EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
MICROBIOLOGY

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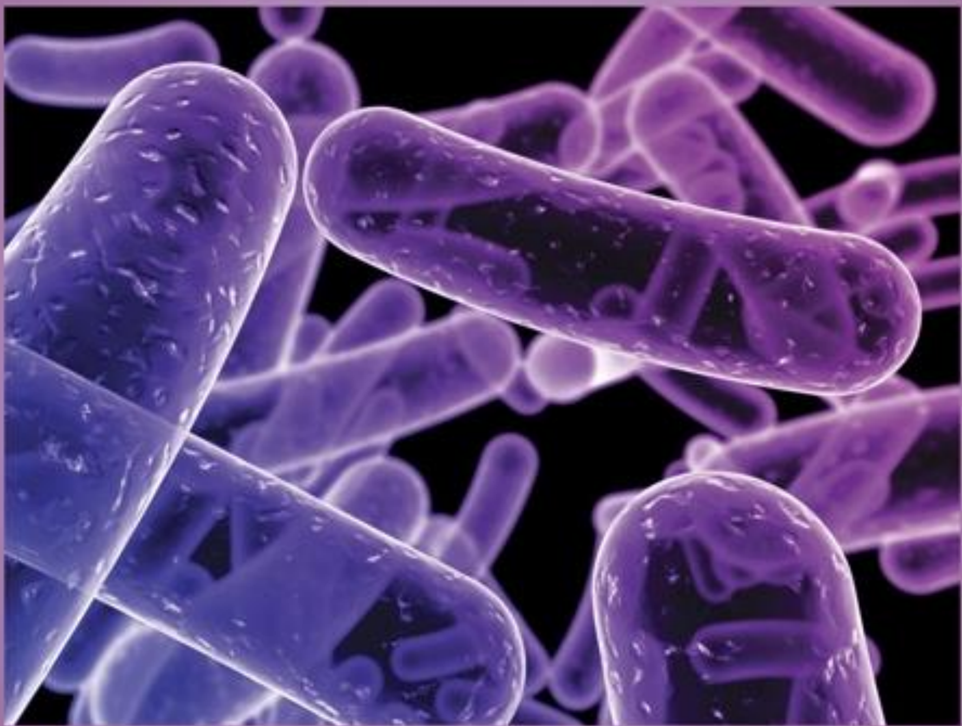

ISSN
2090-0872

WWW.EAJBS.EG.NET

Vol. 14 No. 2 (2022)

Citation: *Egypt. Acad. J. Biolog. Sci. (G. Microbiolog) Vol.14 (2) pp.113- 121 (2022)*
DOI: 10.21608/EAJBSG.2022.260030

Egypt. Acad. J. Biolog. Sci., 14(2):113-121(2022)

Egyptian Academic Journal of Biological Sciences
G. Microbiology

ISSN: 2090-0872
https://eajbsg.journals.ekb.eg/

Investigate the Bacterial and Fungal Infections That Cause Respiratory Tract Infections and Study Their Resistance to Modern antibiotics

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ARTICLE INFO
Article History
Received:19/7/2022
Accepted:25/9/2022
Available:27/9/2022

