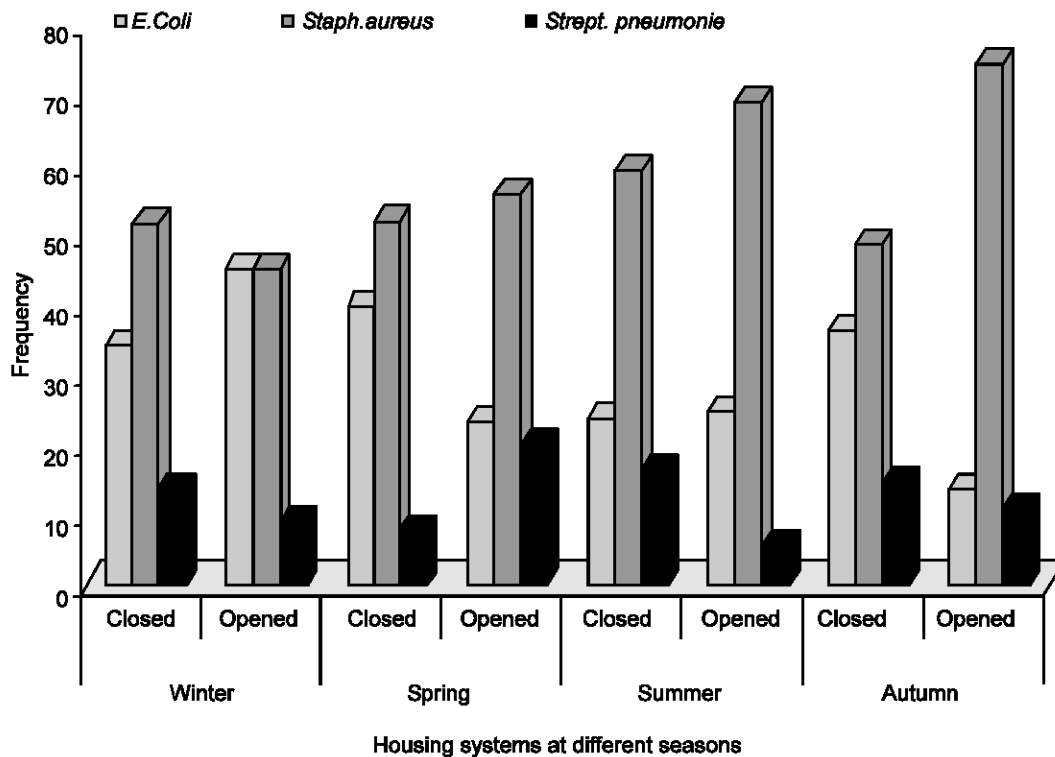


Fig. 4: Frequency distribution of the bacterial isolates in the examined air samples at different seasons

in spring. Yeast (38.46%), *Aspergillus niger* (26.92%), and *Mucour* (19.23%) predominated in closed houses, Yeast (60.87%) and *Aspergillus niger* (8.69%) predominated in open houses in summer. Yeast (40%), *Aspergillus niger* and *Candida albicans* (12%)

predominated in closed houses, *Candida albicans* (59.1%) and Yeast (9.09%) predominated in open houses in autumn.

Figure 6, the frequency distribution of fungal and mycotic isolates in the examined water samples at different

