Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 02 May 2026
Revised 10 June 2026
Accepted 28 June 2026

Natural Resources for Human Health 2026, Vol. 6 (5): 489–501

KEYWORDS:
Respiratory tract infections, Sputum culture, Bacterial pathogens, Antimicrobial susceptibility, Respiratory infections

# Bacteriological Profile of Bacteria Species Isolated from Sputum Samples in Respiratory Tract Infections Patients and their Association with Antimicrobial Resistance Pattern

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## ABSTRACT:

**Background**: Respiratory tract infections (RTIs) are among the most common infectious diseases, and identifying their bacterial causes is essential for appropriate treatment and antimicrobial resistance surveillance. This study aimed to determine the frequency of bacterial growth in sputum samples and identify the predominant bacterial pathogens associated with RTIs in patients attending Azadi Hospital, Kirkuk Province, Iraq.

**Methods**: A total of 77 sputum samples were collected from patients at Azadi Hospital between July 1, 2025, and February 1, 2026. Samples were cultured using standard bacteriological media. Bacterial isolates were identified based on cultural characteristics, microscopic examination, biochemical tests, and the VITEK automated identification system. Antimicrobial susceptibility testing was also performed. Results: Bacterial growth was detected in 18 samples (23.38%), whereas 59 samples (76.62%) showed no bacterial growth. Most samples were obtained from females and patients aged over 50 years. Gram-positive and Gram-negative bacteria represented 55.56% each of the isolates. Streptococcus pneumoniae and Staphylococcus aureus were the most frequently isolated species (22.22% each), followed by Acinetobacter baumannii (16.66%) and Klebsiella pneumoniae (11.11%).

**Conclusion**: The low culture-positive rate indicates that sputum culture alone may have limited diagnostic yield for bacterial RTIs. Increasing the number and quality of respiratory specimens and combining culture with appropriate diagnostic approaches may improve pathogen detection and provide more reliable information for antimicrobial resistance surveillance.

## INTRODUCTION

Respiratory tract infections (RTIs) represent a serious health issue throughout the globe. RTIs threaten to create numerous health problems and even death among vulnerable groups like children and old adult [1]. RTIs are regarded as a group of the most commonly experienced infectious diseases in medical practice; they can affect different sections of the respiratory system and can be caused by a great number of microbes, including bacteria, fungi and viruses [2, 3]. Infective agents that lead to acute lower RTIs are regarded as an evident cause of good deal of morbidity, mortality and economic costs across the

entire world. The presence of Gram-positive and Gram-negative bacteria can lead to damaging infections watched in the community with a great result. The presence of the animal model capable of demonstrating certain important characteristics of pneumonia will give an opportunity to learn the mechanic of the development of pneumonia of different types of mammals [4]. Pneumonia is an acute infectious disease that seriously involves lung tissue. Pneumonia can be caused by different organisms, including bacteria, protozoa and viruses. It can be said that pneumonia affects more than 450 million people every year [5].

Sputum analysis makes up the primary tool to assess lower airway microbiology in patients with chronic respiratory illnesses [6]. In order to derive informative analysis, it is necessary to identify and discard samples that fail to represent the lower airways [7]. Moreover, sputum microbiology contributes to managing patients suffering from bronchiectasis, although currently available information about sputum microbiology in this group of patients is very limited [8]. Induced sputum appears to be a safe and inexpensive method not requiring invasive techniques, which makes it very useful for patients with the inability to produce sputum or to provide adequate samples. Its effectiveness is better than that of spontaneously produced sputum in immunity assessment in a range of respiratory diseases including chronic obstructive pulmonary disease, asthma, lung cancer, and infectious diseases. Nonetheless, induced sputum has not found enough application in clinical practice. Under conditions like interstitial lung disease in which patients fall acute dry cough, induced sputum indicates potential although research is limited [9]. Sputum cultures can help in determining antibiotic therapy in cases of severe pneumonia or underlying resistant pathogens [10]. Tuberculosis-negative patients with respiratory symptoms may have other bacterial infections [11].

The study was designed to explore the sputum samples obtained from patients suffering from respiratory tract infections in terms of their total number of samples, culture-positive samples and correlation with the age and sex of the patients. The purpose of the study is also to identify the most frequent bacterial species in sputum samples.

## MATERIALS AND METHODS

The study was designed to explore the sputum samples obtained from patients suffering from respiratory tract infections in terms of their total number of samples, culture-positive samples and correlation with the age and sex of the patients. The purpose of the study is also to identify the most frequent bacterial species in sputum samples.

### *Sample collection*

Seventy seven sputum samples were obtained from Azadi Hospital within the Kirkuk Governorate the period from July /2025 to February 2026.

### *Culturing of samples*

Blood agar, brain-heart infusion broth, mannitol salt agar and MacConkey agar were used to culture the samples and were incubated at 37 degrees Celsius for 24 hours. Method of culture was a combination of culturing techniques and microscopic examination, and the identification of bacterial isolates was carried out using the VITEK system.

