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Diagnostic and Clinical Insights Into Respiratory Bacterial Co Infections in COVID-19 A Molecular, Biochemical, and Antimicrobial Resistance Study

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ABSTRACT:
Respiratory infections, particularly those complicated by bacterial co-infections, present a critical challenge during the COVID-19 pandemic. This study aims to investigate the prevalence, bacterial diversity, and antibiotic susceptibility patterns of respiratory bacterial co-infections among COVID-19 patients. Through molecular testing and culture methods, 100 respiratory samples were analyzed to identify bacterial pathogens. Statistical analyses revealed significant gender and age distribution differences among the samples. Pseudomonas aeruginosa, Klebsiella pneumoniae, and Streptococcus pneumoniae were among the frequently isolated bacteria. The study also investigated antibiotic susceptibility patterns, highlighting varying resistance and sensitivity profiles. Findings underscore the importance of understanding bacterial co-infections in the context of COVID-19 for effective patient management.

Keywords: Respiratory infections, Bacterial co-infections, COVID-19, Prevalence, Molecular testing, Antibiotic susceptibility, Patient management, Epidemiology, Diagnostic methods, Antibiotic therapy

INTRODUCTION

The COVID-19 pandemic, caused by the novel SARS-CoV-2 virus, has overwhelmed healthcare systems worldwide and revealed complex interactions between viral infections and bacterial co-infections. While substantial attention has been given to understanding the virology and clinical outcomes of COVID-19, emerging evidence highlights the significant role bacterial co-infections play in influencing disease severity, treatment efficacy, and healthcare resource utilization (Bahl et al., 2021).

Respiratory bacterial co-infections in COVID-19 patients represent a significant concern, adding complexity to the clinical presentation and complicating both diagnosis and treatment. These co-infections can exacerbate the severity of respiratory illness and contribute to longer hospital stays, increased morbidity, and mortality (Scully et al., 2021). The presence of bacterial pathogens alongside SARS-CoV-2 raises concerns about the potential for synergistic pathogenicity, immune dysregulation, and exacerbation of inflammatory responses (Fried et al., 2020). Therefore, accurate and timely diagnosis is essential in managing these co-infections.

In this context, biochemical diagnostics, as outlined in the tables, play a critical role in improving the accuracy of diagnosis and guiding effective therapeutic interventions. Key biochemical markers such as

C-reactive protein (CRP), procalcitonin (PCT), and lactate are valuable tools in identifying bacterial infections and distinguishing them from viral causes, including COVID-19. The elevated levels of these biomarkers, as seen in the study, help identify patients at higher risk for bacterial infections and guide clinicians in initiating targeted antibiotic therapy (Meyer et al., 2021). For example, in cases of bronchopneumonia, high CRP and elevated LDH levels are associated with bacterial infections, particularly with pathogens like *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, as revealed through both culture and PCR testing (Lansbury et al., 2020).

The biochemical parameters, combined with molecular diagnostic techniques like RT-PCR, improve the overall diagnostic sensitivity, helping healthcare providers differentiate between bacterial and viral infections more effectively. This is critical in the context of COVID-19, where bacterial co-infections may be overlooked without comprehensive diagnostic approaches. The ability to detect pathogens quickly and accurately is crucial for improving patient outcomes, minimizing the use of broad-spectrum antibiotics, and reducing the risk of antimicrobial resistance (Tzeng et al., 2021).

Critical analysis of current research on COVID-19 and respiratory bacterial co-infections reveals a complex and variable landscape. While some studies report a relatively low prevalence of bacterial co-infections, others document a considerable burden, particularly among patients with severe or critical illness. Variations in detection methods, patient populations, and healthcare settings contribute to the diverse findings in the literature, highlighting the need for a more comprehensive and context-specific understanding (Meyer et al., 2021).

A clear understanding of the interplay between COVID-19 and respiratory bacterial co-infections is essential for optimizing clinical management strategies, guiding antimicrobial therapy decisions, and minimizing the risk of antimicrobial resistance. Moreover, incorporating biochemical markers into diagnostic strategies, alongside molecular methods, enables a more holistic approach to patient care. Identifying the epidemiological and clinical characteristics of bacterial co-infections can help inform public health interventions aimed at reducing disease burden, improving patient outcomes, and strengthening healthcare systems during the ongoing pandemic (Meyer et al., 2021).

This analysis aims to synthesize the current body of evidence on the relationship between COVID-19 and respiratory bacterial co-infections, with particular emphasis on the role of biochemical and molecular diagnostics in enhancing clinical decision-making. By critically evaluating the existing literature and identifying gaps in knowledge, this study seeks to inform evidence-based management approaches for COVID-19 patients with concurrent bacterial respiratory infections (Lansbury et al., 2020).

AIM AND OBJECTIVES

This study aims to investigate respiratory bacterial co-infections among COVID-19 patients, focusing on prevalence, bacterial diversity, and antibiotic susceptibility patterns. The objectives include:

1. Assessing the prevalence of bacterial co-infections in respiratory samples from COVID-19 patients.
2. Identifying the bacterial species responsible for co-infections using molecular and culture-based methods.
3. Analysing the gender and age distribution of patients with bacterial co-infections.
4. Evaluating the antibiotic susceptibility patterns of isolated bacterial strains.
5. Correlation of Biochemical Markers with Microbiological Findings

MATERIALS AND METHODOLOGY

Study Design and Sample Collection

A cross-sectional study was conducted over a specified period, during which 100 respiratory samples were collected from patients presenting with symptoms indicative of respiratory infections. The samples were obtained from various hospital wards, including ICU, MICU, and general wards.

Molecular Testing

All collected samples underwent molecular testing using real-time polymerase chain reaction (RT-PCR) to identify the presence of respiratory pathogens. This included testing for a range of bacterial species commonly associated with respiratory infections.

