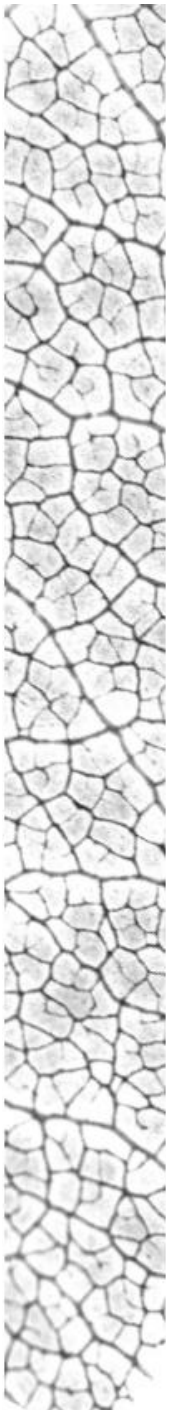

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Microbial examination outcome of sputum at tertiary hospital in Baghdad-Iraq

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Abstract
The lungs are a common site for infection by prevalent pathogens which be faced in worldwide, include bacteria, atypical, viruses, fungi and other infectious agents. Ten million persons became infected with TB and 1.6 million died of this disease. This study was designed to explore the rate of AFB, the most common bacteria and fungi in the sputum specimen obtained from patients with respiratory tract infection. Positive sputum culture was 1155 (5.6%).The prevalence of Gram negative 752 (80.1%) was higher than Gram positive 187 (19.91%). The most common Gram-negative bacteria were: Pseudomonas aeruginosa (19.91%), Moraxella catarrhalis (17.14%), then Klebsiella pneumoniae and Escherichia coli (15.86%), (13.31%) respectively. Streptococcus pneumoniae (15.0%) is at the top of Gram-positive bacteria. A significant greater proportion of TB in West Baghdad than in the East Baghdad. Bacterial isolates exhibited a high frequency of resistance to most of common antibiotics such as: Ticaricillin (TI), Amoxacillin (AMX), Rocephin (CTR), Gentamycin (GEN). Gram negative bacteria population was the most common organisms for RTI then TB and fungi, higher prevalence of microbial infection in men than women and increased of antibiotic resistance especially β-lactam antibiotics.

Keywords: tuberculosis, respiratory infection, sputum, bacterial

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INTRODUCTION

Respiratory tract infections (RTI) are behold as one of the major public health problems, and may cause morbidity and mortality in numerous developing countries (Prajapati et. al. 2011; Manikandan and Amsath2013). The lungs are a common portal for infection by prevalent pathogens which be faced in worldwide (Woerden et. al. 2013), include bacteria, atypical bacteria, viruses, fungi and other infectious agents (Cukic and Hadzic 2016; Papanikolaou and Tsenempi 2020).

Despite tremendous progress in medical sciences and amend health care facilities in progressing countries, TB still must be considered as a health problem affecting large sectors of society, particularly in resource poor setting and developing countries (Khaliq et. al. 2015; Mason et. al. 2017). Globally, Tuberculosis (TB) is the ninth leading cause of death as a single infectious agent (Srivastava et. al. 2015; Safwat et. al. 2020). World Health Organization (WHO) published that,ten million people became infected with TB and 1.6 million died of this disease. (Rai et. al. 2019; WHO. 2019).

Tuberculosis (TB) as an infectious disease, detectable by Mycobacterium tuberculosis bacteria

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(MTB) in the sputum, and airborne a major source of transmission (Hasan et. al. 2015; Ambreen et. al. 2019). Various pathogenic bacteria and fungi are representing as a causative agent for respiratory tract infection which found with/without tuberculosis in sputum, included: *Pseudomonas spp., Streptococcus spp., Proteus spp., Klebsiella spp., Staphylococcus spp., Enterobacter spp., Acinetobacter spp. Haemophilus influenza, Moraxella catarrlis,* and *Actinomyces*(Nagalingam et. al. 2005; Mankandan and Amsath 2013; Hasan et. al.2015). Furthermore, the incidence of pulmonary mycotic infection has increased significantly since the 1960s, particularly due to increasing of risk factors or groups of patients and could fatally dimensions to pulmonary TB. These opportunistic fungi are acquired either by inhalation of contaminated soil or a as part of normal flora (Mwaura et. al. 2013; Kali et. al. 2013; Amiri et. al. 2016).

