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Molecular Study of (Mycoplasma pneumoniae, Streptococcus pneumoniae) in respiratory infections patients in thiqar province

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Abstract

The high death and hospitalization rates caused by bacterial respiratory infections make them a major worldwide health problem. Acute otitis media, bacterial rhinosinusitis, pharyngotonsillitis, bronchitis, and community-acquired pneumonia are among the common respiratory tract illnesses caused by common bacterial respiratory infections, which include Streptococcus pneumoniae, Mycobacterium tuberculosis, Haemophilus influenzae, Chlamydia pneumoniae, Mycoplasma pneumoniae, and Legionella pneumophila.

The study aimed to detect (Mycoplasma pneumoniae) and Streptococcus pneumoniae that caused respiratory infections and determined if it caused this infection. The study included 140 patients (67males and 73 females) who were suffering from respiratory infections, ages ranging from 10-80 years, in a period from 1 September 2023 to 1 February 2024, all of the 140 samples collected from Nasiriyah Teaching Hospital, Center for Chest Diseases and Respiratory Diseases in Nasiriyah, Souq Al-Shuyoukh General Hospital, Alhussein Teaching Hospital, Private Clinics in Shatrah. Specimens included a sputum sample and oropharyngeal swab for direct detecting by polymerase chain reaction (pcr) Diagnosis is based on the presence of the (16s rRNA) gene to detect (Mycoplasma pneumoniae, Streptococcus pneumoniae) patients had with un-cultivated bacteria where 3\140(2.14%) of patients had infection with Mycoplasma pneumoniae and 6\140(4.28%) with Streptococcus pneumoniae

The present study was conducted that the most detected bacterial in female group 6 (75.0%), while in the male group 2 (25.0%), in addition, the most isolated bacteria in female group were both *M. pneumoniae* 3 (50.0%), and *S. pneumoniae* 3 (50%),whil in age groups in first group 3, while non-detected bacteria in both fourth and seventh age group 0,

I. Introduction:

Respiratory tract infections (RTI) are one of the most common diseases in the world . (RTI) are caused by a board range of pathogens, including viruses, bacterial infections, in children and young adults are often caused by the common bacterial infection Mycoplasma pneumoniae, Streptococcus pneumoniae. It's a highly polymorphic (Waites *et al* ., 2017).

The incidence of respiratory infections in the population is related to many factors, among which environmental factors such as air quality, temperature, and humidity have attracted much attention. In particular, air pollution has caused widespread discomfort and concern in developing countries(Chen *et al*., 2023).

In addition bacterial infections (Mycoplasma pneumoniae, Streptococcus pneumoniae, . Staphylococcus aureus, Haemophilus influenza, Chlamydophila pneumoniae, Legionella pneumophila) (Sattar *et al* ., 2021 , Payus *et al* ., 2022)

M.pneumoniae one of the smallest prokaryotic cell kinds of microorganisms that may grow on inanimate things and pass the bacterium filter. It also lacks cell walls Mycoplasma pneumoniae is a prevalent pathogen that accounts for up to 40% of community-acquired pneumonias. It causes upper and lower respiratory tract infections in people of all ages. Diseases caused by Mycoplasma pneumoniae range in severity from minor respiratory tract infections to severe atypical pneumoniae. (Kumar,2018).

M.pneumoniae causes extrapulmonary infections such as pharyngitis, encephalitis, Steven-Johnson syndrome, septic, arthritis, pericarditis, and other signs of autoimmune pneumoniae that develop into severe, life-threatening pneumoniae in addition to respiratory tract infections. (Willyard *et al* ., 2017).

Streptococcus pneumoniae or pneumococcus, is a member of the Streptococcus genus of gram-positive, spherical, alpha-hemolytic bacteria. (Ryan *et a*l ., 2004).They are non-motile, do not generate spores, and are typically seen in pairs (diplococci). S. pneumoniae is a noteworthy human pathogenic bacterium that has been the focus of numerous studies on humoral immunity and

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was dentified as a prominent cause of pneumonia in the late 19th century.The organism first appeared in 1881 and was dubbed the pneumococcus in 1886. (Plotkin *et al* ., 2015).
was initially identified concurrently and separately by French chemist Louis Pasteur and U.S. Army surgeon George Sternberg.The organism's distinctive appearance in Gram-stained sputum led to its naming as Diplococcus pneumoniae in 1920. Since it resembled streptococci a lot, it was called Streptococcus pneumoniae in 1974(Wainer , 2015).