Multiplex RT-PCR testing

Multiplex RT-PCR (Reverse Transcription Polymerase Chain Reaction) is a technique used to simultaneously amplify multiple RNA targets in a single reaction. The principle behind multiplex RT-PCR involves combining reverse transcription and PCR amplification steps to convert RNA into complementary DNA (cDNA) and then amplify specific cDNA targets.

In multiplex RT-PCR, multiple primer sets and fluorescent probes specific to different RNA targets are included in the reaction mixture. The reverse transcriptase enzyme converts RNA into cDNA, and then the DNA polymerase enzyme amplifies the cDNA targets using PCR. Each primer set is designed to target a distinct RNA sequence, and the fluorescent probes allow for the detection and quantification of the amplified products.

The key to successful multiplex RT-PCR is the optimization of reaction conditions, including primer design, annealing temperatures, and cycling parameters, to ensure efficient and specific amplification of all target sequences. Multiplex RT-PCR is widely used in molecular biology research and diagnostic applications for the simultaneous detection of multiple RNA targets, such as viral pathogens or gene expression analysis, providing a rapid and cost-effective method for comprehensive molecular analysis.

Culture and Sensitivity Testing

Parallel to molecular testing, aerobic culture methods were employed to grow and isolate bacterial pathogens from the respiratory samples. The isolates were then subjected to antibiotic susceptibility testing (AST) using standard protocols to determine their sensitivity or resistance to various antibiotics.

Biochemical analysis

The biochemical analysis of respiratory pathogens involved measuring key markers to evaluate inflammation, infection, and renal function. C-Reactive Protein (CRP) levels were quantified using ELISA and nephelometry, serving as indicators of inflammation. Lactate Dehydrogenase (LDH) was assessed through enzymatic assays to reflect tissue damage. Procalcitonin (PCT), measured via immunoassays, acts as a biomarker for bacterial infections and sepsis. The White Blood Cell Count (WBC) was determined using an automated cell counter to assess the immune response. Erythrocyte Sedimentation Rate (ESR) was measured using the Westergren method and automated analyzers to indicate inflammatory processes. Finally, urea and creatinine levels were evaluated for renal function, with urea measured through the urease method and creatinine via the Jaffe reaction. These biochemical markers offer crucial insights into the clinical status of patients with respiratory conditions and will be correlated with microbiological findings for comprehensive analysis.

Data Analysis

The gender and age distribution of the patients, as well as the ward details, were recorded and statistically analysed. Chi-square tests were performed to assess the significance of differences in distribution and

detection rates between the molecular and culture methods. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the PCR and culture methods were calculated.

RESULT

GENDER-WISE DISTRIBUTION OF THE ISOLATES WITH STATISTICAL ANALYSIS

During the study period, 100 samples were collected and subjected to molecular testing for respiratory pathogens. The statistical analysis shows a significant difference in the gender distribution of the samples. The majority of the samples (68%) were from male patients, while 32% were from female patients, and this difference is statistically significant (chi-square $\chi 2=12.96 \chi 2=12.96$, $p < 0.05$).

AGE-WISE DISTRIBUTION OF THE ISOLATES WITH STATISTICAL ANALYSIS

During the study period, 100 samples were collected and subjected to molecular testing for respiratory pathogens. The age-wise distribution of the isolates is presented below:

Age Range	Number of Samples	Percentage (%)
18-30	16	16%
31-40	10	10%
41-50	14	14%
51-60	32	32%
61-70	20	20%
>70	8	8%

The statistical analysis shows a significant difference in the age distribution of the samples. The highest proportion of samples (32%) were from patients in the 51-60 age group, followed by 20% from the 61-70 age group, and this distribution is statistically significant (chi-square $\chi 2=22.3988 \chi 2=22.3988$, $p < 0.05$).

RELATION BETWEEN WARD DETAILS AND DIAGNOSIS OF THE ISOLATES

To analyse the relationship between the ward details and the clinical diagnoses, we can create a contingency table and perform a chi-square test for independence. This test will help us determine if there is a significant association between the wards and the diagnoses.

Diagnosis	ICU	MICU	IICU	RICU	EDICU	EMDG	GMW	Total
Bronchopneumonia	9	2	1	6	1	1	6	26
COPD	6	1	0	4	0	1	8	20
LRTI	8	1	1	5	1	0	6	22
Pleural Effusion	1	0	0	0	0	0	1	2
ARDS	3	0	1	1	0	1	0	6
URTI	1	1	0	0	1	0	1	4
Lobar Pneumoniae	3	1	1	2	1	1	1	10
Bronchitis with Bronchial Asthma	1	0	0	1	0	0	2	4

Chronic Lung Disease	1	0	0	0	0	0	1	2
Interstitial Lung Disease	1	0	0	1	0	0	2	4
Total	34	6	4	20	4	4	28	100

Once the chi-square statistic is calculated, compare it to the critical value from the chi-square distribution table for 54 degrees of freedom at a 0.05 significance level. If the calculated $\chi 2 \chi 2$ is greater than the critical value, we reject the null hypothesis, indicating a significant association between ward and diagnosis.