Many exogenous and endogenous factors may increase the development of any infection to disease (Narasimhan et. al. 2013; Nhamoyebonde and Leslie 2014).The Age and gender have a marked effect in Infectious diseases which are rarely impact males and females equally; the two genders may have different combinations of risk factors (Silveira and Franch 2013; Mason et. al. 2016). Increasing evidence that tuberculosis (TB) was diagnosed in men more than in women (Horton et. al. 2016; Safwat et. al. 2020). Frequently, different methods for obtaining sputum samples are considerable needed to diagnose pulmonary infections Biswas et. al. 2013) and for microbial analysis which is widely used in developing countries (Deka et. al. 2016; Satapara et. al. 2017).

Antimicrobial resistant is a global problem (Budayanti et. al. 2019) as a result of the abuse of antibiotics on both quantity and quality (Fletcher 2015; Hammadi et. al. 2017) in addition to starting immediately antibiotic treatment without waiting results for bacterial culture (Cukic and Hadzic 2016; Budayanti et. al. 2019).

This study was designed to explore the rate of AFB, the most common bacteria and fungi in the sputum specimen obtained from patients with respiratory tract infection.

MATERIALS AND METHODS

Setting and design: AL-Yarmouk Teaching Hospital is the biggest tertiary referral hospital with a capacity of 800 beds. It is located in West of Baghdad named Al-Karkh side; the other side is Al-Rusafa to the East. A retrograde study covered a period of six years, between January 2013 and December 2018. The outcome of the sputum samples study from both out and in-patients suffering from respiratory tract infections. Sputum were collected from both genders and all age groups.

Sputum specimen collection, technique and equipment used were in accordance with WHO program and guidelines (WHO 2019).It is important to check that the sample contains sputum, and not oropharyngeal secretions nor saliva, the good-quality specimens were those with ≤10 squamous epithelial cells and >25 leukocytes per field (ElKorashy and El-Sherif 2014). Usually specimens obtained in early morning due to the secretion aggregation in the lung overnight, providing convenient environment in which bacteria can proliferate; this is particularly important when testing for tuberculosis. Contamination of the sample can result in inappropriate or delayed treatment, to avoid that and before breakfast meal, the patients were asked for rinsing and repeated gargling with tap water and avoided to clean their teeth or use a mouthwash which may kill the bacteria, as well as, samples were collected before antibiotics are started (Biswas et. al. 2013; Shepherd 2017; Gebremeskel, et al, 2016).

All sputum specimens were collected in sterile containers, have dry wide-necked, leak-proof with screw cap labeled with the patient's medical registration number. Usually, patient could go outside or open a window, take a very deep breath and hold the air for 5 seconds, slowly breathe out, then take another deep breath, cough deeply, spit the sputum into the container and promptly screwing of the cap and transported to the laboratory within two hours and processed directly. The specimen amount should be enough for testing, reaches the 5 ml (or more) or about one tea spoon (at least small fingernail) (Minnesota Department of Health2018).

The samples then delivered in a convenient manner to the Microbiology unit at Teaching Laboratories in AL-Yarmouk Teaching Hospital. All samples were analyzed to identify TB by Ziehl-Neelsen (ZN) stain, or bacteria, yeasts and fungi by (Gram'-stain), in addition, these samples were cultured on different media to identify the type of bacteria, antibiotic sensitivity test was done too.