II. Material and Methods

The study had been conducted on (140) confirmed respiratory infections patients (67males and 73 females) ages ranging in 10-80 years study extend from 1 September 2023 to 1 Febraury 2024,. All 140 samples were collected from Nasiriyah Teaching Hospital, Alhussein Teaching Hospital, and Souq Al_shuyoukh General Hospital, Private Clinics in Shatrah. Various specimens, including sputum swab and Oral pharyngeal swab samples for direct detection of *Mycoplasma pneumoniae, , Streptococcus pneumonia* After patient approval for participating in the study. The inclusion criteria are mild-moderate, severe symptoms and acute respiratory distress syndrome (ADRS) patients, According to the diagnosis of specialist doctors, patients suffering from respiratory symptoms include cough, high temperature, muscle fatigue, and headache, samples were collected using a special swab containing nutrients suitable for bacteria using sterile methods and then transferred to the laboratory.

Samples processing: - In the laboratory, prior to DNA extraction, respiratory samples were processed as -Sputum samples: - They were homogenized by adding an equals volume of mucolytic agent (2-mercaptoethanol 0.1M) and vortex vigorously. After that incubation for 30 min at room temperature and vortexing was done. Then, the solution was centrifuged at 10000 g / min for 10 min and the supernatant was removed. The pellet was resuspended in 100 ml of saline water

Extraction of the bacterial DNA

Genomic DNA was extracted from M.pneumoniae and S. pneumoniae by using Geneaid Genomic DNA extraction Kit (china) and after this process, to ensure the DNA was extracted, and its quality was tested, the samples were run on a 1.5 % gel agarose.

III. Result :

Identification of Pathogenic Bacteria by PCR Technique

The current results was identified the among 140 samples of patients with RTI was *S. pneumoniae* 5 (3.57%), and *M. pnuemoniae* 3 (2.14%), while the other samples was negative and infected with other bacteria 132 (94.28%), as in the figure 4-5.

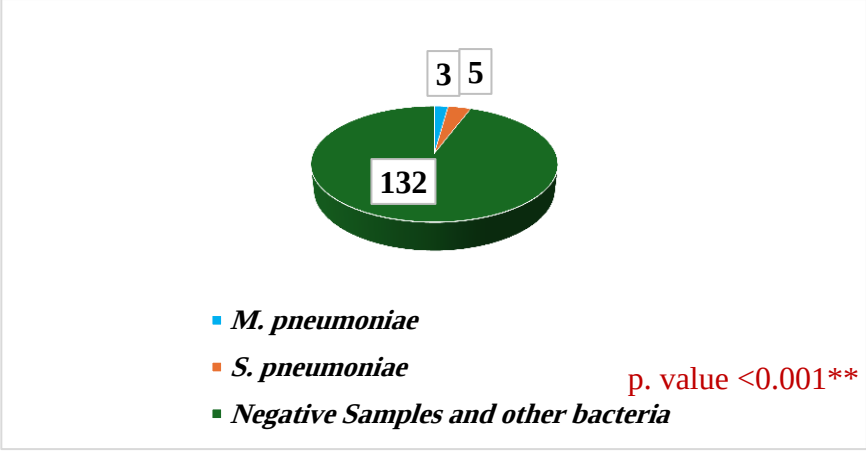

Figure 4-1: Identification of pathogenic bacteria by PCR technique

University of Thi-Qar Journal of agricultural research

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Prevalnce of of Isolated Bacterial According to Sex

The present study was conducted that the most detected bacterial in female group 6 (75.0%), while in the male group 2 (25.0%), in addition, the most isolated bacteria in female group were both *M. pneumoniae* 3 (50.0%), and *S. pneumoniae* 3 (50%), the study also noted a non-significant difference at p. vlalue < 0.05 according to sex, as in the table 4-1 and figure 4-2.

Table 4-1: Prevalnce of of isolated bacterial according to sex

Sex	Male		Female		Total	
	No.	%	No.	%	No.	%
M. pneumoniae	0	0.0	3	50.0	3	37.5
S. pneumoniae	2	100	3	50.0	5	62.5
Total	2	25.0	6	75.0	8	100
CalX^2= 1.60 TabX^2= 3.84 DF= p. value 0.206						