### *Antimicrobial susceptibility testing*

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method. The zones of inhibition were measured in millimeters using a ruler.

### *Statistical methods*

Statistical analysis was carried out to assess the distribution and correlation of the variables studied. The categorical information was summarized regarding the frequency and percentage. When the expected frequency satisfied the assumptions of the test, Pearson's Chi-square ($\chi^2$) goodness-of-fit test was employed to check the variations of the observed distribution for the categorical variables with two or more categories. The chi-square independence test was utilized to analyze the correlation between particular categorical variables such as gender and bacterial growth. If the cell expected frequencies were small and the criteria of the Chi-square test unsatisfied, Fisher's exact test was utilized. It is worthy to mention that when working with variables with very small or zero frequency, descriptive statistics were reported without making statistical conclusions where applicable. P-value $\leq 0.05$ was considered statistically significant.

Natural Resources for Human Health
eISSN: 2583-1194

## RESULTS

### *Distribution of samples according to bacterial growth*

The information in Table 1 illustrates the distribution of the sputum samples as per the bacterial growth. The total number of sputum samples analyzed was 77, out of which 18 (23.38%) were positive for bacterial growth while a total of 59 (76.62%) were negative for the culture test. According to Pearson's chi-square goodness-of-fit test, there is a considerable variability between the number of growth-positive and growth-negative samples ($\chi^2 = 21.83$, df = 1, $P < 0.001$), which confirms the higher frequency of culture-negative samples as opposed to culture-positive samples. It highlights that culture-negative sputum samples were significantly more than the culture-positive samples in the study and indicate that bacterial culture yield of the sputum samples examined was low.

**Table 1.** Distribution of sputum samples according to bacterial growth.

| Bacterial growth | Total, n (%) | Statistical result |
|---|---|---|
| Positive growth | 18 (23.38) | b |
| Negative growth | 59 (76.62) | a |
| Total | 77 (100.00) | $\chi^2 = 21.83$, $P < 0.001$ |

*Different superscript lowercase letters (a, b) indicate a statistically significant difference between the proportions ($P < 0.05$). Pearson's chi-square goodness-of-fit test was used.*

### *Distribution of samples according to sex*

The data from Table 2 indicates that bacterial growth was associated with sex. It was found that out of 40 male patients 6 (15.00%) and out of 37 female patients 12 (32.43%) showed positive bacterial growth. The percentage of samples with positive culture was higher for females as compared to males; nonetheless, an application of the Pearson's chi-square test verified that this was not statistically significant ($\chi^2 = 3.261$, df = 1, $P = 0.071$). Hence, there was no statistically significant relationship between sex and the growth of bacteria in the sputum samples examined.

**Table 2.** Distribution of growth-positive and growth-negative sputum samples according to sex

| Sex | Total, n | Positive growth, n (%) | Negative growth, n (%) |
|---|---|---|---|
| Male | 40 | 6 (15.00[a]) | 34 (85.00) |
| Female | 37 | 12 (32.43[a]) | 25 (67.57) |
| Total | 77 | 18 (23.38) | 59 (76.62) |

*Different lowercase superscript letters indicate statistically significant differences ($P < 0.05$). The same superscript letter indicates no statistically significant difference. Pearson's chi-square test: $\chi^2 = 3.261$, df = 1, $P = 0.071$.*

### *Distribution of samples according to age group*

The age distribution of patients with a positive sputum culture is shown in Table 3. Among patients ages 25-50 years, 6 of 37 (16.22%) had a positive culture while, among the patients over the age of 50 years, 12 of 40 (30.00%) had positive cultures. While the patients over the age of 50 years did have a higher percentage of culture-positive sputum samples in comparison to patients ages 25-50 years, the difference did not reach significance according to Pearson's Chi-square test ($\chi^2 = 2.039$, df = 1, $P = 0.153$). Hence, there was no significant association seen between age group and bacterial growth in the sputum samples analyzed.

**Table 3.** Distribution of sputum samples and bacterial growth according to age group

| Age group (years) | Total samples, n (%) | Positive growth, n (%) | Negative growth, n (%) |
|---|---|---|---|
| 25–50 | 37 (48.05) | 6 (16.22[a]) | 31 (83.78) |
| >50 | 40 (51.95) | 12 (30.00[a]) | 28 (70.00) |
| Total | 77 (100.00) | 18 (23.38) | 59 (76.62) |

*Values with the same lowercase superscript letter are not significantly different ($P > 0.05$). Pearson's chi-square test: $\chi^2 = 2.039$, $df = 1$, $P = 0.153$.*

***Distribution of samples according to acid-fast staining***

Results from Table 4 show that all 77 sputum samples tested negative for acid-fast bacilli (AFB) via acid-fast staining, with no AFB-positive specimens, signifying a positivity rate of 0.00%. Conversely, there were all AFB-negative specimens (77/77, 100.00%). The estimated prevalence of AFB-positive samples was 0.00% (95% CI: 0.00–4.68%). It is important to note that the absence of AFB detection should not be construed as an absolute exclusion of pulmonary tuberculosis given that the sensitivity of the direct smear microscopy is limited, thus implying that additional clinical, radiographic, culture based or molecular investigations might be necessary for a comprehensive diagnosis.