Revealing Respiratory Bacterial Diversity: Insights From Rt-Pcr Analysis

	BACTERIAL PATHOGEN	**NO OF ISOLATES**
POSITIVE-77	*Pseudomonas aeruginosa*	18
	Klebsiella pneumoniae	12
	Bordetella spp	11
	Acinetobacter baumanii	10
	Hemophilus influenza	9
	Streptococcus pneumoniae	9
	Staphylococcus aureus	4
	Moraxella spp	2
	E. cloacae	1
	Streptococcus agalactiae	1
NEGATIVE-23		

The provided table outlines the results of a respiratory pathogen detection assay conducted via RT-PCR for 100 patients. Among the clinical specimens analyzed, 76% (38 out of 100) tested positive for bacterial pathogens, while the remaining 24% (12 out of 100) tested negative. The positive specimens exhibited a diverse array of bacterial species, with Pseudomonas aeruginosa being the most frequently detected (23 isolates), followed by Klebsiella pneumoniae (16 isolates) and Bordetella spp. (14 isolates). Other identified pathogens include Acinetobacter baumanii, Hemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus, Moraxella spp., E. cloacae, and Streptococcus agalactiae. These findings underscore the efficacy of RT-PCR in identifying respiratory bacterial pathogens across a larger sample size, facilitating targeted therapeutic interventions.

Covid-19 And Bacterial Co-Infection Spectrum: Gene Detection And Clinical Implications In Multiplex Pcr Analysis

Infection Type	COVID-19 Gene(s) Detected	Bacterial Pathogen Identified	Genes Detected by Multiplex PCR	Number of Cases	Clinical Relevance	Ward Diagnosis Details
	N gene, S gene	Streptococcus pneumoniae	lytA, ply (pneumolysi	2	Pneumococcal infections worsen	ICU: Severe pneumonia,

			n, autolysin genes)		respiratory outcomes in COVID-19 patients.	respiratory distress.
	E gene	Klebsiella pneumoniae	KPN-specific capsular genes (e.g., wzy, magA)	4	Associated with multi-drug resistance and virulent traits complicating treatment.	ICU: Sepsis, respiratory failure.
	S gene	Pseudomonas aeruginosa	exoA, lasR, oprL (Exotoxin A, lasR, outer membrane protein genes)	12	Increases risk of ventilator-associated pneumonia (VAP) in COVID-19 patients.	ICU/ventilation: Critical pneumonia.
Single Bacterial Infection (37)	N gene	Acinetobacter baumannii	ompA, blaOXA (Outer membrane protein A, carbapenem resistance gene)	6	Highly resistant, complicates co-infection and therapy in COVID-19 patients.	ICU: Acute respiratory failure.
	N gene	Bordetella spp.	ptxS1, fhaB (Pertussis toxin, filamentous hemagglutinin genes)	4	Severe exacerbation of respiratory symptoms in immunocompromised COVID-19 patients.	ICU: Severe pneumonia, respiratory distress.
	E gene	Moraxella spp.	ompCD, copB (Outer membrane protein genes)	2	Chronic respiratory diseases worsened by co-infection with COVID-19.	Ward: Pneumonia, breathing difficulty.
	S gene, E gene	Haemophilus influenzae	hpd, bexA (Haemophilus protein D, capsulation genes)	6	Co-infection exacerbates COVID-19 symptoms.	ICU: Severe pneumonia.

Double Bacterial Infection (18)	N gene	Staphylococcus aureus + Pseudomonas aeruginosa	mecA (methicillin resistance) + exoA, lasR (Exotoxin A, lasR genes)	2	Severe respiratory failure, antibiotic resistance complicates co-infection.	ICU: Ventilator dependency.
	S gene	Streptococcus pneumoniae + Pseudomonas aeruginosa	lytA, ply + exoA, oprL	4	Pneumococcal infections combined with P. aeruginosa worsen respiratory outcomes.	ICU: Severe bilateral pneumonia.
	N gene, E gene	Streptococcus pneumoniae + Acinetobacter baumannii	lytA, ply + blaOXA, ompA	4	Co-infection of resistant bacteria with COVID-19 worsens prognosis, aggressive management required.	ICU: ARDS, sepsis.
	E gene	Enterococcus cloacae + Haemophilus influenzae	vanA (vancomycin resistance) + hpd, bexA	2	Combined resistant and virulent factors result in poor prognosis.	ICU: Sepsis, multi-organ failure.
	S gene, E gene	Klebsiella pneumoniae + Acinetobacter baumannii	magA, blaCTX-M + blaOXA	2	Dual-resistant bacteria complicate treatment, worsening the COVID-19 infection outcome.	ICU: Respiratory failure.
	N gene	Pseudomonas aeruginosa + Bordetella spp.	exoA + ptxS1	2	Severe combined respiratory infection in COVID-19 patients, requiring intensive treatment.	ICU: VAP, respiratory collapse.
	S gene, N gene	Pseudomonas aeruginosa + Haemophilus influenzae	exoA + hpd	2	Severe pneumonia exacerbated by viral and bacterial co-infection.	ICU: ARDS, multi-organ failure.
	N gene, S gene	Streptococcus pneumoniae + Haemophilus influenzae + Moraxella spp.	lytA, hpd, ompCD	2	Triple co-infection leading to critical pneumonia in immunocompromised COVID-19 patients.	ICU: Severe pneumonia, septic shock.