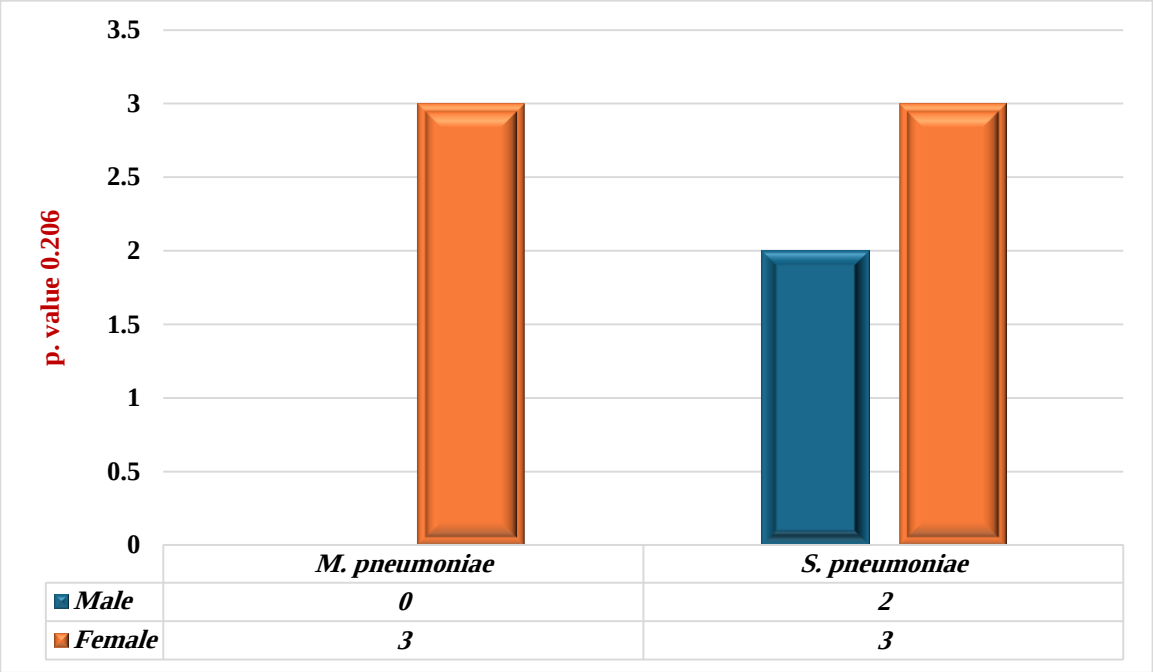

Figure 4-2: Prevalnce of of isolated bacterial according to sex

Prevalnce of of Isolated Bacterial According to Age-groups

University of Thi-Qar Journal of agricultural research

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The present study was conducted that the most detected bacterial in first group 3, while non-detected bacteria in both fourth and seventh age group 0, the study also noted a non-significant difference at p. vlalue < 0.05 according to age groups, as in the figure 4-3.

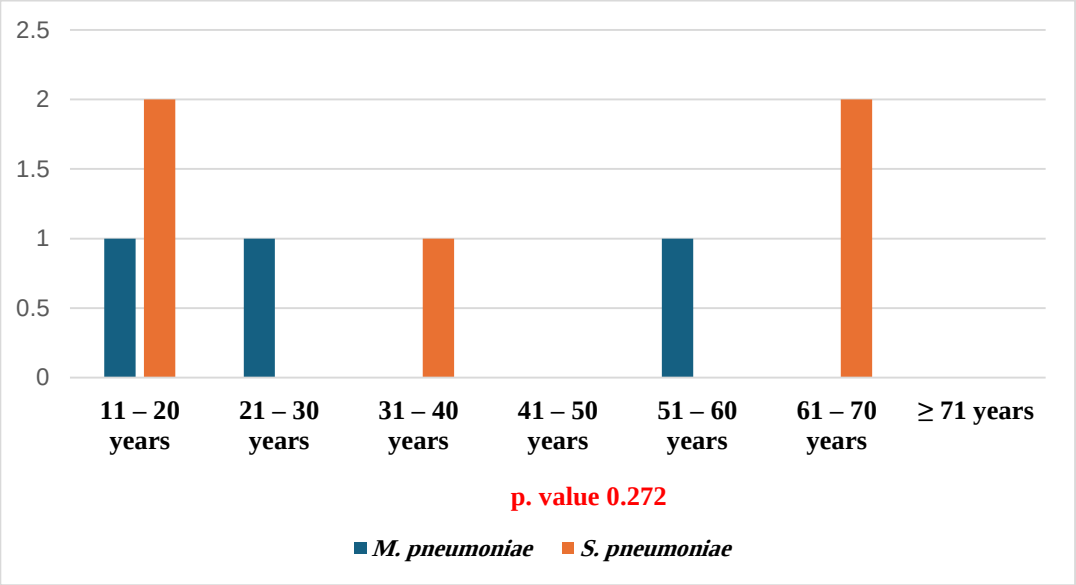

Figure 4-3: Prevalnce of of isolated bacterial according to age groups

Prevalnce of of Isolated Bacterial According to Reidency

The present study was showed that the most detected bacterial in Shatrah and 4 (50%), while in the the lowest detected bacteria in both Nasiryah distract and Suq-alshioukh 2 (25%), the study also noted a non-significant difference at p. vlalue < 0.05 according to residency, as in the table 4-2 and figure 4-4.