***Keywords*:**
Respiratory tract infection, CRP, Hematological parameters, bacteria, yeast, antibiotic.

ABSTRACT

This study was conducted on 110 patients who suffer from respiratory tract infections in Samarra General Hospital. The results of isolation and identification show 110 bacterial and yeast isolates. The results of isolation and diagnosis showed that 32 isolates of Staphlococcus aureus were obtained, with a percentage of 29%. *Klebsiella pneumonia* 27(24.5%), *Pseudomonas aeruginos and Haemophilus influenzae*13 isolates with a percentage of 11.81%. *ten isolates of Streptococcus pneumonia* and *eight of Streptococcus pyogenes* in 9.09% and 7.2% respectively. 7 isolates of *candida albican*. The study showed a significant difference in the incidence of bacterial infection according to gender, the infection rate among males was 73%, from 73 samples out of the total samples, with a number of 78 isolates, As for females, the rate of infection was 27%, from 27 samples, and the numbers of isolates were 32. The ages of the infected patients ranged from 10 to 80 years. The highest number of isolated bacterial and fungal species was within the age groups 50-60 years, and the lowest was above 70 years. C-reactive protein test and some hematological parameters were performed. The results showed different values between patients and the normal range. The results showed that most of the bacterial isolates were resistant to antibiotics, Ampicillin, Oxacillin, Clindamycin, Gentamicin, Ceftriaxone and Trimethoprime while all the isolates were sensitive to Meropenem, Imipenem, Amoxiclave, Ciprofloxacin and Levofloxacin while their resistance to Azethromycin, Erythromycin are variable.

INTRODUCTION

The respiratory system is the link between the outer wall and the body, as it contains a lot of types of bacteria that are part of normal flora (Albrich *et al.*, 2012) and some of them have a role in respiratory injury because they are opportunistic nature in the case of weak body immunity system, Acute respiratory tract infections (ARTIs) are the leading cause of death in low-income countries and rank fifth among the world's leading causes of death, with a global statistic indicating that about three million adults and three million children die each year from this type of infection (Jansen, 2014). Bacteria play a greater role in respiratory infections than viruses and fungi. Most previous studies have agreed that some types of *Streptococcus* are the most common causes of respiratory infections, particularly *S. pneumoniae* bacteria, as they are the primary cause of pneumonia.

These studies did not overlook the role of races and other bacterial species, particularly *Klebsiella pneumonia*, which is an important cause of respiratory injuries because they have multiple virulent factors that facilitate the process of doubling them within the host (Gray & Stevevs, 2009; Ashurst & Dowson, 2018). *Staphylococcus aureus* infection is a major public health problem due to the ferocity of bacteria and the emergence of multi-resistant strains (Durand, 2009). *Staphylococcus aureus* is the most important species that are distinct from other types of staphylococcus that cause disease in humans, through their ability to clot blood plasma, called cops, through the action of the coagulase enzyme (Foster, 2009; González-Martín, *et al.*, 2020). Thus, S. aureus infection is a challenge in terms of diagnosis and treatment strategies (Pérez *et al.*, 2018).

MATERIALS AND METHODS

120 samples (blood, swab, and sputum) were collected from people confirmed to have respiratory diseases and who were hospitalized in Samarra General Hospital. Both genders aged from 10-80 years, in the period from 1/11/2021 to 1/3/2022. Most of the study period was during the fall and winter seasons, a period that is characterized by a number of factors that weaken the respiratory system and prepare for the emergence of infections. Samples (blood, swab, sputum) were taken from the same patient. Sputum and swab are transferred to the laboratory (College of Applied Sciences, University of Samarra) for culturing in the selective and differentiation media and stained by Gram stain and some biochemical tests were done to confirm the diagnosis tests (Mahon *et al.*, 2014). Antibiotic Susceptibility Test The disc diffusion method was used to determine the antibiotic sensitivity of the isolates using the method described by Kirby-Bauer cited by (Vandepitte *et al.*, 2003). The results were compared with the standard diameter of inhibition zones for each antibiotic (CLSI, 2021)). The antibiotic discs used were Meropenem (MEM), Imipenem (IPM), Ampicillin (AM), Amoxiclave (AMC), Ceftriaxone (CRO), Azethromycin (AZM) Oxacillin (OX), Clindamycin (DA), Ciprofloxacin (CIP), Levofloxacin (LEV), Gentamicin (CN), and Trimethoprime (TMP), Erythromycin (E). (Bioanalyse/ India)

Five ml of blood was withdrawn from the patient, and 1 mL was collected in plastic tubes containing anticoagulant EDTA to estimate the WBC of the lymphocyte and granulocyte, RBC, Hb, PCV and PLT using automation automatic analysis (Company/ Minaray BC-3000 plus/ Germany). Four ml were then placed in a centrifuge for 15 minutes (3600 cycles/min) to obtain the serum for CRP analysis. This process was done simultaneously in the hospital and after filling out the questionnaire to the patient.