Triple Bacterial Infection (12)	S gene	Klebsiella pneumoniae + Bordetella spp. + Pseudomonas aeruginosa	magA, ptxS1, exoA	6	Highly virulent bacterial combination leading to respiratory failure in COVID-19 patients.	ICU: Critical bilateral pneumonia.
	N gene, E gene	Klebsiella pneumoniae + Streptococcus pneumoniae + Acinetobacter baumannii	magA, lytA, blaOXA	2	Dual resistant bacteria causing acute respiratory failure with COVID-19.	ICU: Sepsis, respiratory collapse.
	S gene	Staphylococcus aureus + Streptococcus pneumoniae + Haemophilus influenzae	mecA, lytA, hpd	2	Dangerous combination leading to rapid deterioration of respiratory health in COVID-19 patients.	ICU: Severe ARDS, shock.
Quadruple Infection (4)	N gene	Klebsiella pneumoniae + Bordetella spp. + Acinetobacter baumannii + Streptococcus pneumoniae	magA, ptxS1, blaOXA, lytA	2	Multiple resistant pathogens causing severe infection. Prolonged intensive care needed.	ICU: Acute respiratory distress syndrome (ARDS).
	S gene	Klebsiella pneumoniae + Bordetella spp. + Acinetobacter baumannii + Haemophilus influenzae	magA, ptxS1, blaOXA, hpd	2	Multi-pathogen infections worsen prognosis, complicating COVID-19 recovery.	ICU: Multi-organ failure, respiratory support.
Quintuple	E gene	Klebsiella pneumoniae + Bordetella spp. + Pseudomonas aeruginosa + Staphylococcus aureus + Streptococcus	magA, ptxS1, exoA, mecA, cfb	2	Extreme multi-pathogen infection requiring rapid, broad-spectrum treatment.	ICU: Critical, multi-organ dysfunction.

Infection (6)		agalactiae				
	S gene	Acinetobacter baumannii + Haemophilus influenzae + Klebsiella pneumoniae + Bordetella spp. + Pseudomonas aeruginosa	blaOXA, hpd, magA, ptxS1, exoA	2	Life-threatening combination of viral and bacterial pathogens in immunocompromised individuals.	ICU: Multi-organ failure, ventilator support.

This table integrates all the details related to COVID-19 and bacterial co-infections, including gene detections, bacterial pathogens, clinical relevance, and ward diagnosis details across various infection types.

AEROBIC CULTURE GROWTH OF RESPIRATORY PATHOGENS

CULTURE RESULT	BACTERIAL PATHOGEN	NO OF ISOLATES
POSITIVE-16	*Acinetobacter baumanii*	4
	Pseudomonas aeruginosa	2
	Klebsiella pneumoniae	4
	Enterobacter cloacae	2
	Streptococcus pneumoniae	2
	Aspergillus niger	1
	Aspergillus fumigatus	1
NEGATIVE-84		

DETAILED MULTIPLEX RT- PCR AND CULTURE COMPARISON RESULTS

DIAGNOSIS	AEROBIC CULTURE		PCR-REPORT		TOTAL	%
	GROWTH	NO GROWTH	POSITIVE	NEGATIVE		
BRONCHO PNEUMONIA	4	22	18	8	26	26%
COPD	4	16	16	4	20	20%
LRTI	4	18	18	4	22	22%
ARDS	0	6	6	0	6	6%
URTI	0	4	4	0	4	4%
PNEUMONIAE	2	8	10	0	10	10%
BRONCHITIS+BRONCHIAL ASTHMA	0	4	2	2	4	4%
CHRONIC LUNG DISEASE	0	2	0	2	2	2%
PLEURAL EFFUSION	0	2	0	2	2	2%
INTERSTITIAL LUNG DISEASE	2	2	2	2	4	4%

TOTAL	16	84	76	24	100	100 %

The detection of respiratory pathogens is critical for accurate diagnosis and treatment. This report compares the effectiveness of aerobic culture and PCR methods in identifying bacterial pathogens in 100 respiratory samples, covering diagnoses such as Broncho pneumoniae, COPD, LRTI, ARDS, URTI, Pneumonia, Bronchitis with Bronchial Asthma, Chronic Lung Disease, Pleural Effusion, and Interstitial Lung Disease. The data reveals significant differences in detection rates between the two methods. For example, out of 100 samples, Broncho pneumoniae had 18 positive results by PCR but only 4 by culture, COPD had 16 positive results by PCR compared to 4 by culture, and LRTI had 18 positive results by PCR compared to 4 by culture. In total, PCR detected pathogens in 76 samples, while aerobic culture detected pathogens in only 16 samples.

To statistically analyse these differences, a Chi-Square test was performed. The null hypothesis stated that there would be no significant difference in detection rates between the two methods, while the alternative hypothesis posited a significant difference. The Chi-Square test result was 82.56 with 1 degree of freedom and a p-value of less than 0.00001. This indicates a significant difference in detection rates, leading us to reject the null hypothesis.

These findings suggest that PCR is significantly more sensitive than aerobic culture in detecting respiratory pathogens. Given its higher sensitivity, PCR should be considered the preferred method for diagnosing respiratory infections to ensure timely and accurate treatment. Consequently, it is recommended that clinical practices implement PCR as the standard diagnostic tool for respiratory infections and that further research be conducted to confirm these findings across different populations and settings. This analysis underscores the importance of using sensitive and accurate diagnostic methods like PCR to improve patient outcomes in respiratory infections.

Comparative Analysis of Culture and PCR Results for Respiratory Pathogens showing relation in their analysis

This table summarizes the results of culture and PCR testing for respiratory pathogens. It presents the total number of isolates tested (16) and compares the distribution of organisms detected by culture and PCR, along with their similarity percentages. The table showcases the specific organisms identified for each method, highlighting any discrepancies between the two testing approaches. Additionally, it includes a category for samples that tested negative in PCR.

Total isolate (16)	Culture	PCR	Distribution of organism	Similarity
2	*K. pneumoniae*	*K. pneumoniae*	2	100%
12	*A. baumannii*	*H. influenzae*	8	25%
		K. pneumoniae		
		Bordetella		
		A. baumannii		
	E. cloacae	*H. influenzae* *E. cloacae*	4	50%
	Aspergillus niger	*P. aeruginosa*	1	0
	Aspergillus fumigatus		1	
	A. baumannii	*S. pneumoniae*	4	50%

		A. baumannii		
	Pneumococci	*K. pneumoniae*	8	25%
		Bordetella		
		A. baumannii *Pneumococcus*		
	P. aeruginosa	*Bordetella*	2	0
2	*K. pneumoniae*	*Negative*	0	0

This table compares the results of culture and PCR testing for respiratory pathogens. It indicates the number of samples that tested positive and negative using both methods. The P-value of 0.240 suggests no significant difference between culture and PCR results in detecting the condition at the conventional 0.05 significance level.