Direct sputum smear microscopy remains the essential techniques for the diagnosisespecially for acid-fast bacilli (AFB), it is widely available, simple, and inexpensive method for pulmonary TB diagnosis and treatment monitoring (Ambreen et. al. 2019). The patients with suspected pulmonary tuberculosis, early morning sputum samples were collected on three sequential days. The sputum samples were stained with Ziehl-Neelsen stain and examined for Mycobacterium tuberculosis (AFB) using the oil immersion lens and the result were scored as a positive for TB when at least two sputum smears were positive for acid-fast bacilli (AFB) test (Rai et. al. 2019).

For each positive TB result another sample was taken from the patient and was sent to The National Reference TB Laboratory (NTP) Baghdad/Iraq to confirm the result. This reference laboratory is working according to standard protocols and the guidelines provided by the Global Laboratory Initiative (GLI) advancing TB diagnosis, Mycobacteriology Laboratory Manual2014 (Stinson et. al. 2014). According to The

Iraqi National TB Control Program, ten glass slides were collected from The Specialized Chest and Respiratory Disease Center for quality control assurance (Tuberculosis panel test).

Gram's stain technique is the most convenient and available microbiological test for sputum sample which were examined for identification of bacterial infections and fungal elements, in addition, White Blood Cells (WBCs) and Squamous Epithelial Cells (SECs) (Cukic 2013). Samples with more than 25 WBCs, and less than 10 SECs per low power field were considered appropriate for culture. Sputum samples were also subjected to Potassium Hydroxide (KOH) preparations for the detection, identification and descriptive information of fungal elements (Kali et. al. 2013).

Sputum culture and Identification of bacterial isolates: In the Bacteriology laboratory, sputum sample was inoculated on (Blood, McConkey), and Chocolate agar (under CO2) for bacterial identification and Sabouraud Dextrose Agar for fungi. A sterile wire loop was used, being prepared according to the manufacturing companies (Biomark™, India) and (Biorex, UK). The culture plates were incubated for 24-72 hours at 35°-37°C for bacteria, and at 25-30°C for fungi observation and evaluation for growth done every 24 hrs during the first seven days and weekly till 28 days through the formation of colonies. The growth was identified characteristics depend on the: morphological, microscopy and conventional different biochemical tests (Jawetz et. al. 2019).

Biochemical tests: After one day incubation Gram stain and biochemical tests(IMVC) were used to classify the colonies of growth into Gram positive, Gram negative and fungi, (Api 20 Staph, Api 20 Strep,Api 20 E) and Api Candida (Biomereumix-France) were used for bacteria and fungi respectively (Bekkari et. al. 2016).

Antibiograms of isolates and antibiotic susceptibility testing: Out of 939 bacterial isolates 677 were subjected to the antibacterial resistance study using twenty-eight antibacterial discs, conformity by Kirby-Bauer method for susceptibility test, used the disc diffusion technique and was accomplished on Muller-Hinton agar (Biomark™, India) were incubated at 35-37ºC for 18-24 hours under aerobic condition. The inhibition area around disc was measured in accordance to CLSI guidelines 2017 (Patel et. al. 2017). The following antibiotics were used: Ampicillin (AMP),Ceftazidime (CAZ), Erythromycin (E), Piperacillin (PI), Ticaricillin (TI), Ciprofloxacin (CIP), Cefaclor (CEC), Amoxacillin (AMX), Chloromycetin (C), Imipenem (IPM), Ceftriaxone (CRO), Amikacin (AK), Cephalothin (KF), Rocephin (CTR), Tetracyclin (TE), Cefotetan (CTT), Teicoplanin(TEC), Trimethoprim (TR), Cefixime (CFM), Cefoxitin (FOX), Tobramycin (TM), Doxycycline (DOX), Gentamicin (GEN), Gemifloxacin (GEM), Sulfatrim (COT), Azithromycin (AZM), Cefotaxime (CTX) and Aztreonam (ATM) (Al-Razi Diagnostics Center/Iraq and Bioanalys /Ankara-Turkey).