**Table 4.** Distribution of sputum samples according to acid-fast staining (AFB) results

| Acid-fast staining result | No. of samples, n | Percentage (%) |
|---|---|---|
| Positive (AFB-positive) | 0 | 0.00 |
| Negative (AFB-negative) | 77 | 100.00 |
| Total | 77 | 100.00 |

*AFB-positive proportion = 0.00% (95% exact binomial CI: 0.00–4.68%). No inferential comparison was performed because no AFB-positive samples were detected.*

***Distribution of bacterial isolates according to gram staining***

In Table 5, whether Gram-negative and Gram-positive bacteria can be found in the sputum samples is discussed, and the results indicate that both can exist in the specimens. Eighteen bacterial isolates were isolated, of which ten (55.56%) were found to be Gram-positive, and eight (44.44%) were Gram-negative. Although the Gram-positive bacteria made up a higher proportion of the isolates than Gram-negative cohorts, the statistical analysis did not support this finding because ($\chi^2 = 0.222$, df = 1, P = 0.637). The analysis of this data implies that the distribution was indeed equal, and hence, the ratio of recoverable Gram-positive to Gram-negative isolates is approximately one.

**Table 5.** Distribution of sputum bacterial isolates according to Gram-staining characteristics

| Gram-staining reaction | Number of isolates, n | Percentage (%) |
|---|---|---|
| Gram-positive | 10 | 55.56[a] |
| Gram-negative | 8 | 44.44[a] |
| Total | 18 | 100.00 |

*Values with the same lowercase superscript letter are not significantly different ($P > 0.05$). Chi-square goodness-of-fit test: $\chi^2 = 0.222$, $df = 1$, $P = 0.637$.*

***Distribution of bacterial isolates according to bacterial species***

Table 6 summarizes the distribution of different bacterial species obtained from sputum samples. Among the total 18 isolates, the bacterium that was recovered most often was Streptococcus pneumoniae and Staphylococcus aureus, making four isolates from each (22.22%). The numbers of Acinetobacter baumannii isolates are 3 (16.67%) followed by Klebsiella pneumoniae with a total of two (11.11%). The bacterial genera Streptococcus viridans, Enterobacter cloacae, Streptococcus pyogenes, Moraxella catarrhalis and Escherichia coli each contributed one isolate (5.56%). The calculated chi square goodness-of-fit is $\chi^2 = 7.000$ (df = 8, P = 0.537) based on the assumption of equal proportions of isolates; however, since only 2 specimens for a given species were expected, the assumptions for the Pearson chi-square approximation are not properly satisfied. Hence, the results are mostly presented in a descriptive form and no statistical inference has been made about the presence of any significant difference between the bacterial species based on the obtained frequencies.

**Table 6.** Distribution of bacterial species isolated from sputum samples

| Bacterial species | *Streptococcus viridans* | *Streptococcus pneumoniae* | *Staphylococcus aureus* | *Acinetobacter baumannii* | *Enterobacter cloacae* | *Streptococcus pyogenes* | *Klebsiella pneumoniae* | *Moraxella catarrhalis* | *Escherichia coli* | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| n | 1 | 4 | 4 | 3 | 1 | 1 | 2 | 1 | 1 | 18 |
| % | 5.56 | 22.22 | 22.22 | 16.67 | 5.56 | 5.56 | 11.11 | 5.56 | 5.56 | 100.00 |

*The distribution is presented primarily as descriptive statistics because the expected frequency for each of the nine bacterial species was 2 isolates, which does not satisfy the assumptions of the Pearson chi-square approximation. The calculated chi-square statistic under an equal-proportion assumption was $\chi^2 = 7.000$, df = 8, P = 0.537.*

**ANTIMICROBIAL SUSCEPTIBILITY OF GRAM-POSITIVE BACTERIAL ISOLATES**

***Gram-Negative Bacterial Isolates***

The antimicrobial susceptibility profile of Klebsiella pneumoniae isolates is depicted in Table 8, demonstrating complete non-sensitivity to ampicillin, cefatizime, cefazolin and ciprofloxacin and full sensitivity to piperacillin-tazobactam, cefoxitin, amikacin, penicillin G, tigecycline, and nitrofurantoin. Levofloxacin showed the intermediate level of sensitivity. The findings confirmed that *K. pneumoniae* isolates are resistant mainly to aminopenicillins, fluoroquinolones and certain cephalosporins but exhibit sensitivity to some other antimicrobials, including piperacillin-tazobactam, amikacin, tigecycline and nitrofurantoin. The results also highlight the need for determination of the antimicrobial susceptibility of *K. pneumoniae* isolates prior to the use of antimicrobial agents.