RESULTS AND DISCUSSION

In this study, 120 samples (sputum, swab, blood) were collected from patients with respiratory infections who were hospitalized at Samarra General Hospital. The samples that had a growth on bacterial and fungal media were 100 samples, which is estimated at (83.3%) and samples that did not show growth are 20 samples, which is estimated at (16.7%). 110 isolates Single and mixed were obtained from a total of 100 samples, as in Table 1.

The isolates were distributed into 50 isolates (45.45%) of Gram-positive bacteria, 53 (48.18%) of Gram-negative bacteria, and 7 (6.36%) of yeasts, as shown in Figure 1.

Table 1: Samples from patients with a respiratory infection.

Samples types	Number	%	Growing samples	%	Isolates no.	%
sputum	45	37.5	48	48	58	52.8
swab	75	62.5	52	52	52	47.2
total	120	100	100	83.3	110	100

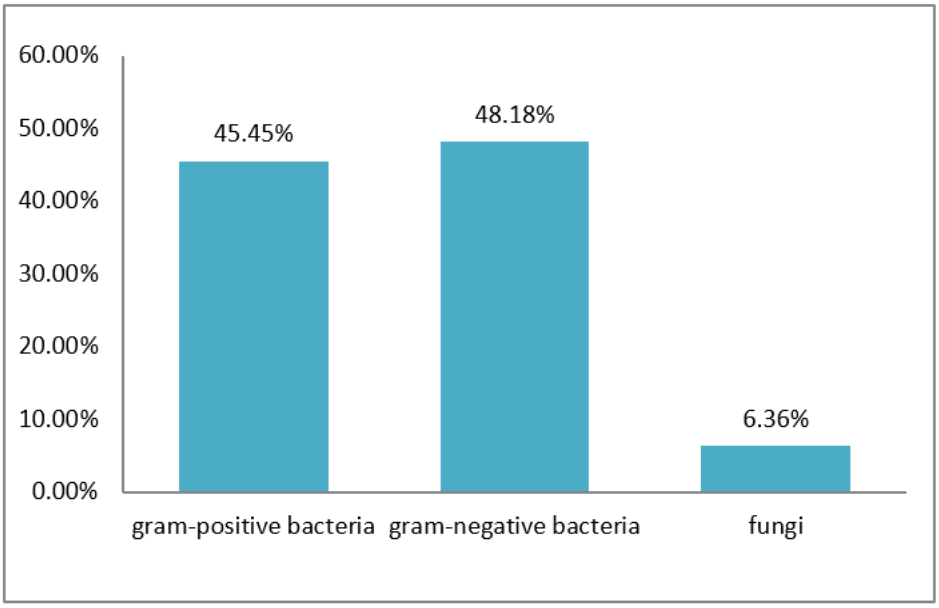

Fig. 1: Types of microbes obtained from respiratory tract infection

Bacterial isolates were diagnosed as mentioned in (Mahon *et al*., 2014). The bacterial species shown in Table 2 and were obtained in Figure 2.

Table (2): The pathogenic bacteria that isolates from respiratory tract infection

Isolates / Tests	*S. aureus*	*S. pneumoniae*	*S. pyogenes*	*k. pneumoniae*	*P. aeruginosa*	*H. influenzae*
Gram stain	+	+	+	-	-	-
Catalase test	+	-	-	+	+	+
Oxidase test	-	-	-	-	+	+
coagulase	+	-	-	NT	NT	NT
Bacitracin	ND	ND	S	ND	ND	ND
Optochin	ND	S	ND	ND	ND	ND
urease	-	-	-	V	V	-
IMVIC	NT	NT	NT	--++	---+	V- V+
Hemolysis	β	α	β	γ	β	V
Motility	-	-	-	-	+	-
X.V factor	NT	NT	NT	NT	NT	+