CULTURE	PCR		**P-VALUE**
	POSITIVE	NEGATIVE	
POSITIVE	14	2	**0.240**
NEGATIVE	62	22	

DETAILED COINFECTION WITH RESPIRATORY PATHOGENS IN SARS-COV-2

Covid Positive- 20	PCR-(+ve)	PCR-(-ve)	Culture –(+ve)	Culture– (-ve)
	14	6	2	18
Covid Negative-80				

Using the chi-squared distribution with 1 degree of freedom, we can find the p-value for $\chi 2=15\chi 2=15$. Using a chi-squared distribution table or an online chi-squared calculator, we find that the p-value for $\chi 2=15\chi 2=15$ with 1 degree of freedom is very small, less than 0. 0001. The p-value is less than 0.0001, indicating a significant difference between the culture and PCR tests in detecting the condition.
The PCR test shows high sensitivity (87.5%) in correctly identifying positive cases of respiratory bacterial infection but moderate specificity (73.8%) in identifying negative cases. The Positive Predictive Value is low (18.4%), indicating a higher likelihood of false positives, while the Negative Predictive Value is high (91.7%), indicating accurate exclusion of infection in negative cases. Overall accuracy is 76%.

To analyze this data, let's calculate the following	
Sensitivity (True Positive Rate): The proportion of actual positives that are correctly identified by the PCR test.	87.5%
Specificity (True Negative Rate): The proportion of actual negatives that are correctly identified by the PCR test.	73.8%
Positive Predictive Value (PPV): The proportion of positive PCR test results that are true positives.	18.4%
Negative Predictive Value (NPV): The proportion of negative PCR test results that are true negatives.	91.7%
Overall Accuracy: The proportion of all tests that are correctly identified	76%

by the PCR test.	

BIOCHEMICAL AND MICROBIOLOGICAL CORRELATIONS ACROSS RESPIRATORY CONDITIONS

The tabulated data provides a comparative analysis of **biochemical markers** and **microbiological findings** across various respiratory conditions. Below are the key relationships:

Diagnosis	CRP (mg/L)	LDH (U/L)	PCT (ng/mL)	Lactate (mmol/L)	WBC (cells/µL)	ESR (mm/hr)	Urea (mg/dL)	Creatinine (mg/dL)	Culture Growth	Culture Pathogens	PCR Positive	PCR Pathogens
Bronchopneumonia	High (20-200)	Elevated (300-600)	High (>0.5)	Slightly elevated (1-2)	Elevated (12,000-18,000)	High (30-60)	Mildly elevated (20-40)	Normal to mildly elevated (0.8-1.2)	4	Pseudomonas aeruginosa, Klebsiella pneumoniae	18	Pseudomonas aeruginosa, Bordetella spp, Haemophilus influenza
COPD	Slightly elevated (10-40)	Slightly elevated (250-450)	Low (<0.25) or normal	Normal (0.5-1)	Slightly elevated (10,000-12,000)	Elevated (20-40)	Mildly elevated (20-40)	Normal (0.6-1.2)	4	Streptococcus pneumoniae, Staphylococcus aureus	16	Haemophilus influenza, Moraxella spp
LRTI	Moderate (10-100)	Elevated (300-500)	Moderate (0.25-0.5)	Slightly elevated (1-2)	Elevated (12,000-15,000)	High (20-50)	Normal to slightly elevated (20-30)	Normal (0.6-1.2)	4	Klebsiella pneumoniae, Bordetella spp	18	Streptococcus pneumoniae, Pseudomonas aeruginosa

ARDS	High (100-200)	Very high (>500)	High (>0.5)	Elevated (2-4)	Elevated (15,000-20,000)	Very high (50-70)	Elevated (30-50)	Elevated (1.2-2.0)	0	None detected	6	Streptococcus pneumoniae, Acinetobacter baumanii
URTI	Low to moderate (5-40)	Slightly elevated (200-300)	Low (<0.25)	Normal (0.5-1)	Mildly elevated (9,000-12,000)	Elevated (20-30)	Normal	Normal	0	None detected	4	Moraxella spp, Haemophilus influenza
Pneumonia	High (20-200)	Elevated (300-600)	High (>0.5)	Elevated (1.5-3)	Elevated (12,000-18,000)	High (30-50)	Elevated (30-50)	Normal to mildly elevated (0.8-1.2)	2	Klebsiella pneumoniae, Acinetobacter baumanii	10	Streptococcus pneumoniae, Bordetella spp
Bronchitis + Bronchial Asthma	Slightly elevated (5-30)	Normal to elevated (200-300)	Low (<0.25)	Normal (0.5-1)	Normal to slightly elevated (9,000-11,000)	Mildly elevated (15-30)	Normal	Normal	0	None detected	2	Staphylococcus aureus, Haemophilus influenza
Chronic Lung Disease	Normal to slightly elevated (5-20)	Normal to slightly elevated	Low (<0.25)	Normal (0.5-1)	Normal (4,500-10,000)	Mildly elevated (10-20)	Normal	Normal	0	None detected	0	None detected

		(200-300)										
Pleural Effusion	Slightly elevated (10-50)	Normal to slightly elevated (200-300)	Low (<0.25)	Normal to slightly elevated (0.5-1)	Normal to slightly elevated (6,000-10,000)	Mildly elevated (10-20)	Normal	Normal	0	None detected	0	None detected
Interstitial Lung Disease	Slightly elevated (10-50)	Elevated (300-500)	Low (<0.25)	Normal (0.5-1)	Normal to slightly elevated (6,000-10,000)	Elevated (20-30)	Slightly elevated (20-40)	Normal to mildly elevated (0.8-1.2)	2	Klebsiella pneumoniae, Pseudomonas aeruginosa	2	Moraxella spp, Haemophilus influenza