Table 1. Results of sputum examination over sixyears' time of the study

Year	TB		Bacteria		Fungi		Total examined sputum sample
	Sputum sample	Positive	Sputum sample	Positive	Sputum sample	Positive	
2013	1136	16	408	178	1544	7	1551
2014	4230	43	546	317	4776	24	4800
2015	2502	37	721	112	3223	14	3237
2016	3368	30	592	80	3960	16	3976
2017	2995	29	475	119	3470	14	3484
2018	3037	14	499	92	3536	13	3549
Total	17268	169	3241	898	20509	88	20597
P-value	0.0038		0.0001		0.905		

Table 2. Positive sputum for TB, bacteria, and fungus distributed according to gender

Year	Gender	+ve TB	+ve Bacteria	+ve Fungi
2013	Male	9	94	6
	Female	7	84	1
2014	Male	30	165	15
	Female	13	152	9
2015	Male	23	67	7
	Female	14	45	7
2016	Male	15	44	10
	Female	15	36	6
2017	Male	14	78	10
	Female	15	41	4
2018	Male	8	58	9
	Female	6	34	4

Statistical analysis: Data were submitted to grouping, tabulation and analysis using Statistical Package for the Social Sciences (SPSS) version 24 software. The results expressed in frequencies and percentages, Chi-square test was used to explore the association between variables, p-value of 0.05 less than was considered as significant value.

RESULTS

During the six-year study period a total of 20597 sputum sample of LRTI cases (Direct smear, cultured on microbial agars and Sensitivity test) were analyzed in Microbiology unit.

Table 1 showed a total sputum sample analyzed were 20597, among them 1155 were positive (5.6%) and 19442 (94.4%) were negative. For TB 17268 sample were examined, 169 sample were positive at a rate of (1.1%), (898) sample were positive for bacterial growth out of 3241 sample gave a rate of (27.7%), and only 88(0.4%) were positive for fungal growth. There was a significant relationship of positive results of TB and bacterial growth in different years of the study (P-value 0.0038, 0.0001respectively) but not with fungal growth (P=0.905).Noticeably the highest sputum samples were collected and the highest ever for positive result were recorded in 2014.

The distribution of positive microbial findings according to gender is illustrated in (**Table 2)**. Out of

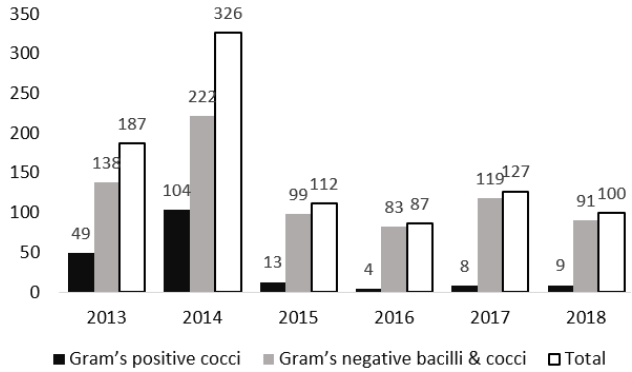

Fig. 1. Gram's stain differentiation of bacterial growth according to the years of the study