ND, no data available; NT, not tested; V: variable; S: sensitive

Candida albicans were diagnosed based on their morphological and cultural characteristics. Colonies appeared after two days of incubation and at a temperature of 28°C, with a creamy color, a convex surface, a rounded shape, regular edges, and an average diameter of 1.5 mm. Microscopically, oval cells were observed when examined with a dye of Lactophenol cotton blue. C. Albicans has the ability to ferment both glucose and maltose with the gas formation in the Durham tube, while it can ferment sucrose without producing gas, it is unable to ferment lactose, lactose and dextrose determining the positive result of fermentation by changing the color of the medium from red to yellow (Collee *et al*., 1996).

When investigating bacteria and yeast in 110 specimens for patients of different gender and different ages, from respiratory tract infection patients attending Samarra Hospital, we obtained 32 isolates 29%, belonging to *S. aureus*, this finding was consistent with (Elabbadi *et al*., 2021). These bacteria are common in hospital-acquired infections (Brook *et al*., 2013). 27 of which belong to *K. pneumonia* 24.5% of these results are close to (Egbe *et al*,.2011) who isolated this bacteria by 30% and indicated that *K.pneumoniae* is dominant in the infection of the respiratory system. And it was less than (Ahmed *et al*., 2021), who isolates these bacteria with a percentage of 40.5%. As shown in figure (2) 13 isolates are *P.aeruginosa* 11.81%. These results were consistent with the findings of (Azzab et al.2016), where *P.aeruginosa* was isolated by 15%, *H. influenzae* by 11.81%. The results of the current study are consistent with the findings of (HAli, 2016) who isolated 10.20%, of these bacteria. Ten isolates of *S.pneumoniae* with a percentage of 17%, (Scott *et al.*, 2000) confirmed the role of these bacteria in the occurrence of respiratory infections. These bacteria naturally inhabit the respiratory passages but can enter the lungs by inhalation and it is one of the most common causes of pneumonia. (Brueggemann *et al.*, 2021). Eight of *S.pyogenes* 7.2%. These bacteria possess many virulence factors that help them spread by breaking down connective tissues, resisting the body's defenses and thus increasing their virulence, such as the M-protein (Musher *et al*.,2021). Also, these results agreed with what was reached by (Shakoor *et al*., 2019), who showed in his study that this bacteria's possession of the capsule is of great importance in the pathogenesis of bacteria, as it acts as a physical barrier that helps it to survive for as long as possible through resistance The body's immunity in addition to its role in resisting the process of phagocytosis. Seven isolates of *C. albicans* isolates with a percentage of 6.36%, these results do not agree with the findings of (Shehab, 2005) who isolate *C. albicans* with a percentage of 22%. The genus *C. albicans* is one of the pathogens that affect the lower respiratory tract and cause tissue damage.

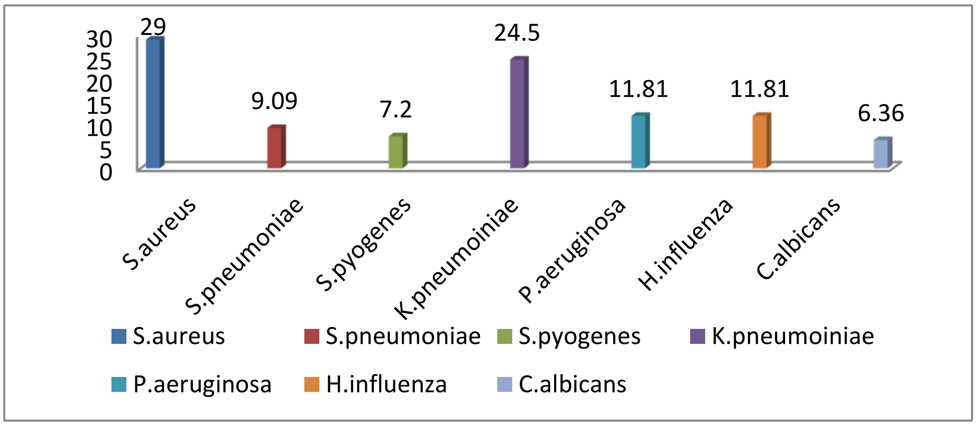

Fig. 2: Percentage of the types of microbes isolated from the respiratory system

Relationship Between Gender and Bacterial and Yeast Infections:

Table (3) showed a difference in the incidence of microbial infection according to gender. The infection rate in males was 70.9%, As for females, the rate of infection was 29.1%. This is due to the female immune system being stronger than the male because estrogen hormone stimulates the immune system, while testosterone works to suppress the immune system, as well as smoking, which causes inflammation of the respiratory tract (Barnes, 2010).

Table (3) Distribution of microbial pathogens according to gender

Pathogens	Total No.	Gender			
		Female		Male	
		No.	%	No.	%
K. pneumonia	27	8	7.27	19	17.27
S. aureus	32	10	9.09	22	20
C. albicans	7	2	1.81	5	4.54
P. aerogenosa	13	3	2.72	10	9.09
S. pyogen	8	3	2.72	5	4.54
S. pneumoniae	10	2	1.81	8	7.27
H. influenzae	13	4	3.63	9	8.18
Total No.	110	32	29.1	78	70.9

Relationship of age to bacterial infections and yeasts

The results showed a difference in the percentage of bacterial infections according to different age groups. The ages of the infected patients ranged from 10 to 80 years, in which the highest number of isolated bacterial and fungal species was within the age groups 50-60 years and the lowest was in the groups 70 years and over. The results showed that the infection rate of Gram-positive bacteria was low, and this is due to the natural presence of most Gram-positive bacteria such as Staphylococcus & Streptococcus, a normal flora in the upper respiratory tract, and these results were consistent with (Roy et al., 2020). (Where it shows a clear impact on the occurrence of bacterial and fungal infections of the lower respiratory tract associated with cases of immune weakness. Table (4) shows the percentage of age groups for respiratory tract infection.

Table (4): relation between age groups and the percentage of infection.

Age group	No. %	*K. pneumonia*	*C. albicans*	*H. influenzae*	*S. aureus*	*S. pneumonia*	*S. pyogenes*	*P. aeruginosa*	No.	%
20-10	(%9)9	5	-	1	3	-	-	-	9	%8.18
30-20	(%16)16	4	2	4	5	-	2	1	18	%16.36
40-30	(%20)20	3	-	2	6	4	2	3	20	%18.18
50-40	(%13)13	4	-	3	3	3	1	2	16	%14.54
60-50	(%31)31	7	4	2	11	3	2	4	33	%30
70-60	(%8)8	3	-	-	3	-	1	1	8	%7.27
70<	(%3)3	1	1	1	1	-	-	2	6	%5.45
	(%100)100	27	7	13	32	10	8	13	110	100

Some Hematological and Immunological Parameters:

The results of the current study showed a difference between patients and the normal range in the level of WBC, LYM, and GRA tests in patients with respiratory infection as shown in Table (5), and there are no significant differences in the level of HGB, MON, RBC.

The increase of white blood cell count (WBC) is caused by infection with one of the pathogenic bacteria, as it leads to stimulating the production of WBC in the bone marrow. A high number of white blood cells may indicate that the immune system is working properly, while a low white blood cell count indicates that there is an injury or a condition that destroys cells faster than they are formed, or that the body is producing fewer than a normal number of body cells, as about 100 billion white blood cells are usually produced White blood cells per day, and there are between 4,000-11,000 of them in approximately every microliter of blood, and in general, white blood cells make up about 1% of all blood cells (Zhang *et al*., 2022). As shown in Table (5) the level of lymphocytes id decreased from than normal range. These results are consistent with what of (Zhu *et al*., 2021)), who indicated in their study that the decrease in the level of lymphocytes is associated with severe bacterial infection, as well as compatible with what was reached by (Kabak.2021). and Hocanlı,. Where they indicate that lymphocytopenia occurs in people with respiratory tract infection, lymphocytes can be used as an auxiliary laboratory marker in the diagnosis of patients with a respiratory infection (Shen *et al.,* 2014)