1. High CRP and PCT Correlate with Bacterial Infections

- Conditions with elevated CRP (>20 mg/L), such as Bronchopneumonia, Pneumonia, and ARDS, are strongly associated with bacterial pathogens like *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Streptococcus pneumoniae* detected in cultures or via PCR.
- Procalcitonin (PCT) is consistently high (>0.5 ng/mL) in conditions dominated by bacterial infections (e.g., Bronchopneumonia and ARDS), indicating active bacterial invasion.
- Viral or non-infectious conditions (e.g., URTI, Chronic Lung Disease) show low or normal PCT levels (<0.25 ng/mL), reflecting minimal bacterial involvement.

2. LDH and Lactate Indicate Tissue Damage Severity

- Elevated LDH (>500 U/L) is seen in severe infections such as ARDS, indicating substantial tissue damage caused by pathogens like *Acinetobacter baumannii* and *Streptococcus pneumoniae*.
- Moderately elevated LDH (300–500 U/L) in conditions like Bronchopneumonia and LRTI correlates with bacterial pathogens (*Klebsiella pneumoniae, Bordetella spp.*), suggesting localized or less severe tissue injury.
- Increased lactate levels (2–4 mmol/L) in ARDS point to metabolic stress and hypoxia, which are characteristic of severe infections.

3. Elevated WBC and ESR Reflect Systemic Inflammatory Response

- High WBC counts (>12,000 cells/µL) in Bronchopneumonia, Pneumonia, and ARDS correlate with active bacterial infections.

- ESR values are particularly high (>50 mm/hr) in conditions with confirmed bacterial growth, indicating prolonged or severe inflammation.
- Conditions with mild WBC and ESR elevations, such as Bronchitis + Asthma and URTI, align with viral or chronic inflammatory processes.

4. Culture Positivity Reflects Severity and Complements PCR Detection

- Culture Growth:
  - Conditions like Bronchopneumonia, COPD, and LRTI demonstrate consistent culture positivity, identifying *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*.
  - Severe conditions like ARDS showed no culture growth despite PCR positivity, suggesting the presence of hard-to-culture pathogens.
- PCR Detection:
  - PCR was particularly useful in detecting additional pathogens (*Bordetella spp.*, *Moraxella spp.*, *Haemophilus influenzae*) in conditions like URTI and LRTI, where culture results were limited.

ANTIMICROBIAL SUSCEPTIBILITY PATTERN FOR CULTURE ISOLATES

Out of 100 clinical specimens being tested, 16/100 pathogens were identified at the species level comprising of non-fermenters which include: A. baumannii (4), and P. aeruginosa, (2). Enterobacterales which includes K. pneumoniae (4), E. cloacae (2), and Gram-positive cocci which includes Pneumococci (2)

DETAILED REPORT ON ANTIMICROBIAL SUSCEPTIBILITY AND COINFECTION ANALYSIS IN THE CONTEXT OF COVID-19

In a comprehensive study involving various bacterial isolates and their susceptibility patterns, along with an examination of coinfection rates with respiratory pathogens in COVID-19 patients, several key insights have emerged.

1. Antimicrobial Susceptibility Patterns:

Non-Fermenters: The analysis of 16 non-fermenter isolates revealed extensive multidrug resistance. All isolates were resistant to multiple antibiotics, including CEFEPIME, CEFOPERAZONE/SULBACTAM, CEFTAZIDIME, IMIPENEM, and MEROPENEM, among others. This broad resistance pattern highlights a significant challenge in managing infections caused by these bacteria. The only antibiotic showing intermediate susceptibility was Colistin. The high level of resistance observed is potentially linked to increased antibiotic use and hospital-acquired infections, which have been exacerbated by the COVID-19 pandemic

Enterobacterales: Similarly, Enterobacterales isolates demonstrated substantial resistance to antibiotics such as CEFEPIME, MEROPENEM, and PIPERACILLIN/TAZOBACTAM. Sensitivity was observed to AMIKACIN, TRIMETHOPRIM/SULFMETHOXAZOLE, and TIGECYCLIN, while Colistin showed intermediate susceptibility. The multidrug resistance in these isolates could also be influenced by factors associated with COVID-19, including increased antibiotic usage and hospital environments conducive to the spread of resistant strains.

Pneumococci: In contrast, the single pneumococcal isolate showed a broad sensitivity to a range of antibiotics, including PENICILLIN-G, AMPICILLIN, and TETRACYCLINE, but was resistant to CEFPODOXIME. This suggests that pneumococcal infections are less affected by the resistance trends seen in other bacterial groups, providing more reliable treatment options.

2. Coinfection Analysis with Respiratory Pathogens:

The study also examined coinfection rates among COVID-19 patients: COVID-Positive Patients: Out of 20 COVID-positive patients, 14 were PCR-positive, and 6 were PCR-negative. Among these, 2 were culture-positive, and 18 were culture-negative. This data was analyzed using a chi-squared distribution with a result of $\chi^2 = 15$, yielding a p-value less than 0.0001, indicating a significant difference between the culture and PCR tests.

PCR Test Performance: The PCR test demonstrated a high sensitivity of 87.5%, accurately identifying a significant proportion of positive cases. However, its specificity was moderate at 26.2%, indicating a notable rate of false positives. The Positive Predictive Value (PPV) was low at 18.4%, suggesting that a substantial proportion of positive PCR results might be false positives. Conversely, the Negative Predictive Value (NPV) was high at 91.7%, reflecting reliable exclusion of infection in negative cases. The overall accuracy of the PCR test was reported as 36%, influenced by the high false-positive rate.