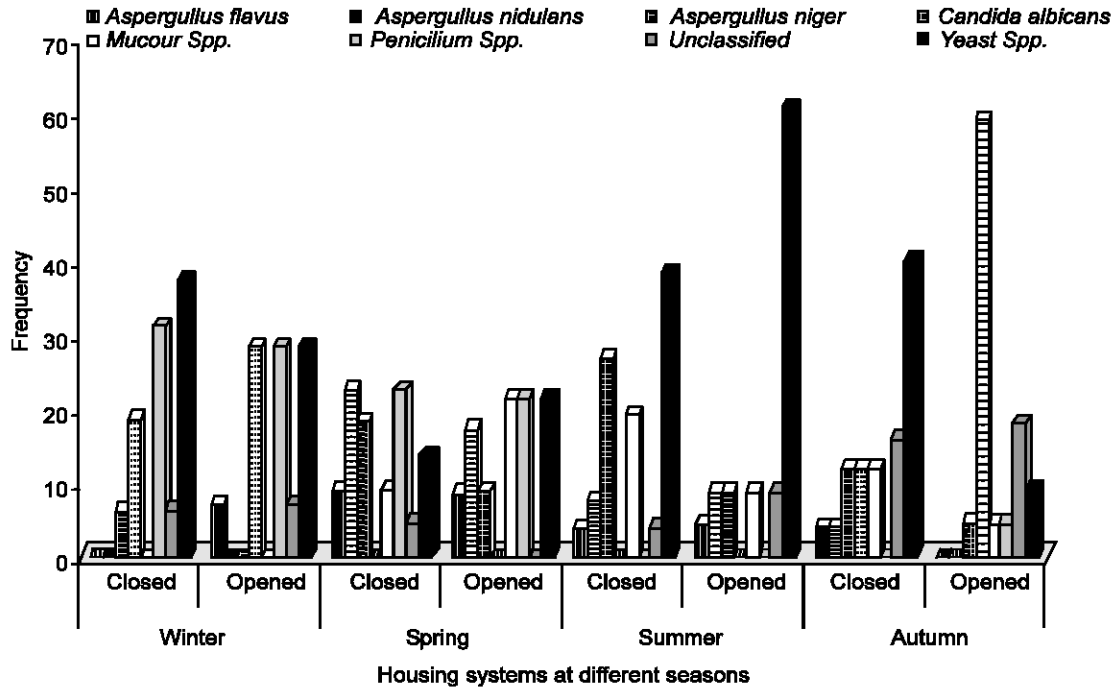


Fig. 5: Frequency distribution of the fungal isolates in the examined litter samples at different seasons