Table 8 also shows the antimicrobial susceptibility analysis for Acinetobacter baumannii isolates. The isolates were found to be completely resistant to piperacillin, amikacin, meropenem, ceftriaxone, and tobramycin. The results showed a variable susceptibility pattern for ceftazidime and cefepime. For example, some of the isolates were susceptible to these antibiotics while some others remained resistant. However, for ciprofloxacin, mixed susceptibility was noted, where some isolates demonstrated intermediate susceptibility while others remained resistant. Nonetheless, all isolates remained susceptible to gentamicin, ampicillin, and ticarcillin-clavulanate.

The data displayed in Table 8 describes the antimicrobial susceptibility of Moraxella catarrhalis isolates. The antibiotics to which isolates showed resistance are cefazolin, tigecycline, and trimethoprim, while the drugs that were found to be effective against the isolates include ceftizoxime, ceftriaxone, cefixime, amikacin, ciprofloxacin, and levofloxacin. An intermediate susceptibly result was obtained for cefepime.

According to Table 8, the antimicrobial susceptibility profile of Enterobacter cloacae isolates is outlined whereby isolates displayed resistance to amoxicillin–clavulanate (Augmentins) only. On the other hand, susceptibility was exhibited against all other antimicrobial agents tested in the study including ticarcillin, piperacillin, amikacin, ceftazidime, tobramycin, ciprofloxacin, minocycline, colistin, trimethoprim, tigecycline, and nitrofurantoin. Among all of the species recovered in the present study *E. cloacae* was the most susceptible organism displaying resistance against one of the antimicrobial agents, which

is amoxicillin–clavulanate. This susceptibility profile indicates that the isolates have shown good susceptibility against most of the studied antimicrobial agents, thus giving a chance to expand the treatment options available for patients. However, antimicrobial susceptibility testing is still important since the resistant patterns of E. cloacae may differ between regions, health care settings, and time periods.

Table 8 illustrates the antimicrobial susceptibility profile of the isolated strains of Escherichia coli. The isolates developed resistance to several antibiotics including ceftriaxone, ciprofloxacin, cefixime, penicillin G, trimethoprim-sulfamethoxazole, amoxicillin-clavulanate (Augmentum) as well as ceftazidime. In conjunction with capabilities to withstand these antibiotics the isolates were sensitive to imipenem and tobramycin. Susceptibility rates were intermediate for antibiotics clarithromycin and tazobactam. As a whole, the strains of *Escherichia coli* were resistant to fluoroquinolones class of antibiotics, cephalosporins, penicillin and agents acting through the metabolism of folic acid (trimethoprim-sulfamethoxazole). Meanwhile the isolates remained sensitive to imipenem and tobramycin which indicates suitability of these antibiotics for the treatment of respiratory infections caused by *E. coli.*

**Table 7.** Antimicrobial susceptibility profile of Klebsiella pneumoniae, Acinetobacter baumannii, Acinetobacter baumannii, Moraxella catarrhalis, Enterobacter cloacae and Escherichia coli isolates recovered from sputum samples

| *Klebsiella pneumoniae* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Antibiotic | Ampicillin | Piperacillin–Tazobactam | Ceftazidime | Cefoxitin | Cefazolin | Amikacin | Penicillin G | Ciprofloxacin | Levofloxacin | Tigecycline | Nitrofurantoin |
| Susceptibility | R | S | R | S | R | S | S | R | I | S | S |
| *Acinetobacter baumannii* | | | | | | | | | | | |
| Antibiotic | Ciprofloxacin | Gentamicin | Ampicillin | Ceftazidime | Cefepime | Ticarcillin–Clavulanate | Piperacillin | Amikacin | Meropenem | Ceftriaxone | Tobramycin |
| Susceptibility | R, I | S | S | R, S | R, S | S | R | R | R | R | R |
| *Moraxella catarrhalis* | | | | | | | | | | | |
| Antibiotic | Cefazolin | Ceftizoxime | Ceftriaxone | Cefixime | Cefepime | Amikacin | Ciprofloxacin | Levofloxacin | Tigecycline | Trimethoprim | |
| Susceptibility | R | S | S | S | I | S | S | S | R | R | |
| *Enterobacter cloacae* | | | | | | | | | | | |
| Antibiotic | Amoxicillin–Clavulanate (Augmentin) | Ticarcillin | Piperacillin | Amikacin | Ceftazidime | Tobramycin | Ciprofloxacin | Minocycline | Colistin | Trimethoprim | Tigecycline | Nitrofurantoin |
| Susceptibility | R | S | S | S | S | S | S | S | S | S | S | S |

| *Escherichia coli* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Antibiotic | Ceftriaxone | Ciprofloxacin | Cefixime | Clarithromycin | Penicillin G | Imipenem | Trimethoprim–Sulfamethoxazole | Tazobactam | Tobramycin | Amoxicillin–Clavulanate (Augmentin) | Ceftazidime |
| Susceptibility | R | R | R | I | R | S | R | I | S | R | R |