Correlation with COVID-19:

The high level of multidrug resistance observed in non-fermenters and Enterobacterales could be correlated with the increased use of antibiotics and the heightened risk of hospital-acquired infections associated with COVID-19. The significant differences observed in diagnostic testing, with a high sensitivity but moderate specificity and low PPV for PCR, suggest challenges in accurately diagnosing respiratory infections in COVID-19 patients. This underscores the need for careful interpretation of PCR results and potentially the use of complementary diagnostic methods to improve accuracy.Overall, the findings highlight the complex interplay between COVID-19 and bacterial infections, emphasizing the importance of antibiotic stewardship and the use of multi-faceted diagnostic approaches to manage and treat infections effectively.

COMPARATIVE ANALYSIS OF ANTIBIOTIC SUSCEPTIBILITY PATTERNS IN RESPIRATORY PATHOGENS AND THEIR RELATION TO SARS-COV-2

The antibiotic susceptibility patterns observed in this study reveal critical insights when compared to other recent studies on respiratory pathogens, particularly in the context of the SARS-CoV-2 pandemic.

Non-Fermenters

In this study, non-fermenters exhibited high resistance to levofloxacin, meropenem, ciprofloxacin, and ceftriaxone, with complete resistance to levofloxacin and meropenem. This is consistent with findings from an earlier study by Rawson et al. (2021), which reported increased resistance in non-fermenters such as Acinetobacter baumannii during the COVID-19 pandemic, attributed to the overuse of broad-spectrum antibiotics (Rawson et al., 2021).

Enterobacterales

The observed resistance patterns in Enterobacterales to cefoperazone/sulbactam and piperacillin/tazobactam align with results from a study conducted by Lansbury et al. (2020), which identified similar resistance trends among Enterobacterales isolated from COVID-19 patients. Their study also noted high sensitivity to amikacin and tigecycline, mirroring our findings (Lansbury et al., 2020).

Pneumococci

For pneumococci, our findings of complete resistance to cefpodoxime but high sensitivity to other antibiotics such as penicillin-G and linezolid are corroborated by a study conducted by Hsu et al. (2021). This study highlighted the preservation of pneumococcal susceptibility to beta-lactams and macrolides, despite the increased empirical use of these antibiotics during the pandemic (Hsu et al., 2021).

Relation to SARS-CoV-2

The relationship between antibiotic susceptibility patterns and SARS-CoV-2 co-infections underscores the importance of tailored antibiotic stewardship programs. As noted in the studies by Rawson et al. (2021) and Lansbury et al. (2020), the empirical use of antibiotics in COVID-19 patients has likely contributed to the observed resistance patterns. This overuse can lead to higher resistance rates, complicating the treatment of secondary bacterial infections in these patients. Comparing our results with other studies reveals a consistent trend of increased antibiotic resistance among non-fermenters and Enterobacterales, and preserved sensitivity among pneumococci to specific antibiotics. These findings highlight the critical need for precise and judicious antibiotic use, particularly in the context of the COVID-19 pandemic, to manage co-infections effectively and mitigate the escalation of antimicrobial resistance (Tzeng et al., 2021).

DISCUSSION

The findings from this study reveal a significant gender disparity in the prevalence of respiratory infections among COVID-19 patients, with a higher incidence observed in male patients (68%) compared to female patients (32%) which is statistically significant (chi-square χ^2=12.96, $p < 0.05$). This aligns with existing literature, which suggests that males are more susceptible to severe respiratory infections, potentially due to biological and behavioral factors such as higher smoking rates and occupational exposures (Bahl et al., 2021; Scully et al., 2021). Biochemical markers, including C-reactive protein (CRP) and lactate, were also found to be elevated in male patients, suggesting a stronger inflammatory response, which may contribute to the severity of infection. Elevated CRP levels are commonly associated with an acute inflammatory response, which could exacerbate respiratory conditions in males (Bahl et al., 2021).

In terms of age distribution, this study identified the highest incidence of infections in the 51–60-year age group (32%), followed by the 61–70-year group (20%) this distribution is statistically significant (chi-square χ^2=22.3988, $p < 0.05$). These findings are consistent with global trends, where older adults are more vulnerable to severe respiratory infections due to age-related decline in immune function and comorbidities (Fried et al., 2020). Older adults also exhibited higher CRP and lactate levels, indicating a more severe inflammatory response, which may contribute to poor prognosis and higher mortality in this age group (Fried et al., 2020). These findings highlight the importance of prioritizing older populations for prevention and early intervention strategies, especially during the ongoing COVID-19 pandemic.

The comparison between RT-PCR and traditional culture methods revealed a notable difference in sensitivity. RT-PCR identified bacterial pathogens in 76% of samples, while culture methods detected pathogens in only 16% of cases. The statistical significance of this difference (chi-square $\chi^2 = 82.56$, $p < 0.00001$) strongly supports the superior diagnostic capability of RT-PCR for identifying bacterial infections in COVID-19 patients. The biochemical markers, including elevated CRP and procalcitonin (PCT) levels, were often associated with bacterial co-infections, which PCR was able to detect more accurately compared to traditional culture methods. This highlights the importance of RT-PCR as a diagnostic tool for rapid and precise pathogen identification, which is crucial in managing respiratory infections in COVID-19 patients (Meyer et al., 2021).

The diversity of pathogens detected by RT-PCR, including *Pseudomonas aeruginosa, Klebsiella pneumoniae, and Bordetella spp.,* reflects the complex nature of bacterial infections in COVID-19 patients. The presence of these pathogens complicates treatment regimens and necessitates tailored antimicrobial therapies. The biochemical markers, such as elevated lactate and CRP, indicate systemic

inflammation, often seen in patients with severe bacterial infections, further justifying the use of PCR in clinical practice (Meyer et al., 2021; Lansbury et al., 2020).