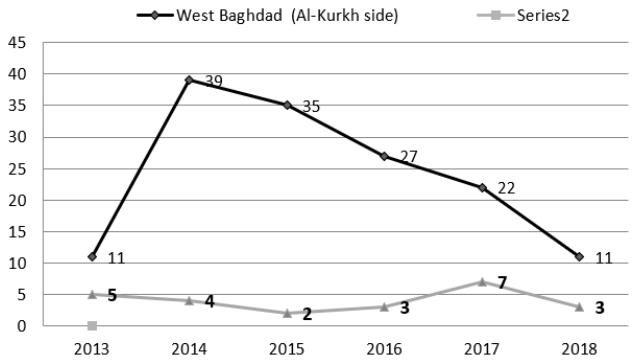

Fig. 2. TB positive sputum along the two sides of Baghdad

Table 3. Types of bacteria isolated during the period of the study

Bacteria	2013	2014	2015	2016	2017	2018	Total
Staphylococcus aureus	15	14	2	2	6	4	43
Streptococcus pneumoniae	34	87	11	2	2	5	141
Enterococci	-	3	-	-	-	-	3
Escherichia coli	11	12	24	20	34	24	125
Pseudomonas aeruginosa	20	49	16	27	37	38	187
Klebsiella pneumoniae	20	52	19	13	23	22	149
Enterobacter cloacae	3	7	8	2	6	3	29
*Proteus*spp.	1	4	4	4	6	2	21
Acinetobacter baumannii	3	7	21	7	6	1	45
Serratiamarcescens	1	6	2	1	-	-	10
Citrobacter spp.	4	3	3	2	7	1	20
Moraxella catarrhalis	75	82	2	2	-	-	161
Stenotrophomonasmaltophilia	-	-	-	2	-	-	2
Burkholderiacepacia	-	-	-	2	-	-	2
Pantoeaspp	-	-	-	1	-	-	1
Total positive culture	187	326	112	87	127	100	939

1155 positive results, the highest percentages were in males 662 (57.31%) in comparison to females 493 (42.68%).

There is a decline in the number of bacterial isolates throughout the six years of this study. Out of total 939 bacterial isolates, the prevalence of Gram negative 752(80.1%) which was higher than Gram positive 187(19.91%).The most common Gram-negative bacteria out of the total 939 isolates were *Pseudomonas aeruginosa* (19.91%), followed by *Moraxella catarrhalis* (17.14%), then *Klebsiella pneumoniae* and *Escherichia coli* (15.86%), (13.31%) respectively. Heavy growth of *Streptococcus pneumoniae* (15.01%) as a Gram positive was also seen. Furthermore, the total rates of bacterial isolates were at its highest rate 326 (34.71%) in 2014.The prevalence of Gram negative was (68.1%) 222/326. The most common one was *Moraxella catarrhalis* 82/222 (36.93%), then *Klebsiella pneumonia* 52/222 (23.42%) and *Pseudomonas aeruginosa* 49/222 (22.02%) (**Figure 1**) and (**Table 3**).

As plotted in **Figure 2**, a significantly greater proportion of TB in West Baghdad (Al-Karkh side)than in the East Baghdad (Al-Rusafa side) (85.8% vs.14.2%, P< 0.05).Moreover, the highest result for TB in Al-Karkh side 39/145 in 2014, whereas Al-Rusafa side recorded 7/24 positive in 2017, (26.89%) and (29.16%) respectively.

Out of 939 bacterial isolates,102(15.1%) G+ve and 575(84.9%) G-ve were subjected to different types of antibiotics in the culture media and the outcome were put in (**Table 4**).It was divided streamlined (2013-2018) as follows: 163 (43, 120), 190 (40, 150), 106 (8, 98), 72 (2, 70), 101 (6, 95) and 45 (3, 4) (Gram positive, Gram negative) respectively. The highest ranked to show resistance against varieties of selected antibiotics was: *E.coli* then *K. pneumoniae* and *Ps. aeruginosa* as a Gram negative, and *Strept. pneumoniae* as a Gram positive.

Bacterial isolates exhibited a high frequency of resistance to most of common antibiotics such as: Ticaricillin (TI), Amoxacillin (AMX), Rocephin (CTR), Gentamycin (GEN), Tetracyclin (TE), Piperacillin (PI), and moderate frequency of resistance for Ampicillin (AMP), Erythromycin (E), Amikacin (AK). While the rest results showed less frequency of resistance to the other antibiotic used in this work.

DISCUSSION

The lungs are one of the most common portals for infection by viruses, bacteria, fungi, protozoa and other infectious agents (Woerden et. al. 2013). Bacterial and fungal infections secondary to pulmonary tuberculosis has gained more relevance recently due to its ability to survive within the tubercular infection for decades and to the increased use of broad-spectrum antibiotics. Immunosuppressive drugs lead to weakening of immune response, then reactivation of old TB (Kali et. al.2013; Hasan et. al. 2015).

