Section: Miscellaneous

Original Research Article

COMPREHENSIVE DETECTION OF VIRAL AND BACTERIAL ETIOLOGIES IN ADULT ACUTE RESPIRATORY TRACT INFECTIONS USING MULTIPLEX REAL-TIME PCR AND CONVENTIONAL CULTURE IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Acute respiratory tract infections (ARTIs) are a major global health burden, particularly in low- and middle-income countries. Accurate and timely identification of causative pathogens is essential for effective clinical management and infection control. This study aimed to comprehensively evaluate the viral and bacterial etiologies of ARTIs in adult patients using multiplex real-time PCR alongside conventional culture methods in a tertiary care hospital in India.

Materials and Methods: A cross-sectional study was conducted on 135 adult patients presenting with clinical symptoms of ARTI. Throat swabs were collected and subjected to both conventional bacterial culture and multiplex PCR using the Fast Track Diagnostics Respiratory Pathogen 21 PLUS kit (BioMérieux, Luxembourg), targeting 21 respiratory pathogens. Standard microbiological techniques and antibiotic susceptibility testing were employed for culture-positive samples.

Results: Multiplex PCR demonstrated high sensitivity in detecting a broad range of pathogens, including Influenza A (H1N1), Influenza B, RSV, Rhinovirus, Human Coronaviruses, and Parainfluenza viruses, as well as bacterial agents such as Streptococcus pneumoniae, Haemophilus influenzae, and Staphylococcus aureus. Co-infections involving multiple viral or viral-bacterial combinations were frequently observed. The majority of patients were inpatients (94%) with varying comorbidities. Seasonal variations and departmental distribution were also analyzed.

Conclusion: The integration of multiplex PCR with conventional methods significantly enhances diagnostic yield in ARTIs, providing rapid and comprehensive pathogen detection. The findings highlight the burden of mixed infections and the need for molecular diagnostics in guiding targeted therapy, reducing empirical antibiotic use, and supporting infection control practices. This study contributes valuable epidemiological insights into adult ARTIs in a tertiary care setting.

Keywords: Acute Respiratory Tract Infections (ARTIs), Multiplex Real-Time PCR, Respiratory Pathogens, Co-infection, Molecular Diagnostics.

INTRODUCTION

Acute respiratory tract infections (ARTIs) are one of the primary causes of illness and mortality globally, particularly in low- and middle-income countries. They account for a sizable number of hospital visits, especially among the elderly, immunocompromised people, and those with pre-existing medical

disorders.[1] ARTIs can cause moderate upper respiratory tract symptoms as well as severe lower respiratory tract infections such pneumonia, bronchitis, and bronchiolitis.[2] ARTIs are caused by a variety of viruses and bacteria, and identifying the causative pathogen is crucial for optimal clinical care, infection control, and epidemiological surveillance.[3] Traditionally, ARTIs have been diagnosed primarily using culture-based techniques, microscopy, and antigen detection assays. While these approaches are still useful, they have inherent drawbacks, such as limited sensitivity, lengthy turnaround times, and the inability to detect fastidious or non-culturable organisms.[4] Furthermore, many respiratory diseases, particularly viruses, have similar clinical presentations, making it difficult to discriminate between distinct etiologies based just on symptoms. This diagnostic uncertainty frequently results in the overuse or misuse of antibiotics, adding to the growing issue of antimicrobial resistance.[5] Recent advances in molecular diagnostics, notably multiplex real-time polymerase chain reaction (PCR), have transformed the field of respiratory infection identification. These assays enable the simultaneous identification of numerous viral and bacterial agents from a single clinical material with excellent sensitivity, specificity, and quick turnaround time.[6] Multiplex PCR has emerged as a helpful method for early and reliable identification of respiratory infections, allowing clinicians to apply prompt and tailored treatments. Furthermore, it sheds light on co-infection patterns, which are widely acknowledged as major factors to disease severity and consequences.[7]