Table 4-2: Prevalnce of of isolated bacterial according to residency

	Nasiryah		Shatrah		Suq-alshioukh		Total	
	No.	%	No.	%	No.	%	No.	%
M. pneumoniae	1	50.0	2	50.0	0	0.0	3	37.5
S. pneumoniae	1	50.0	2	50.0	2	100	5	62.5
Total	2	25.0	4	50.0	2	25.0	8	100
CalX^2=1.60 TabX^2= 5.49 DF= 2 p. value 0.449								

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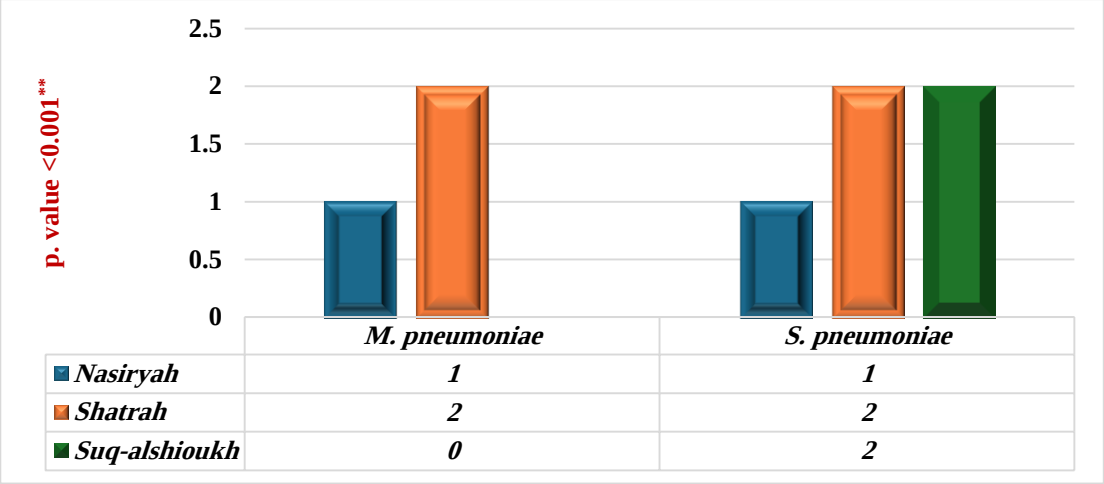

Figure 4-4: Prevalnce of of isolated bacterial according to residency

Gel electrophoresis

The extracted DNA from sputum samples was electrophoresis by gel for detection the involved bacteria in the current study as in figure 4-10.

Detection of *Mycoplasma pneumoniae*

The present study detected *M. pneumonia* from patients suffering from RTI, as in figure 4-11, and figure 4-12.