Our findings were acceptable when scored lower percentage of positive growth(5.6%) and high percentage of negative culture (94.4%) when compared with that reported in Sarajevo, Bosnia and Herzegovina study,(20,2%) (79,8%) respectively (Cukic and Hadzic 2016), on the other hands, our study recorded high prevalence of bacterial growth (Gram negative and Gram positive) and lower percentage of AFB bacilli (TB) then fungi when compared with the other studies in 2013, 2014 showed (2.3%), (25.9%) Gram negative

Table 4. Pattern of bacterial resistance to different antibiotic (n=677 isolate submitted for sensitivity test out of 939)

	Staph. aureus	*Strept. pneumoniae*	*E. coli*	*Ps. aeruginosa*	*K. pneumoniae*	*E. cloacae*	*Proteus* spp	*A baumannii*	*S. marcescens*	*Citrobacter*	*M. catarrhalis*
AMP	5	9	24	11	20	6	7	14	3	2	9
CAZ	-	7	9	10	13	4	1	1	1	4	3
E	12	23	-	-	-	-	-	-	-	-	29
PI	2	1	30	20	26	5	2	13	2	11	-
TI	1	-	42	51	16	4	9	17	2	11	4
CIP	2	3	9	13	17	3	1	7	-	7	18
CEC	2	-	10	7	16	1	1	10	-	4	1
AMX	9	8	42	12	39	11	7	12	2	5	34
C	1	1	5	7	8	1	3	11	1	-	1
IPM	1	4	9	14	3	1	1	14	1	-	5
CRO	2	1	6	8	8	2	-	5	-	3	-
AK	-	-	9	9	6	1	4	17	-	21	-
KF	1	-	8	6	6	6	5	1	-	1	2
CTR	-	1	41	18	20	3	8	19	1	4	1
TE	4	4	30	21	21	3	6	19	2	3	18
CTT	-	-	12	4	9	1	2	4	3	-	-
TEC	-	1	5	2	9	-	-	5	1	-	-
TR	1	1	5	5	8	-	1	1	-	1	-
CFM	2	-	15	12	10	6	1	5	-	3	-
FOX	-	1	3	8	6	5	2	4	-	1	-
TM	-	1	7	3	3	1	2	1	-	1	-
DOX	-	-	4	4	3	-	1	-	-	1	-
GEN	3	6	17	36	29	6	7	24	3	4	10
GEM	1	1	2	2	9	-	1	2	1	-	1
COT	1	1	2	2	12	-	2	2	-	1	2
AZM	-	1	16	9	15	1	3	18	2	3	-
CTX	1	1	2	3	6	3	-	1	-	1	-
ATM	1	-	7	7	3	-	2	1	-	1	-

rods, (9.30%), (11.1%) Gram positive cocci, respectively and in 2016 (44.18%), (6.51%), TB and fungi respectively (Mwaura et. al. 2013; ElKorashy and El-Sherif 2014; Amir et. al.2016). The sensitivity of a diagnostic test depends on the quality of the sputum samples obtained; thus, poor quality sputum samples can lead to missed diagnoses for tuberculosis or other microbial infections (Datta et. al.2017). Microscopy (direct smear) is inexpensive and it's the most frequently used laboratory test globally. In addition, reference have been made that sputum culture results commonly correlated with the smear results, but not always correlate with clinical parameters and this depending on the laboratory technicians that trained in national TB diagnostic guidelines and others (Campbell and Forbes 2011; Cukic 2013).

For host-related factors, several studies described that the higher prevalence of TB infection were found to be significantly associated with male gender than female (Khaliq et. al. 2105; Srivastava et. al. 2015; Singh et. al. 2018; Rai et. al. 2019; Safwat et. al. 2020) and this in consistence with our results in this study. TB epidemiology was affected by age and sex throughout life because each gender has different risk factors for TB, elderly, poor, malnourished and other environmental factors (Singh et al. 2018), in addition, incarcerated men are a vulnerable for TB (Dara et. al. 2015; Mason et. al. 2017), on the other hands, Lönnroth et. al. and Dodd et. al. (2015) confirmed to considered men as a high-risk group for TB and should more subjected to routine diagnostic and/or screening services (Lönnroth et. al. 2013; Dodd et. al. 2015)