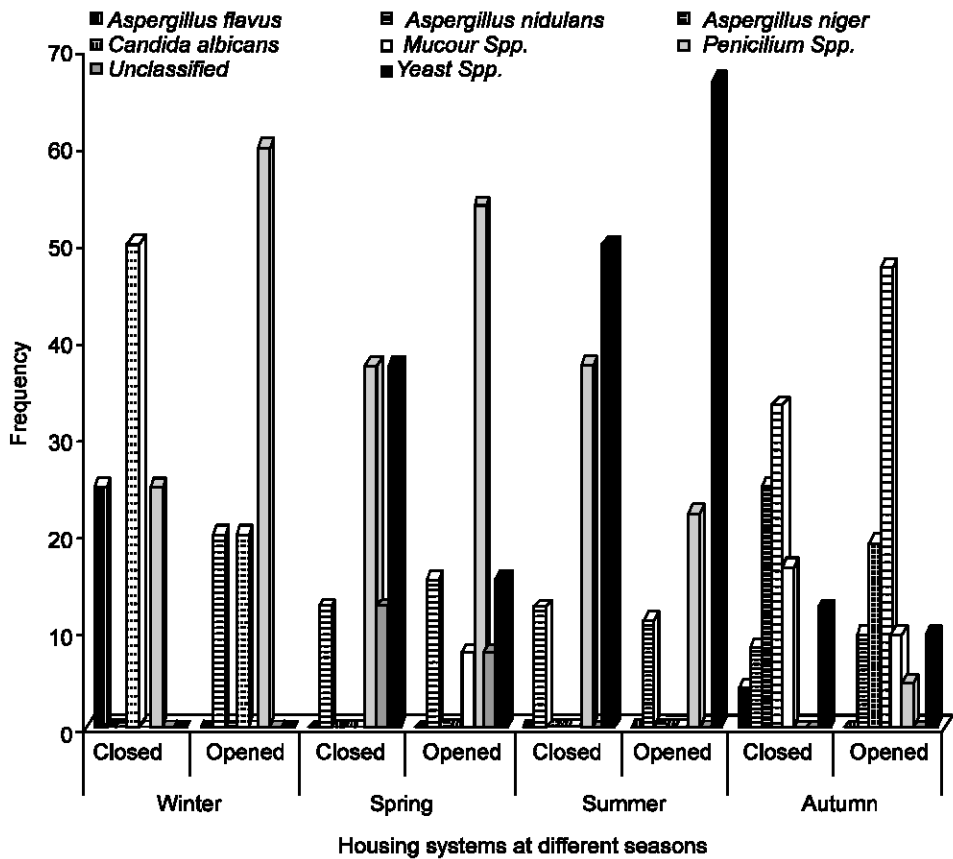


Fig. 6: Frequency distribution of the fungal isolates in the examined water samples at different seasons

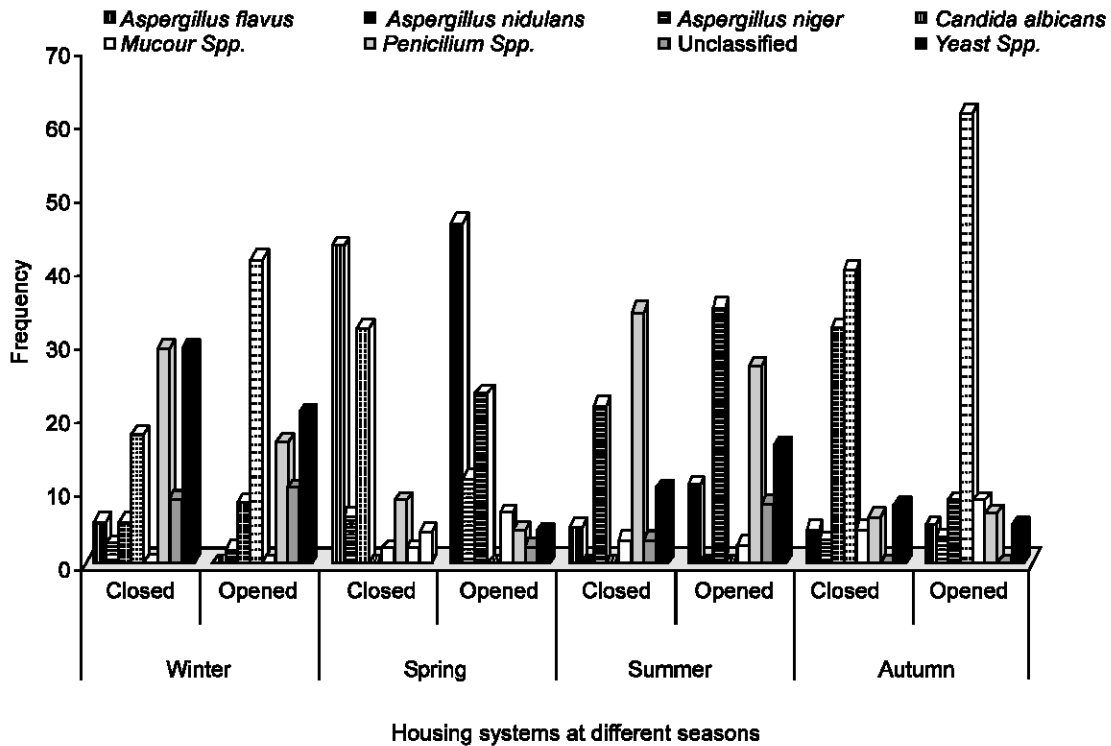


Fig. 7: Frequency distribution of the fungal isolates in the examined swab samples at different seasons

seasons showed that *Candida albicans* (50%), *Aspergillus flavus* (25%) and *Penicillium Sp* (25%) predominated in the closed houses, *Penicillium Sp* (60%), *Candida albicans* (20%) and *Aspergillus nidulans* (20%) predominated in open houses in winter. *Penicillium Sp* (37.5%) predominated in closed houses, *Penicillium Sp* (53.85%), *Aspergillus nidulans* and *Yeast* (15.38%) predominated in open houses in spring. *Yeast* (50%) (66.6%) and *Penicillium Sp* (37.5%) (22.2%) predominated in closed houses and open houses respectively in summer. *Candida albicans* (33.3%), (47.62%) and *Aspergillus niger* (25%) (19.05%) predominated in closed houses and open houses respectively in autumn.