India, like many developing countries, bears a significant burden of ARTIs, yet comprehensive data on the etiological spectrum of respiratory infections utilizing molecular diagnostics are scarce.[8] Most prior research have focused on pediatric groups or used traditional methodologies that do not cover the entire range of respiratory infections.[9] Adult patients, particularly those with comorbidities or hospitalized for other illnesses, are a susceptible population that is frequently underrepresented in respiratory monitoring programs.[10] A better understanding of the infections common in this population is critical for refining diagnostic techniques, guiding empirical treatment, and improving patient outcomes.[11] In this respect, the current study was conducted to evaluate the range of viral and bacterial pathogens responsible for acute respiratory tract infections in adult patients at a tertiary care hospital. We used a dual diagnosis strategy, combining classic culture methods with multiplex PCR with the Fast Track Diagnostics Respiratory Pathogen 21 PLUS kit (BioMérieux, Luxembourg). This allowed for the detection of a wide range of respiratory viruses, including Influenza A (including H1N1), Influenza B, Respiratory Syncytial Virus (RSV), Rhinovirus, Parainfluenza viruses, Human Metapneumovirus, and Coronaviruses, as well as bacterial pathogens like Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, and Chlamydia pneumoniae. This study also sought to examine the demographic profiles, seasonal trends, and co-infection patterns of individuals presenting with ARTI. The findings are expected to add to the epidemiological understanding of respiratory infections in the region, aid in evidence-based clinical decision-making, and assist hospital infection control programs. Furthermore, by comparing the diagnostic yield of multiplex PCR to traditional culture methods, the study emphasizes the importance of molecular diagnostics in improving pathogen identification and determining the future of infectious illness diagnosis.

MATERIALS AND METHODS

Study Design and Population

This cross-sectional investigation was carried out at a tertiary care hospital during a set length of time. A total of 135 patients with clinical symptoms indicating acute respiratory tract infections (ARTIs) were included. Patients were selected from a variety of departments, including pulmonology, medicine, respiratory medicine, cardiology, nephrology, gastrointestinal, ENT, and neurology. Outpatient and inpatient cases were both considered, with inpatients accounting for the vast majority (94%).

Specimen Collection

Throat swabs were collected aseptically from all patients using sterile swabs and transferred immediately into 3 mL of HIMEDIA's HiViral™ Viral Transport Medium (VTM). Samples were labeled and transported under cold chain conditions to the molecular diagnostics laboratory for further processing.

Conventional Bacterial Culture and Identification

Samples were initially inoculated on blood agar and MacConkey agar plates and incubated aerobically at 37°C for 24–48 hours. Suspected colonies were further identified using standard microbiological techniques:

- **Streptococcus pneumoniae**: Identified by alpha-hemolytic colonies on blood agar, Gram-positive diplococci with capsule, optochin sensitivity (≥14 mm zone of inhibition), and bile solubility. Inulin fermentation was also tested.
- **Staphylococcus aureus**: Identified by golden yellow colonies, positive catalase and coagulase tests, and mannitol fermentation.
- **Haemophilus influenzae**: Growth dependent on X and V factors, confirmed by satellitism test.
- **Pseudomonas aeruginosa**: Identified by characteristic pigment production and positive oxidase test.
- Other isolates such as Acinetobacter baumannii, Klebsiella pneumoniae, and Escherichia coli were identified based on colony morphology,

Gram staining, and standard biochemical reactions.

Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar according to CLSI guidelines.

Nucleic Acid Extraction

Nucleic acid extraction was performed using the QIAsymphony® SP automated system (QIAGEN, Germany). Samples were loaded into the sample rack along with internal control (IC) preparation, consisting of carrier RNA, molecular biology grade water, and buffer AVE.

Each extraction batch included:

- Sample prep cartridges
- Magnetic particles
- Lysis, wash, and elution buffers
- Proteinase K
- 8-rod covers and reagent troughs

The four-step extraction protocol (lyse, bind, wash, and elute) yielded purified RNA in elution tubes after approximately one hour.

Multiplex PCR for Respiratory Pathogen Detection

The Fast Track Diagnostics Respiratory Pathogen 21 PLUS kit (Fast Track Diagnostics, BioMérieux, Luxembourg) was used for detection of respiratory pathogens. The panel included 21 viral and bacterial targets grouped across six primer-probe mixes:

- **Viruses detected**: Influenza A (including H1N1), Influenza B, RSV A & B, Rhinovirus, Human Coronavirus (229E, OC43, NL63, HKU1), Parainfluenza viruses 1–4, Human Metapneumovirus A & B, Adenovirus, Human Bocavirus, Enterovirus, Parechovirus.
- **Bacteria detected**: Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Chlamydia pneumoniae, Mycoplasma pneumoniae.

Each PCR reaction included 12.5 µL buffer, 1 µL enzyme, 1.5 µL primer-probe mix, and 10 µL of extracted template RNA. Amplification was carried out using the Rotor-Gene Q real-time thermocycler (QIAGEN). Cycling conditions were:

- Reverse transcription: 42°C for 15 minutes
- Initial denaturation: 94°C for 3 minutes
- 40 cycles of denaturation at 94°C for 8 seconds and annealing/extension at 60°C for 34 seconds

A specimen was considered **positive** when the cycle threshold (Ct) value was less than 36.