Many studies showed higher rate of bacterial isolation in comparison to the current study, according to (Mansoor et al.), where the rate of organisms isolated, they were 25.8% *Klebsiella pneumoniae*, 23.5% *Streptococcus pneumoniae* and 18% *Pseudomonas aeruginosa* (Mansoor et. al. 2017), this is supported by other study of Promite et al. (2017) who stated that about 17 respiratory tract infection (RTI) patients were infected by *Klebsiella pneumoniae* 42.5%, 30% by *Escherichia coli* and 27.5% by *Pseudomonas aeruginosa* (Promite et. al. 2017), Whereas, (Anvari et al.) issued that the most prevalent bacteria isolated from the respiratory secretions was *Acinetobacter* (Anvari et. al. 2014), while, (Cukic and Hadzic), detected that *Streptococcus pneumoniae* was significantly the most common bacterium isolated (Cukic and Hadzic 2016) in addition to (Budayanti et. al.) published that *Klebsiella pneumonia* was dominated pathogens (30.86%) (Budayanti et. al. 2019), and this coincides with (Altiner et al.) research who found that *Streptococcus pneumonia, Haemophilus Influenzae, Haemophilus Parainfluenzae*, and *Moraxella catarrhalis* were typical pathogens which isolated from sputum samples (Altiner et al. 2009), in contrast, our study registration showed higher percentages than the results of a study conducted in 2014 in Egypt and in 2015 in Basra-Iraq when projected that the commonest isolate was *Pseudomonas aeruginosa* (11.1%) and (35.1%)

followed by *Klebsiella pneumoniae* (7.4%) and (10.8%) respectively (ElKorashy and El-Sherif 2014; Hasan et. al. 2015). Our finding was congruent with (O'Neill 2016) and with a study conducted in Dhaka-Bangladesh, found that the causative pathogens of RTIs are *Streptococcus pneumoniae, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli.* (Promite et al. 2017), On the other hand, current results are similar to that previously reported in London, UK by (Zhang et al.) who clarified that the LRT have until recently been considered to be a sterile environment, but sputum samples showed similar bacterial profile when repeated culture and suggested either these bacteria were mostly colonizing the lower airways, or Innate immune responses may be defective in addition to the Logistic regression analysis showed that the long duration of pulmonary infection was a significant risk factor for bacterial colonization (Zhang et. al. 2012).

This study manifest fungi co-infection with TB or bacterial Gram which observed in 88/1155 (7.61%) of patients with pulmonary infections, this result was lower than three India's studies in 2013, 2015 and 2016 recorded (40%, 35% and 62%) respectively, (Kali et. al. 2013; Adhikari et. al. 2015; Kalyani et. al. 2016), in addition a study in 2015 in Valencia-Spain published a total of 252 patients were included in the study, 89(35.3%) had persistent positive cultures for fungi elements Máiz et. al. 2015), the current findings was congruent with a study conducted in 2017 by (Satapara et al.) in Gujarat-India where (8%) the cultured sputum samples on Sabouraud agar were positive for fungus (Satapara et. al. 2017). Fungal pulmonary infection has been emerging lately as a result of a widely used broad-spectrum antibiotics and steroids and the Immune deficiency or suppression in TB patients made them easily vulnerable to opportunistic fungal infections (Kalyani et. al. 2016).