Antimicrobial resistance was a key finding in this study, particularly among non-fermenters like *Acinetobacter baumannii and Pseudomonas aeruginosa*, which exhibited high levels of resistance to commonly used antibiotics such as meropenem and levofloxacin. These findings are in line with studies showing increased antibiotic resistance in hospital-acquired infections during the COVID-19 pandemic, likely due to the overuse of broad-spectrum antibiotics (Rawson et al., 2021; Tzeng et al., 2021). Biochemically, these resistant pathogens often lead to elevated levels of lactate and WBC counts, which are indicative of systemic infection and sepsis. The presence of such pathogens is associated with poor prognosis and complicates the management of infections (Rawson et al., 2021).

In contrast, Streptococcus pneumoniae isolates showed a more favorable susceptibility profile, suggesting that pneumococcal infections can still be effectively managed with standard antibiotic therapies. This finding is reflected in the biochemical data, where patients with Streptococcus pneumoniae infections often had lower inflammatory markers such as CRP and lactate, indicating a less severe infection (Hsu et al., 2021). This highlights the importance of accurate pathogen identification and antimicrobial susceptibility testing in guiding treatment decisions, particularly in the context of antibiotic resistance.

The study found that a significant proportion of COVID-19 patients had bacterial co-infections, with Pseudomonas aeruginosa, Klebsiella pneumoniae, and Bordetella spp. being the most common pathogens detected. The high sensitivity (87.5%) and moderate specificity (73.8%) of the PCR test in identifying bacterial co-infections suggest that while PCR is highly effective in detecting pathogens, false positives may occur. Biochemical markers, particularly elevated CRP and PCT levels, support the presence of bacterial infection, but they also highlight the need for careful interpretation of PCR results to avoid overdiagnosis (Lansbury et al., 2020). The low Positive Predictive Value (PPV) of 18.4% suggests that a substantial number of positive PCR results may not correspond to true infections, underscoring the need for corroborative clinical and laboratory assessments (Lansbury et al., 2020). The biochemical markers such as lactate and CRP levels can provide important clinical context to interpret PCR results accurately and guide treatment decisions.

Biochemical markers such as CRP, LDH, and PCT levels play a vital role in diagnosing the severity of infections and assessing the risk of complications such as sepsis or ARDS. Elevated CRP and LDH levels are indicative of a robust inflammatory response, often seen in bacterial co-infections in COVID-19 patients, and correlate with poor clinical outcomes. The study found that patients with high CRP and lactate levels often required more intensive care, highlighting the relationship between biochemical markers and clinical outcomes in bacterial co-infections (Hsu et al., 2021). Elevated lactate levels are particularly significant in sepsis, where tissue hypoxia and organ dysfunction occur, necessitating rapid therapeutic interventions (Tzeng et al., 2021).

In conclusion, the integration of biochemical and microbiological data enhances our understanding of the complex nature of respiratory infections in COVID-19 patients. Elevated CRP, PCT, and lactate levels provide critical information on the inflammatory and immune response, while PCR testing offers precise pathogen identification. Together, these diagnostic modalities can guide clinical decision-making, ensuring timely and appropriate treatment for bacterial co-infections in COVID-19 patients.

CONCLUSION:

This study provides valuable insights into the complex interplay between COVID-19 and bacterial co-

infections, highlighting the significant role of both biochemical markers and microbiological diagnostics in the clinical management of these patients. Our findings underscore the heightened risk of severe respiratory infections in male patients and older adults, aligning with existing research on age- and gender-related vulnerabilities. The superior sensitivity of RT-PCR over traditional culture methods in detecting bacterial pathogens emphasizes its critical role in rapid and accurate diagnosis, allowing for more targeted antimicrobial therapy.

Additionally, the antimicrobial resistance patterns observed, particularly in Acinetobacter baumannii and Pseudomonas aeruginosa, highlight the ongoing challenges of managing hospital-acquired infections during the COVID-19 pandemic, exacerbated by overuse of broad-spectrum antibiotics. Biochemical markers such as CRP, lactate, and PCT are essential in assessing the severity of infection and guiding treatment decisions, as they correlate with poor clinical outcomes, especially in patients with bacterial co-infections.

Overall, this study emphasizes the importance of an integrated diagnostic approach that combines molecular, biochemical, and microbiological data to improve patient outcomes. As COVID-19 continues to present significant challenges globally, further research is needed to refine diagnostic and therapeutic strategies, particularly in vulnerable populations, to mitigate the impact of bacterial co-infections and improve clinical management during and beyond the pandemic.

Limitations Of The Study

This study has several limitations. First, the sample size was small, which may limit the generalizability of the findings. Additionally, as a retrospective study, it is subject to selection bias and incomplete data, which could affect the results. The biochemical markers used (CRP, PCT, lactate) are not specific to bacterial infections, which may complicate the interpretation of the data, especially in the context of viral infections. The study also focused on a limited set of pathogens, which may not fully represent the broader spectrum of antimicrobial resistance. Moreover, the lack of longitudinal follow-up limits our understanding of the long-term impact of bacterial co-infections in COVID-19 patients. Finally, the regional and temporal scope of the study may reduce the applicability of the findings to other regions or healthcare settings. Further large-scale, multi-canter studies are needed to validate these results and improve clinical management strategies for co-infected COVID-19 patients.

CONFLICT OF INTEREST

All authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that no other relationships or activities could appear to have influenced the submitted work. Hence all authors declare no interest in conflicts in the submitted journal.

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