Data Analysis: All microbiological and molecular results were compiled and analyzed using Microsoft Excel. Demographic variables such as age, sex, department of admission, and inpatient/outpatient status were evaluated in relation to pathogen detection. Descriptive statistics were used to assess distribution patterns and frequency of infections.

RESULTS

A total of 135 patients with respiratory tract infections were included in this study. The majority were male (n = 88, 65.18%), with females constituting 34.81% (n = 47). Among male patients, 57 (64.77%) were positive for respiratory pathogens, whereas 37 (78.72%) of female patients tested positive.

Age-wise Distribution:

The highest positivity rate was observed in the 56–65 years age group (82.35%), followed by the 46–55 years group (76.66%) and the 76–85 years group (69.23%). The lowest incidence (40%) was seen in the 26–35 years age group. A detailed age-wise distribution is presented in [Table 1].

Table 1: Age wise distribution of the positive cases.

S. No	AGE (in years)	No. of samples	No. of positive samples	% of positive
1.	16-25	6	4	66.66%
2.	26-35	10	4	40%
3.	36-45	16	9	56.25%
4.	46-55	30	23	76.66%
5.	56-65	34	28	82.35%
6.	66-75	26	17	65.38%
7.	76-85	13	9	69.23%
8.	Total.	135	94	

Patient Distribution

Patients were admitted across several departments, with the pulmonology department accounting for the majority of cases (62.96%). Among 85 pulmonology cases, 61 (64.89%) tested positive. Department-wise details are given in [Table 2].

Table 2: Department wise distribution of the patients

Department	No. Of cases (n=135)	No.of. Positive cases (n= 94)
Pulmonology	85 (62.96%)	61 (64.89%)
Medicine	23 (17.03%)	15 (15.95%)
Respiratory medicine	11 (8.14%)	7 (7.44%)
Cardiology	6 (4.44)%	4 (4.25%)
Nephrology	4 (2.96%)	3 (3.19%)
Gastroenterology	3(2.22%)	1 (1.06%)
HPBLT	2 (1.48%)	1 (1.06%)
ENT	1 (0.74%)	1 (1.06%)
Neurology	1 (0.74%)	1 (1.06%)
TOTAL	135	94

Diagnostic Test Results

Of the 135 samples, 86 (63.70%) were positive by multiplex PCR, which included 17 culture-positive samples. Eight samples were negative by PCR but positive by culture. Overall, 94 samples (69.62%) were positive by either method [Table 3]

Table 3: Results of Fasttrack Diagnostic Multiplex PCR respiratory panel and Bacterial culture

MPPCR	Bacterial Culture positive	BacterialCulture negative	Total
Positive	17	69	86
Negative	8	41	49
Total	25	110	135

Distribution of Pathogens Detected by Multiplex PCR: In the current investigation, an analysis of the 86 samples that tested positive by multiplex PCR revealed a high prevalence of viral infections. The majority of patients (55/86, 63.95%) tested positive for a single viral infection. Mixed viral and bacterial infections were found in 16 patients (18.60%), emphasizing the significance of co-infections in respiratory tract diseases. Six instances (6.97%) were found to have several viral infections, while seven samples (8.13%) contained only one bacterial pathogen. Two samples (2.32%) had several bacterial infections [Table 4]. These data highlight that viral infections, particularly single-virus cases, are the most common cause of respiratory tract infections in the study group, although bacterial and mixed infections, while less common, are nonetheless clinically significant.

Table 4: Distribution of Pathogens Detected by Multiplex PCR

S.NO	Pathogens	Total no.of positive samples (n=86)	% of positive samples
1.	Single virus	55	63.95%
2.	Multipleviruses	6	6.97%
3.	Single bacteria	7	8.13%
4.	Multiplebacteria	2	2.32%
5.	Mixed virus +Bacteria	16	18.60%

Distribution of Viruses Detected by Multiplex PCR: Among the 86 samples that tested positive for respiratory pathogens, a total of 61 viral pathogens were identified through multiplex PCR. The majority of cases (55/61; 90.16%) involved a single viral infection. Influenza A (H1N1) was the most frequently detected virus, accounting for 15 cases (24.59%), followed by Rhinovirus in 9 cases (14.75%). Other notable single-virus detections included Influenza A (non-H1N1) and RSV A & B, each found in 7 samples (11.47%), and Influenza B in 5 cases (8.19%). Less commonly identified viruses included Parainfluenza 3, various human coronaviruses (HKU, OC43, 229E), Adenovirus, Boca virus, Parainfluenza 4, and Human Metapneumovirus A & B. Co-infections involving two viruses (dual viral infections) were observed in 5 samples (8.19%), while one sample (1.63%) showed triple viral infection involving Influenza A, RSV A & B, and Boca virus [Table 5]. This distribution underscores the predominance of Influenza A (H1N1) in respiratory infections within the studied population.