The level of granulocytes is increased. The results indicate a strong correlation between the level of GRA and bacterial infection, and this is consistent with the findings of (Daix *et al*,.2021), which indicated high levels of GRA in the case of bacteria-related infection.

Table (5): the level of Some hematological and immunological parameters

Hematological parameters	Patients levels	Normal range
WBC (10^3 /µl)	11.08±4.39	(9.5-3.5)
LYM (10^3 /µl)	2.68±1.4	5.0-1.0
MON (10^3 /µl)	0.94±0.4	1.0-0.1
GRA (10^3 /µl)	7.06 ±3.01	8.0 -2.0
RBC (10^6/µl)	4.87±0.72	6.0 -4.0
HGB (gm/dl)	13.37±2.25	17.0-11.0
PLT (10^3 /µl)	284.77±72.61	400 -150
CRP (mg/l)	12.61±4.09	10 >

The results in **Table (5)** showed an increase in the level of CRP-reactive protein concentrations in patients with respiratory tract infection, as its concentration increases with the increase in the severity of the infection. CRP has been associated with the level of inflammation. The CRP levels can activate and enhance phagocytosis, thus cleansing the pathogenic microorganisms invading the body. CRP levels were used for early diagnosis of pneumonia, patients with severe pneumonia had high levels of CRP. It is an important indicator for diagnosing and evaluating severe infectious lung diseases. these results are consistent with (Matsumoto et al. 2019), as it was shown in his study that the increase in CRP levels and the severity of pulmonary infection correlates with the progression of the disease associated with lung injury and disease severity.

Most studies have shown that CRP >10 mg/dL indicates severe bacterial infections. However, studies showed that CRP was the only independent variable for the association between viral and bacterial infections. Recently, studies on the quantitative evaluation of CRP as a diagnostic marker of bacterial infection (Kaya *et al*., 2013) have also been demonstrated.

Antibiotics Resistance:

The results have displayed that the bacteria isolated from respiratory tract infections have a high degree of resistance to most of the antibiotics under investigation. As shown in **Table (6).** In recent years, it has been emphasized that there is a remarkable increase in the incidence of infection by antibiotic resistance bacteria in different parts of the world. For example (Mayer,2005) indicated that *P. aeruginosa* infections are difficult to treat as this organism displays a high level of intrinsic antibiotic resistance. Additionally, he found that treatment with fluoroquinolone antibiotics led to dramatic increases in the MIC which may be associated with treatment failure. Antibiotic resistance in Enterobacteriaceae and some gram-positive cocci undergo a remarkable change in characters with the widespread occurrence of resistance transfer factors (RTF). RTF may transfer to drug-sensitive strains by conjugation in much the same way and with much the same type of kinetics as F transfer in *E. coli*. (Small *et al*., 1993).

Table (6): The resistance of bacterial isolates to antibiotics

Bacterial isolates	MEM	IPM	CIP	LEV	AMC	AM	CRO	AZM	OX	DA	CN	TMP	E
K. pneumonia	R	S	S	S	R	R	R	R	R	R	R	R	R
P. aerogenosa	R	S	S	S	S	R	R	R	R	R	R	R	R
H. influenzae	S	S	S	S	S	R	R	S	R	R	R	R	R
S. aureus	S	S	S	S	S	R	R	S	R	R	R	R	R
S. pyogen	S	S	S	S	S	R	R	S	R	R	R	R	S
S. pneumoniae	S	S	S	S	S	S	R	S	R	R	R	R	S

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