The proportion of patients reported in West Baghdad (Al-Karkh side) was higher than that of East Baghdad (Al-Rusafa side), this could be attributed to the location of AL-Yarmouk Teaching Hospital in Al-Karkh side and hence most of visitors and patients attend the hospital were from this Al-Karkh side. There are regions in this side especially at the periphery suffer from, low level of education, financial and social problems, overcrowding residency, and poor housing status, all these factors and more are undermining the lung's defense and increased the risk for Tuberculosis. (Osman et al.) and (Nhamoyebonde and Leslie) were suggested other risk factors for tuberculosis including malnutrition, smoking, alcoholism, silicosis, diabetes and male sex (Osman et. al. 2013; Nhamoyebonde and Leslie2014).This was supported by researchers who thought that improving: housing habitat, decreased crowding, better hygiene and sanitation, use of clean water, better nutrition and isolated of infectious TB patient in sanatoria, may contributed to decline TB notification (Srivastava et al. 2015, Singh et al. 2018), in contrary, Bergen-Norway study in 2019 demonstrated, culture positivity at 6 months for pulmonary TB cases, was not associated with socioeconomic status in these continuation study phase (Ambreen et. al. 2019).

The susceptibility patterns were varied from one bacterial isolate to another depending on the drug. The susceptibility test against 11 geneses of bacterial isolates out of 15 and 28 commercially used antibiotics was used. The current study shows highly resistance to the most antibiotics, specially to Beta lactam: Ticaricillin (TI), Amoxicillin (AMX), Piperacillin (PI) and moderate frequency of resistance for Ampicillin (AMP), also Aminoglycosides: Amikacin (AK), Gentamycin (GEN), Cephalosporin: Rocephin(CTR), Macrolides: Erythromycin (E) and Tetracycline (TE). However, poor-quality of sputum specimens during collection, with the high rate of contaminations by normal flora may cause misdiagnosis of the bacterial types then errors in define antibiotics for sensitivity testing (Subramanyam et. al. 2013; Froment et. al. 2014) It is interesting to find that the results of this study ware close to the results of other studies reported by several researchers (Mansoor et. al.2017; Samad et. al. 2017; Farooq et. al. 2019), despite our findings was not corroborative by two researchers reported that most isolates were susceptible to Aminoglycosisdes (Amikacin) (Manikandan and Amsath2013, ElKorashy and El-Sherif 2014), another study conducted by Anvari et al. reported that *Acinetobacter*is the most prevalent Gram negative bacteria was isolated from respiratory secretions and represented the highest resistance to Ceftriaxone (Anvari et. al.2014), surprisingly a study conducted in 2017 It reported that *P. aeruginosa* and *K. pneumoniae* were resistant to Carbapenem, Cephalosporin, Trimethoprim and Ciprofloxacin antibiotics (Promite et. al. 2017).

Antimicrobial susceptibility is influenced by the origin of infection, periodic alterations, and epidemiological factors according to the regions (Agmy et. al. 2013; Anvari et. al. 2014), however, there are enormous opportunities of environmental factors which increase the antibiotic resistant, the improper use and exposures to broad spectrum antibiotics result the preface to grow resistance genes and the rapid increase in the isolation of resistant strains. (Fletcher 2015; Farooq et. al.2019), in the same context, the transmission of resistant bacteria among the patients and from health staff workers to patients and vice versa, also, lack guidelines for adequate and reasonable use of antimicrobial, as well as, lack of easy-to-use auditing tools for restriction (Aly and Balkhy 2012; Manikandan and Amsath2013). The study from (Samad et al.) showed, that *pseudomonas aeruginosa* develops resistance against almost all antibiotics by several mechanisms like, multi-drug resistance efflux pumps, resistance genes, biofilm formation, aminoglycoside modifying enzymes and

mutations in different chromosomal genes. Samad et al. (2017), therefore, careful selection of antimicrobial therapy has become essential to prevent exacerbation of antigen-resistant isolates (Nagalingam et al. 2005) and avoid the clinicians to implement “blind therapy” before laboratory diagnoses are available and health sectors should educate and instruct public with socialization on appropriates usage of antimicrobial agents.

CONCLUSIONS

In conclusion TB is still an important health problem, more prevalent in West Baghdad (Al-Karkh side). Gram negative bacteria remain the most common organisms for RTI. Fungal pathogens as a secondary or co-existing with Gram bacteria and/or TB infection was noticed. Increasing resistance to antibiotic especially β-lactam and Aminoglycoside is a major concern.

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