Table 5: Distribution of viruses among positive samples

Types of virus	Name of the virus	No of samples	% of positive samples
SINGLE VIRUS (n=55)	Influenza A , H1N1	15	24.59%
	Rhinovirus	9	14.75%
	Influenza A	7	11.47%
	RSV A & B	7	11.47%
	Influenza B	5	8.19%
	Parainfluenza 3	3	4.91%
	Corona HKU	2	3.27%
	Corona OC43	1	1.63%
	Corona 229E	1	1.63%
	Adenovirus	1	1.63%
	Boca virus	1	1.63%
	Parainflu.4	1	1.63%
	Human metapneumovirus A& B	2	3.27%
Dual virus (n=5)	Influenza A,HINI, Adenovirus	1	1.63%
	Influenza A, HINI , Rhinovirus	1	1.63%
	Influenza A , RSV A & B	1	1.63%
	Influenza A, Corona NL 63	1	1.63%
	RSV A &B ,Parechovirus	1	1.63%
TRIPLE VIRUS (n=1)	Influenza A + RSV A&B+ Boca	1	1.63%
	Total	61	

Distribution of Bacteria Detected by Multiplex PCR: Out of the 86 positive samples, 9 samples (10.46%) showed bacterial etiology, as detected by the multiplex PCR panel. Among these, single

bacterial infections were identified in 7 cases (77.77%). Staphylococcus aureus was the most commonly detected single bacterial pathogen, observed in 6 out of the 9 samples (66.66%), followed by Streptococcus pneumoniae in 1 sample (11.11%). Dual bacterial infections were observed in 2 cases (22.22%). These included co-infections of Streptococcus pneumoniae with Staphylococcus aureus, and Streptococcus pneumoniae with Haemophilus influenzae, each detected in one sample [Table 6].

Table 6: Distribution of Bacteria Among Positive Samples

Types of Bacteria	Name of the Bacteria	No. of samples	% of positive samples
Single bacteria	Staphylococcus aureus	6	66.66%
	Streptococcus pneumoniae	1	11.11%
Dual bacteria	Streptococcus pneumoniae+ Staphylococcus aureus	1	11.11%
	Streptococcus pneumoniae + Haemophilus influenzae	1	11.11%

Distribution of Mixed Viral and Bacterial Infections Among Positive Cases: Out of the 135 total samples tested, 16 samples (11.85%) were positive for mixed viral and bacterial infections as detected by multiplex PCR. Among these, Influenza A H1N1 co-infected with Streptococcus pneumoniae was the most frequently identified combination, accounting for 31.25% of the mixed cases. Other notable combinations included Influenza A H1N1 with Staphylococcus aureus (12.5%), Rhinovirus with Streptococcus pneumoniae (6.25%), and Influenza B with various bacteria. A few complex co-infections involving a single virus and dual bacterial pathogens were also observed, including combinations with Streptococcus pneumoniae, Staphylococcus aureus, and Haemophilus influenzae [Table 7].

Table: 7 Distribution of Mixed Viral and Bacterial Pathogens Among Positive Samples

Types of Pathogens	Name of the virus / Bacteria	No. of samples	% of samples
Single virus + single bacteria	Influenza A, HINI, Streptococcus pneumoniae	5	31.25%
	Influenza A,H1N1, Staphylococcus aureus	2	12.5%
	Influenza A,H1N1, Mycoplasma pneumoniae	1	6.25%
	Influenza B , Streptococcus pneumoniae	1	6.25%
	Influenza B, Chlamydia pneumoniae	1	6.25%
	Rhinovirus +Streptococcus pneumonia	1	6.25%
	H.metapneumovirus A &B , Mycoplasma pneumoniae	1	6.25%
Single virus +Dual bacteria	Influenza B , Streptococcus pneumoniae, Staphylococcus aureus	1	6.25%
	Rhinovirus +Streptococcus pneumoniae + Staphylococcus aureus	1	6.25%
	Corona OC43 +Streptococcus pneumoniae+ Staphylococcus aureus	1	6.25%
	Adenovirus+ Streptococcus pneumoniae + Hemophilus influenzae	1	6.25%