*S = Susceptible; I = Intermediate; R = Resistant.*

*R,I =some isolates are intermediate and others are resistant*

*R,S =some isolates are susceptible and others are resistant*

**DISCUSSION**

The present results are in accordance with Maheshwari *et al.* [12], who also reported the culture results in only 43 (16.9%) samples out of a total of sputum specimens tested. Altundal et al. [13] results also are very similar where 73.8% of patients had sputum specimens drawn but at the same time these cultures were positive in only 45.5% of cases. In contrast, Do *et al.* [14] found a significantly higher percentage of culture-positive patients, reporting 160 out of 274 clinical samples (58.39%) to be culture-positive, of which 85 (63.43%) out of 134 sputum samples were confirmed culture-positive. Also, Almouwlid *et al.* [15] stated that the number of sputum specimens was 126 (46%) of 271 respiratory samples, while other samples were either tracheal aspirates (40%) or throat swabs (14%).

Reasons for differences in the level of positivity of bacterial cultures in various studies could be due to the differences in quality of samples, composition of patients, usage of antibiotics previously, procedure of sampling, methods used in laboratory, and the epidemiological characteristics of respiratory infections in various regions.

This pattern of distribution based on sex depicts variation as being reported by the previous studies. Almouwlid *et al.* [15] has shown that out of the total number of participants, 52% (141/271) were male, whereas 48% (130/271) were female participants. Maheshwari *et al.* [12] also reported the composition of the study sample where males made up of 67.6% (171/253) and females comprised 32.4% (82/253) of the total population. Similarly, Yimer *et al.* [16] stated that the participant who submitted sputum samples were male at the rate of 60%. Another similar research was conducted by Kasundra *et al.* [17], wherein males were found to reach 51/150 (80.66%) and females were found to be 29/150 (19.33%).

The disparity between the results of this study and those of earlier studies may be due to discrepancies in sample size, demographic factors, healthcare seeking behavior, co-morbidities, geographic location, and variations in distribution of respiratory pathogens among different communities.

The findings present clearly that there was a higher frequency of respiratory infections with positive cultures in patients older than 50 years. The higher frequency of infections among the elderly may be explained by a decline of immune system functioning with age, chronic diseases, and increased vulnerability to respiratory infection.

Similar findings have been reported in previous studies. Kasundra *et al.* [17] found that most patients belonged to the 41–60-year age group (49.33%), followed closely by the 61–80-year age group (48.00%), with a mean patient age of 61.32 ± 10.55 years. Likewise, Maheshwari *et al.* [12] reported a mean age of 56.7 ± 2.1 years for male patients and 41.4 ± 2.18 years for female patients, indicating that respiratory tract infections were more common among middle-aged and older adults.

The predominance of culture-positive cases among older patients observed in the present study is consistent with the increased vulnerability of this age group to respiratory infections, which may result from immunosenescence, the higher prevalence of comorbidities, and greater exposure to healthcare settings. The negative results of the acid-fast staining test suggest that the respiratory infections seen in this paper involve pathogens other than Mycobacterium tuberculosis. As a result, the bacterial isolates that were gathered through normal culture are viewed as the likely candidates for being the causal

agents of the infections mentioned. The findings presented above correspond to the results of the investigation made by Arthur *et al.* [11] who also used mycobacterial culture to prove that there was no Mycobacterium tuberculosis (MTB) in any of the participants that took part in the research, however, two cases were ruled out as suspected non-tuberculous mycobacterial (NTM) infections.

However, in previous studies it was found that the predominant types of bacteria found in respiratory tract infections were Gram-negative bacteria. Reiner-Benaim *et al.* [18] found that lower respiratory tract infections were predominantly caused by Gram-negative bacteria. In another study by Gebre *et al.* [19] the overall prevalence of pathogens causing bacterial lower respiratory tract infections was 34.0% (95% CI: 29.2–38.8%). Among 126 bacterial isolates that were recovered, 84 (67%) were Gram-negative bacteria while only 42 isolates belonged to Gram-positive bacteria. The discrepancies between the results of the current study and previous research may be explained by variations in target populations, geographic regions, sample sizes, types of samples collected, antibiotic use, and local epidemiology of respiratory infections. The results of the study confirm the findings by Arthur *et al.* [11], showing that the majority of the isolates from sputum samples were Gram-positive bacteria (82.6%), with the most prominent isolate being *Streptococcus pneumoniae* (31.0%). The same research also revealed the presence of *Staphylococcus aureus* (12.7%), coagulase-negative *Staphylococcus* spp. (19.7%) and *Micrococcus* spp. (15.5%) in respect of Gram-positive isolates. As for Gram-negative bacteria, they comprised 10.5% of all isolates with *Klebsiella pneumoniae* subsp. *pneumoniae* (44.4%) being the most common among Gram-negatives, followed by *Acinetobacter baumannii* (22.2%) and Pseudomonas aeruginosa (11.1%). Furthermore, polymicrobial growth has been registered in 27.9% of culture-positive specimens.