Figure 7, the frequency distribution of fungal and mycotic isolates in the examined swab samples at different seasons showed that, *Yeast* (29.41%), *Penicillium Sp* (29.41%) and *Candida albicans* (17.64%) predominated in the closed houses, *Candida albicans* (41.67%) and *Yeast* (20.83%) predominated in open houses in winter. *Aspergillus flavus* (43.48) (46.51%) and *Aspergillus niger* (32.16%) (23.26%) predominated in closed houses and open houses respectively in spring. *Penicillium Sp* (34.48%) and *Aspergillus niger* (21.55%) in closed houses, *Aspergillus niger* (35.14%) and *Penicillium Sp* (27.03%) predominated in open houses in summer. *Candida albicans* (40.32%) and *Aspergillus niger* (32.26%) predominated in closed houses, *Candida*

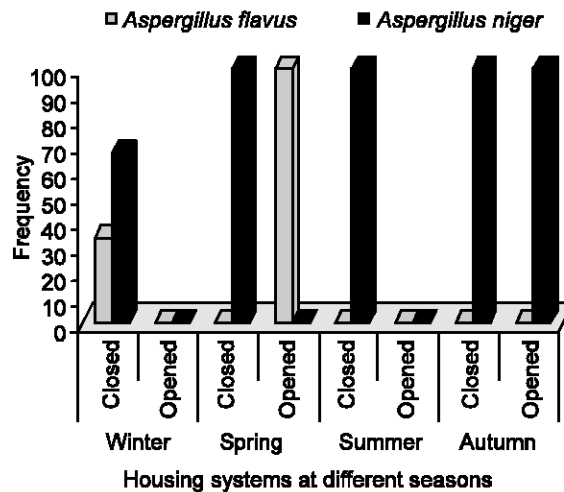


Fig. 8: Frequency distribution of the fungal isolates in the examined air samples at different seasons

*albicans* (61.4%) predominated in open houses in autumn.

Figure 8, the frequency distribution of fungal and mycotic isolates in the examined air samples at different seasons revealed that *Aspergillus niger* (66.6%), *Aspergillus flavus* (33.33%) predominated in the closed houses, no fungal growth was detected in open houses. *Aspergillus niger* (100%) predominated in closed houses, *Aspergillus flavus* (100%) predominated in open houses in spring. *Aspergillus niger* (100%)



predominated in closed houses, no fungal growth was detected in open houses. *Aspergillus niger* (100%) predominated in closed houses, *Aspergillus niger* (100%) predominated in open houses.

**Conclusion:** The results revealed that the microbial (bacterial and fungal) prevalence was varying from one type of sample to another (litter, water, swabs and air), from one season to another (winter, spring, summer and autumn) and from one housing system to another (naturally ventilated houses or open house system and artificially ventilated houses or closed house system). The bacterial prevalence generally was predominating in litter, swabs, air and water samples respectively in winter, spring and autumn. On the contrary; in summer the highest prevalence for bacterial isolates was in swabs, air, water and litter. The fungal prevalence generally was predominating in swabs, air, water and litter samples respectively in winter, spring, summer and autumn.

Also the results revealed that the highest rate of bacterial isolation in closed houses was carried out during autumn, summer, winter and spring respectively, and in open house system the highest isolation rate was carried out during summer, winter, autumn and spring respectively. On the other hand highest rate of fungal isolation in closed houses was carried out during winter, summer, spring and autumn respectively and in open house system the highest isolation rate was carried out during autumn, spring, winter and summer respectively.

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