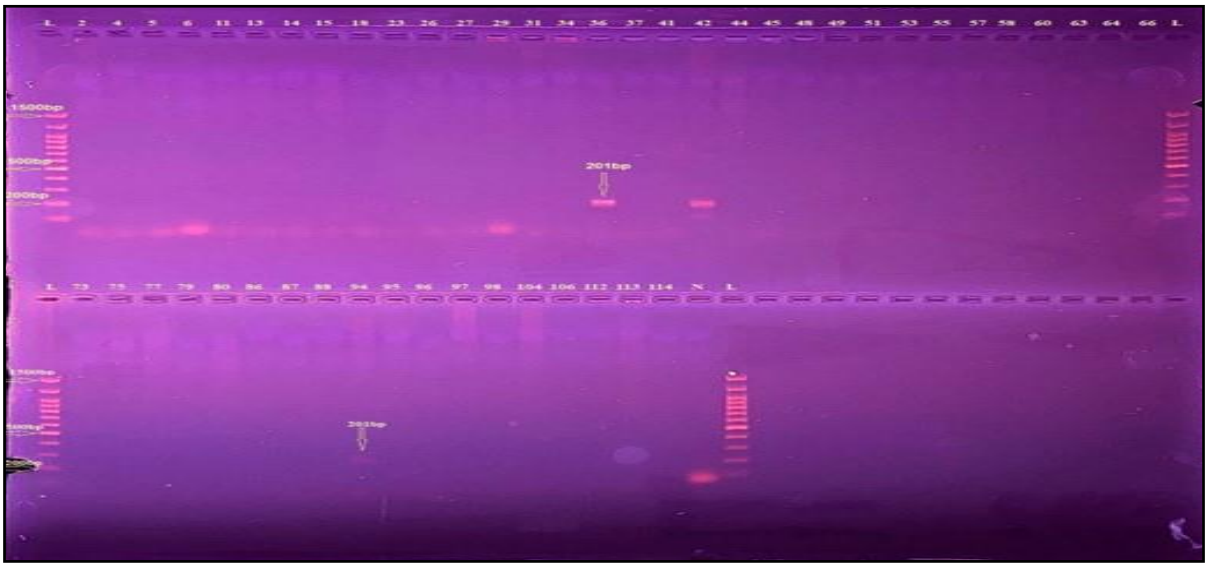

Figure 4-11: Gel electrophoresis for PCR product of (Mycoplasma pneumoniae 1primer) show no mentioned results with different isolates, (Agarose 1,5%, 15min. at 90 volts then lowered to 75 volts for 45min.). Visualized under U.V light after staining with ethidium bromide. Lane L: DNA ladder (100-1500bp), Lanes (1-20) represented negative results and lane N represent negative control.

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Detection of *S. pneumoniae*

The present study detected *s. pneumonia* from patients suffering from RTI, as in figure 4-15.

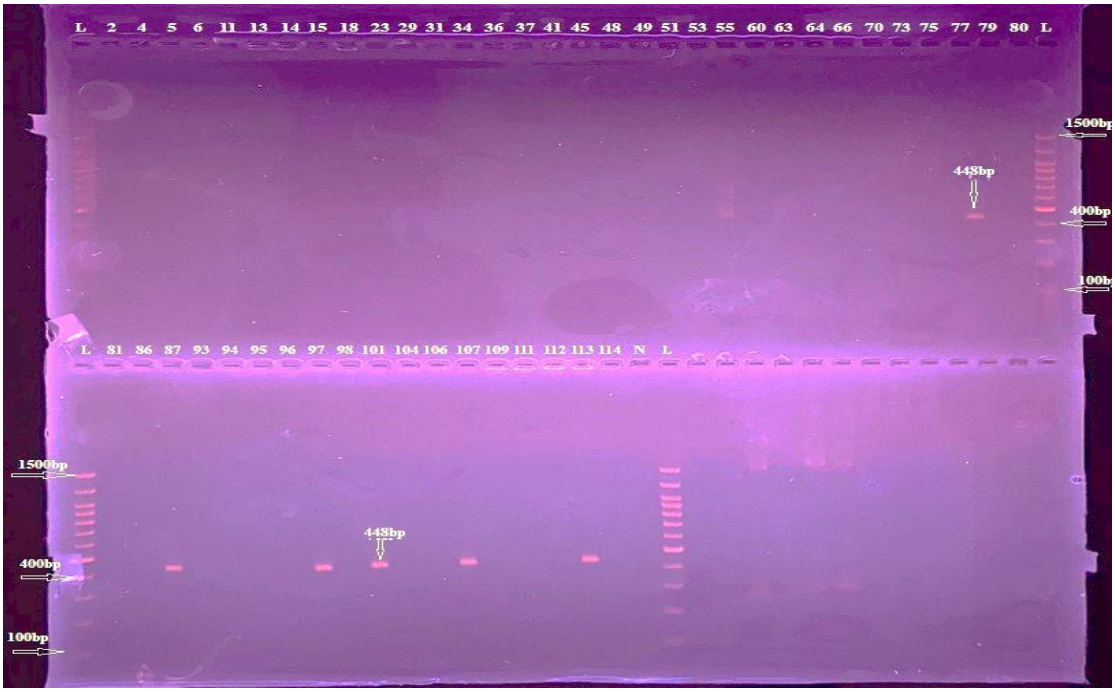

Figure 4-15: Gel electrophoresis for PCR product of (Streptococcus pneumonia primer) show 448 bp Primer annealing temperature at (57°C), (Agarose 1,5%, 15min. at 90 volts then lowered to 75 volts for 45min.). Visualized under U.V light after staining with ethidium bromide. Lane L: DNA ladder (100-1500bp), Lanes (77,87,97,101,107, and 113) represented positive results, the rest of represented negative results, and lane N represent negative control.