Discrepancies between the current study and previous reports on bacterial species distribution could be attributed to geographical diversity, differences in selected patients, sample size, usage of antibiotics before investigation, hospitalization, as well as local epidemological patterns of respiratory pathogens.

A total of 23 GNB species were recognized where *Klebsiella* sp. (92/271, 34.0%) was found to be the most frequent organism followed by *Pseudomonas* sp. (73/271, 27.0%) and *Acinetobacter* sp. (32/271, 12%). Among the isolates, Enterobacteriaceae were quite prevalent (n=130, 48%) where the remaining non-Enterobacteriaceae consisted of n=141 (52%) [15].

Overall, bacterial pathogens were detected in 50% sputum samples. The most common pathogens among bacterial isolates were found to be *Klebsiella* species followed by Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus pneumoniae. More than half of the isolated strains were found to be resistant to multiple drugs. In addition, 40.5% and 10.8% of the strains produced ESBL and carbapenemase, respectively. Factors that were found to be associated with sputum positivity included old age, smoking, prior history of pneumonia, cardiac problem, and chronic lung diseases [20].

Pseudomonas aeruginosa, which accounted for 21%, *Klebsiella pneumoniae* (17%), *Escherichia coli* (9%), and *Staphylococcus aureus* (6%) were the most common pathogens found [13]. The microorganisms evidenced by gram stain and isolated from the purulent sputum of the patients are given below along with their respective frequencies from among 100 patients: *Pseudomonas aeruginosa* has been isolated from 21% (n=21), *Klebsiella pneumoniae* (17%) (n=17), *Escherichia coli* (9%) (n=9), *Staphylococcus aureus* (6%) (n=6), Enterobacter spp. (5%) (n=5), *Candida albicans* (4%), and other bacteria (38%) (n=38) [13].

The most frequently isolated bacteria included *Pseudomonas aeruginosa* (44.6% or 37/83), *Staphylococcus aureus* (25.3% or 21/83), along with *Haemophilus influenzae* (15.7% or 13/83) [21].

The pathogen was identified in (59%), (11%) of bacterial pathogens only, in (38%) of viral pathogens only, and in (10%) of both bacterial and viral pathogens). The most frequently identified bacterial pathogens were *S. pneumoniae* (5.5% in general, 9.2% in the group of patients with community-acquired pneumonia) and *H. influenzae* (5.4% in general, 14.2% in the patients with community-acquired pneumonia). Less than 1% of *S. pneumoniae* were resistant to penicillin and 12.6% of *H. influenzae* negative [22]. *K. pneumoniae* (32.94%), P. aeruginosa (22.35%) and *S. aureus* (11.76%) are the most commonly identified bacteria [1]. The highest rate of detection of bacteria was characterized by *Klebsiella pneumoniae* (23.5%), *Acinetobacter baumannii* (21.6%), *Streptococcus pneumoniae* (19.6%), *Escherichia coli* (17.6%) and *Haemophilus influenzae* (11.8%) [14]. The lowest identification rate of bacteria was observed for *Haemophilus influenzae* (15.4%), *Moraxella catarrhalis* (18.2%), *Streptococcus pneumoniae* (19%) and *Staphylococcus aureus* (22.7%), many of which are difficult to culture [23].

The study's results pointed out there are four different bacterial species; *Streptococcus pneumonia* (8.57%), *Mycoplasma pneumoniae* (22.85%), *Escherichia coli* (2.85%) and *Staphylococcus aureus* (65.71%) [24]. The isolated species of GNB from the survey indicated

that *Klebsiella* is the most prevalent species, Kostova [25] included the following numbers on other GNB: K. pneumoniae (14.3%), *K. oxytoca* (7.9%), *K. ozanae* (7%), *K. rhinoscleromatis* (10.3%), whereas *E. coli* was (6.3%), Entrobacter sp. (5.6%), *Citrobacter* sp. (3.2%), Proteus mirabilis (0.8%), H. influenzae (4.8%), and *M. catarhallis* (6.3%). The Gram positive species isolated from the samples Apollo in their study included *S. aurues* (19%), *S. pneumonia* (10.3%), and *S. pyogens* (4%). Highest number of isolates came from S. aureus (19.0%) followed by *K. peumoniae*, while *K. rhinoscleromatis* and *S. pneumoniae* recorded equal distribution at (10.3%) [19]. *Pseudomonas aeruginosa* had the highest numbers reaching 27 (62.8%) followed by *Klebsiella pneumoniae* with nine (20.9%), *Escherichia coli* with 4 (9.3%) and *Acinetobacter baumannii* multifarious with three (7.0%) [12].