Profile of Culture-Positive Bacteria and Viruses Detected by Multiplex PCR: Among the 25 culture-positive bacterial isolates, Acinetobacter baumannii was the most frequently identified pathogen, accounting for 48% of the isolates. This was followed by Klebsiella pneumoniae (24%), Haemophilus influenzae (8%), and Pseudomonas aeruginosa (8%). Less commonly detected organisms included Staphylococcus aureus (4%), Escherichia coli (4%), and Streptococcus pneumoniae (4%). In terms of viral pathogens, 84 viruses were detected from 86 cases using multiplex PCR. Influenza A H1N1 emerged as the predominant virus, accounting for 29.76% of the viral detections, followed by Rhinovirus (14.28%). Influenza A (non-H1N1) and Respiratory Syncytial Virus (RSV A & B) each contributed 11.90%, while Influenza B was detected in 9.52% of cases. Less frequently identified viruses included Parainfluenza virus type 3 (3.57%) and Coronavirus HKU-1 (2.38%). These findings underscore the dominant role of Influenza A H1N1 and Rhinovirus in respiratory tract infections within the study population.

Profile of Bacteria Detected by Both Multiplex PCR and Culture: Among the 55 bacterial pathogens identified through both multiplex PCR and culture, Streptococcus pneumoniae was the most frequently detected, accounting for 25% of the isolates. This was closely followed by Staphylococcus aureus (24%) and Acinetobacter baumannii (22%). Klebsiella pneumoniae constituted 11%, while Haemophilus influenzae made up 7% of the total. Less commonly detected organisms included Mycoplasma pneumoniae (3%), Chlamydia pneumoniae (2%), and Escherichia coli (2%) (Fig 1). These results highlight the significant burden of S. pneumoniae and S. aureus in respiratory infections, emphasizing their clinical relevance in both community and hospital settings.

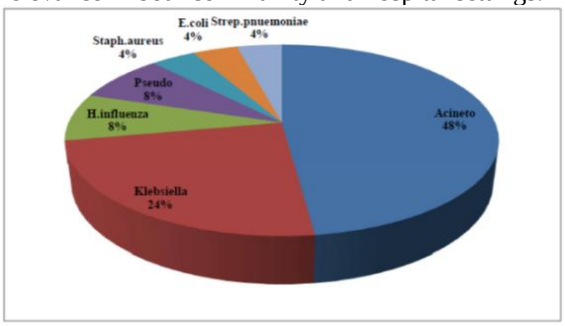

Figure 1: Profile of Bacteria detected in culture and PCR

Distribution of Viruses Among Clinical Conditions: Out of the 84 viral detections, the majority were observed in patients diagnosed with pneumonia (32 cases), followed by those with asthma (9 cases) and COPD (4 cases). Additional cases were distributed among individuals with combined COPD and pneumonia (5), COPD with HIV (2), and other miscellaneous conditions (30), which included interstitial lung disease, acute respiratory distress syndrome (ARDS), sepsis, and unexplained fever. Influenza H1N1 was the most frequently identified virus in pneumonia cases (12 out of 32) and also appeared in COPD and combined cases. Rhinovirus and RSV A & B were also commonly detected, particularly in asthma and miscellaneous cases. Rare viruses such as Boca virus were exclusively identified in pneumonia cases, while Corona OC43, 229E, NL63 and HPIV-4 were detected in only isolated cases, underscoring the wide viral diversity present in respiratory infections.

Distribution of Bacteria Among Clinical Conditions: Among the 55 bacterial detections, the highest number were identified in patients with pneumonia (11 cases), followed by those with COPD (9 cases) and COPD with pneumonia (6 cases). A notable number of detections (21 cases) were also found in patients with miscellaneous clinical conditions, such as interstitial lung diseases and systemic infections. Streptococcus pneumoniae was the most frequently observed pathogen, particularly in pneumonia and COPD+ pneumonia cases. Staphylococcus aureus and Haemophilus influenzae were also prominent, often associated with COPD and asthma. Acinetobacter baumannii, detected mainly via culture, showed a broad distribution across various conditions, including HIV-associated cases. Other notable pathogens included Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Mycoplasma pneumoniae, and Chlamydia pneumoniae. These findings highlight the polymicrobial etiology of respiratory illnesses, especially in chronic and immunocompromised individuals.