Statistical Analysis

The data of the current study was statistically analysis by using SPSS version 26, based in using both descriptive and non-parametric chi-square at p. value < 0.05.

IV. **Discussion**:

DNA was extracted from 1٤0 samples from Respiratory infections patients to detect Mycoplasma pneumoniae using the PCR technique using specific target sequence primers (١٦srRNA gene). The results show that only 3\140(٢.14%)samples give a positive result for Mycoplasma pneumoniae Some studies showed results near to the results of the present study where in the study was conducted in Iraq by Rabab, (2022) in AL - Diwanyiah city, he reports the rate of M. pneumoniae is (5%) This is lower than the findings reported previously in AL - Diwanyiah city (10.9%) (Al-Amarah, 2016), in Basra (19.4%) (Al-Ghizawi *et al*., 2007), in Suleimani city (28.8%) (Faraj et al., 2014), and another study in Marrakech by (Daoudi, N et al 2019)give results almost similar (3.55%) and another study in Sweden (21%) by (Nilsson *et al* .,2008) The prevalence of M. pneumoniae varies depending on whether, a study was performed during an endemic or epidemic year, the laboratory detection method used, or the study participants (Foy *et al*., 2014).al. reported that, among hospitalized children in the United States, M.pneumoniae was the most common bacterial cause of communityacquired pneumonia, accounting for 8% of cases and among hospitalized adults in the United States, M. pneumoniae was identified in ≈ 2% of cases (Williams *et al*., 2015)

The prevalence differences in our study compared with other studies might be attributable to a difference in enrollment criteria, the age group of participants. In my study, Generally, M. pneumonia causes mild respiratory infection, usually managed by primary-care physicians. However, recent studies have suggested that some patients with M. pneumoniae may develop severe

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respiratory manifestations requiring ICU admission, in our investigation, we found 2 patients required intensive care (Izumikawa *et al*., 2014)

Mycoplasma pneumoniae is a human pathogen responsible for upper and lower respiratory tract infections, which causes up to 40% of cases of community-acquired pneumoniae (Kumar,2018). to sex The percentage of *Mycoplasma pneumoniae* infections was (100%) in female, and this study did not record any *M. pneumoniae* infections in males. Some studies showed results near to the results of the present study where in the study was conducted in China (Liu *et al* ., 2023)***M. pneumoniae*** most infection in female group (٤٠%)and, in China in Chengdu (Zhang *et al*.,2021) was most infection in female group of ***M. pneumoniae*** whilst the rate of *M. pneumoniae* in males was (0%).

Detect S.pneumoniae using the PCR technique using specific target sequence primers(16srRNA) The results show that only 5\140(3.75%) samples give a positive result for S pneumoniae this result lower than the study in Najaf city (12.3%) (Motaweq and Naher, 2017). and Aljanaby, (2010) in AL - Najaf city, he reports the rate of S. pneumoniae is (24.44%). Also, these results lower than those previously by Hassen, (2005) in Baghdad city, he finds that the ratio of S. pneumoniae is (29%) and a study in lower than the Sweden the ratio is (27%) (Abdeldaim et al., 2010). and lower than the study done in Baghdad and Al-Anbar (Ramadi) governorates is (21.4%) (Al-Bayatee, 2012)

This may be due to similarity and difference in the method and isolation conditions of S. pneumoniae in addition to mediums which are used in these studies, as well as a method of sampling can change the ratio of bacteria isolation *S. pneumoniae* colonizes the mucosal surfaces of the upper respiratory tract which includes the nose, nasal cavity, pharynx, and larynx, although colonization within the nasal passage is often asymptomatic, but under permissive conditions it is access to the airways can result in lower respiratory tract infections with further dissemination causing invasive pneumococcal disease (i.e. otitis media, bacteremia and meningitis) (Thevaranjan *et al*., 2016)

The most prevalent causal pathogen identified in CAP is S. pneumoniae reaching more than 10%, the lethality of hospitalizedpatients with pneumonia is relatively high despite potent antibiotic treatment, pathogen-host interaction in severe pneumonia may evoke an increase in pulmonary endothelial permeability, resulting in life-threatening acute lung injury, virulence factors of S. pneumoniae, including specific toxins as well as an uncontrolled host immune response, may induce lung barrier dysfunction (Gutbier *et al*., 2017).

V. Refrains

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