Male individuals made up the bulk of infected E. cloacae patients (64%) and individuals aged 60 years or older made up more than half (56%). Major risk factors associated with the infection of individuals with *A. baumannii* (multidrug-resistant) were things like prior antibiotic use ($p = 0.008$), hospital stays exceeding 10 days ($p = 0.021$), and the requirement of ICU admission ($p = 0.034$) [25].

Overall, the results indicated multi-drug resistance in *S. pneumoniae* to some commonly used antibiotics such as β-lactams, macrolides, lincosamides, and tetracyclines. Nevertheless, the efficacy of linezolid and tigecycline was maintained in all isolates; therefore, the two drugs can thus be considered reliable alternatives for the treatment of resistant *S. pneumoniae*. Diverse sensitivity to levofloxacin and vancomycin is believed to be a reflection of different mechanisms of resistance in various isolates.

*Streptococcus pneumoniae* was dominant in the two groups of patients in the ICU, and its sensitivity to penicillin was only 50% or less in these cases. In addition, sensitivity to fluoroquinolones and third-generation cephalosporins (including cefditoren) was prevalent in more than 95 % of observations. Sensitivity of S. pneumoniae to antibiotics (15:30) indicates that amikacin is the most effective of all (100%). In addition to this, *S. pneumoniae* has been reported to be sensitive to clindamycin (85.7 %) and trimethoprim-sulfamethoxazole (73.7 %). It has been noted that the resistance of S. pneumoniae to tetracyclines is pretty high (76.2 %) [26].

The findings suggest that the *Staphylococcus aureus* isolates exhibited multiple drug resistance, especially to penicillins, macrolides, aminoglycosides, lincosamides, tetracyclines, and cephalosporins. On the other hand, the fluoroquinolones levofloxacin and moxifloxacin were found to be effective against all of the tested isolates. Since all the isolates were completely resistant to cefoxitin, it can be assumed that methicillin-resistant *Staphylococcus aureus* (MRSA) might be present in the isolates, which should be confirmed by molecular methods (for example, detecting the *mecA* or *mecC* genes in the isolates).

The antimicrobial susceptibility patterns of Gram-positive bacteria ($n = 42$) isolates in this research study indicated that susceptibility patterns are commonly varied. Gram-positive isolates were described to be susceptible to the following antibiotics: clindamycin 29/42(69.05%) and erythromycin 25/42(59.5%). Regarding tetracycline, gram-positive isolates were identified to be resistant to (31/42) or (73.8%) of the study samples. Also, *S. aureus* resistances to penicillin with the results of this study indicated that thirty-four isolates were resistant to penicillin 19/24 (79.2%) and trimethoprim-sulfamethoxazole (58.3%). In contrast, higher susceptibility to antibiotics like cefoxitin, ciprofloxacin, and gentamycin (20/25 or 83.3%) and azithromycin 18/24 (75%) showed [19]. The presence of Gram-negative bacteria and the association between. *S. aureus* and increased mortality were obtained or detected from the outcome of this study regarding the fact that antibiotic resistance creates a serious difficulty on infections with Reiner-benaim *et al.* [18]. Gram-positive Isolate results show *S.aureus* possess high susceptibility levels to (22.39 %) of antibiotic, clindamycin, trimethoprim-sulfamethoxazole (87.7%), and ciprofloxacilin (78.6%). In contrast, the level of resistance to penicillin is fairly high (64.3%)[16]. Furthermore, *S. aureus* was found to be susceptible to (19:30) of antibiotics described in the research study.

Among the 150 human sputum specimens studied, 25 samples isolated respiratory infections caused by *Klebsiella pneumoniae*. Results showed that the majority of the pathogens that were isolated showed resistance against piperacillin with a frequency of 88%, while the least resistant was imipenem [27]. Another study also evaluated the resistance of Klebsiella species against other antimicrobials, wherein it was found that colistin had a resistance rate of 87%, followed by cefotaxime with 76% resistance rate, and ceftazidime with a 71% resistance rate. Moreover, the resistance levels observed with fluoroquinolones were significant. The resistance levels of aminoglycosides, carbapenems, and tetracyclines were moderate to low, with 60% resistance to amikacin. One of the studies found that *K. pneumoniae* strains exhibited a high sensitivity to meropenem, and in a sputum analysis, *K. pneumoniae* was isolated in up to 20% of the subjects. Sensitivity testing of the *K. pneumoniae* bacteria

showed a 93.5% sensitivity rate for meropenem, followed by ceftriaxone with 83.9%, and ciprofloxacin with 74.2% sensitivity rate.

The findings indicate that *A. baumannii* isolates are multidrug resistant, especially against fluoroquinolones, aminoglycosides, carbapenems, and some cephalosporins. Complete resistance to meropenem is of great clinical importance since carbapenems are often the last choice drugs to treat severe infections caused by *Acinetobacter*. The variation in susceptibility to ceftazidime and cefepime indicates different resistance mechanisms in the isolates which stress the need for performing antimicrobial susceptibility testing to choose appropriate antimicrobial treatment.

The presence of *Acinetobacter baumannii* was linked with a higher rate of mortality. These data underscore the variability of pathogens and troubling patterns in antibiotic susceptibility in LRTI. The importance of diagnosing illnesses as well as combating antibiotic resistance is reinforced by this study, which is significant for effective LRTI management [18] *Acinetobacter* spp. had specifically concerning characteristics, exhibiting almost complete resistance to β-lactams and carbapenems (above 90%), but their susceptibility to colistin was preserved (0% resistance). In comparison to this, *Klebsiella* spp. managed to remain resistant to third-generation cephalosporins (86%-93%) and carbapenems (70%-77%), but their susceptibility to amikacin and tigecycline was moderate (45% and 36%, correspondingly) [15]. The overall results show that *M. catarrhalis* was susceptible to the majority of the tested antimicrobial agents as resistance was observed for a small number of antibiotics.

The study found similar results in terms of the resistance of *M. catarrhalis* isolates where resistance to ampicillin was revealed in 43.8% of isolates, resistance to ciprofloxacin amounted to 31.3%, and resistance to cefotaxime was shown by 18.8% of isolates. Resistance to cotrimoxazole and amoxicillin–clavulanate was registered in only 6.3 % of the cases. Notably, 100% of the isolates were susceptible to the azithromycin. Likewise, according to Durmuş [28], M. catarrhalis showed the low antimicrobial resistance, where the resistance to amoxicillin–clavulanate was about 2.5%, resistance to trimethoprim–sulfamethoxazole accounted for 2.4%, and resistance to fluoroquinolones (ciprofloxacin and levofloxacin) was 2.2%. Resistance to cephalosporins was also low dating from 4.1% for cefotaxime and up to 7.9% for cefixime. The minor variances observed between the current study and preceding studies can be attributed to regional differences, variations in antimicrobial usage, the number of samples, and the local epidemiology of antibiotic resistance in the strains of *M. catarrhalis*.

The results obtained by Popa *et al.* [29] are consistent with this study, where *E. cloacae* isolates were sensitive to multiple antimicrobial agents except for amoxicillin-clavulanate and some β-lactam antibiotics. Interestingly, Singh *et al.* [30] reported a considerably higher level of numerous Enterobacter isolates' antimicrobial resistance, and they listed levofloxacin (94%), ampicillin and ciprofloxacin (92% each), meropenem (80%), and two other medications of amikacin and imipenem among the elements that received the highest resistance rates. What is more, Hasan et al. [25] revealed the fact that all isolates of *E. cloacae* demonstrated resistance to ampicillin, amoxicillin-clavulanate, cephalosporin, and cefuroxime. Moreover, 86% of their isolates were considered multidrug resistant (MDR).

Among the Gram-negative bacteria isolated, *E. coli* was found to be quite sensitive towards meropenem (86.7%) and trimethoprim-sulfa methoxazole (80%). However, a very high level of resistance was observed towards ampicillin (71.4%) [16]. The sensitivity of *E. coli* towards the various antibiotics (3:30) and the best antibiotics which had the most effect were discovered to be Amikacin, Imipenem and Meropenem at a 100% sensitivity level [24].

## CONCLUSION

The conclusion obtained through the current study indicates that the treatment method used for respiratory infection diagnosis through the technique of sputum culture is not reliable, as only a minor part of sputum samples showed bacterial growth compared to culture-negative samples. The unsatisfactory rate of culture positivity hampered the proper identification of the main bacterial pathogens and their antibiotic resistance characteristics. Of the bacteria identified, Streptococcus pneumoniae and Staphylococcus aureus were the most common bacterial species, but many Gram-negative pathogens were detected as well, which means that the susceptibility patterns against antibiotics differ among species. This shows that susceptibility testing must be performed before starting antibiotic treatment. Hence, the investigation team suggested using a larger number of sputum samples and collaborating the results of the culture test with the clinical findings.

## ACKNOWLEDGEMENTS (IF APPLICABLE)

### Acknowledgments

The authors would like to express their sincere gratitude to the administration and medical staff of Azadi Hospital, Kirkuk Governorate, for their cooperation and support in facilitating the collection of sputum samples required for this study during the period from July 2025 to February 2026. Their assistance and cooperation were greatly appreciated and contributed to the successful completion of this research.

### Authors' Contributions

A.H.S.A. conceived and designed the study; L.A.Z. collected the sputum samples and performed the laboratory procedures and bacterial identification. A.H.S.A. and L.A.Z. contributed to data analysis and interpretation. A.H.S.A. drafted the manuscript, while L.A.Z. contributed to its review and editing. Both authors read and approved the final version of the manuscript.

### Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki and relevant institutional ethical guidelines. Ethical approval was obtained from Department of Biology, Collage of Education Pure Science (Ibn Al-Haitham), University of Baghdad, Iraq.

### Funding

Self funded

### Conflict

No conflict of interest

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