Seasonal Distribution of Respiratory Infections: The seasonal analysis of respiratory infections revealed a higher incidence during the winter months, with 77.35% (41/53) of patients testing positive. This was followed by the rainy season, during which 72.54% (37/51) of samples showed pathogen positivity. In contrast, the summer season recorded the lowest positivity rate at 51.61% (16/31). These findings indicate a clear seasonal trend, with winter and rainy seasons being more conducive to the spread of respiratory infections. However, no specific seasonal variation was noted in the distribution of individual viruses. The overall data suggests that environmental and climatic factors might influence the prevalence of respiratory pathogens, especially during colder and more humid months [Table 8].

Table 8: Seasonal distributions among the study group

S. No	Season	Total No. of patients	Total No. of positive patients	% of positive patients
1.	Winter	53	41	77.35%
2.	Summer	31	16	51.61%
3.	Rainy	51	37	72.54%
4.	Total	135	94	

Antibiotic Sensitivity Pattern: Antibiotic susceptibility testing was performed for culture-positive bacterial isolates. Among 12 isolates of Acinetobacter baumannii, 8 (66.66%) were resistant to carbapenems, indicating a high prevalence of multidrug resistance. Of the 6 Klebsiella pneumoniae isolates, 2 were identified as ESBL producers, while 3 (50%) exhibited resistance to carbapenems. One of the two Pseudomonas aeruginosa isolates (50%) was also found to be carbapenem-resistant. A single Escherichia coli isolate demonstrated resistance to both AmpC β-lactamase and carbapenems. The two Haemophilus influenzae isolates cultured were sensitive to penicillin and ceftriaxone but showed resistance to quinolones and cotrimoxazole. It is important to note that antibiotic susceptibility could not be assessed for pathogens such as Streptococcus pneumoniae (n=13), Staphylococcus aureus (n=12), and Haemophilus influenzae (n=2), which were detected by multiplex PCR but did not grow in culture.

DISCUSSION

Respiratory tract infections (RTIs) remain a major global health burden, contributing significantly to both morbidity and mortality. They encompass a broad spectrum of upper and lower tract illnesses, ranging from the common cold, pharyngitis, sinusitis, to more serious infections like pneumonia and acute bronchitis. While clinical symptoms such as fever, cough, chest pain, dyspnea, wheezing, and tachypnea often overlap, differentiating among the various etiologies remains challenging in the absence of laboratory confirmation, particularly in primary care settings.[12] RTIs are primarily viral in origin, accounting for approximately 80% of cases, with bacteria contributing to the remainder.[13] Major viral pathogens include rhinovirus, influenza viruses, respiratory syncytial virus (RSV), adenoviruses, coronaviruses, and parainfluenza viruses, while key bacterial agents include Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, and Chlamydophila pneumoniae.[14,15]

The last two decades have witnessed the emergence of several novel respiratory viruses such as Influenza A H5N1, SARS-CoV, MERS-CoV, human metapneumovirus (hMPV), human bocavirus (HBoV), and new strains of coronaviruses including NL63 and HKU1.[16] These developments, coupled with frequent outbreaks and seasonal epidemics, have underscored the need for improved diagnostic strategies. Traditional laboratory methods such as direct fluorescent antibody assays (DFA) and viral cultures, while historically useful, have limitations including slow turnaround times, need for technical expertise, and inability to detect emerging viruses without targeted reagents.[17] The advent of polymerase chain reaction (PCR), particularly real-time and multiplex PCR, has revolutionized viral diagnostics by enabling the simultaneous detection of multiple pathogens with high sensitivity and specificity.[18] Multiplex PCR assays such as the FastTrack Diagnostics Respiratory Pathogen 21 have made it feasible to detect a wide array of pathogens from a single sample, aiding in timely clinical decision-making. These molecular methods also support antimicrobial stewardship by identifying viral etiologies, thereby reducing unnecessary antibiotic use and the risk of antimicrobial resistance (AMR), while guiding antiviral therapy and improving infection control.[19]

In our study, which aimed to characterize the viral and bacterial etiology of respiratory tract infections in adults, samples including throat swabs, sputum, BAL, pleural fluid, and tracheal aspirates were analyzed using multiplex PCR and conventional culture methods. Viral pathogens were detected in 62.22% of patients, and bacterial agents were identified in 40.74% either by culture or PCR. Influenza A (H1N1) was the most frequently detected virus (29.06%), followed by rhinovirus (13.95%). Among bacteria, Streptococcus pneumoniae (25.45%) was most prevalent, followed by Staphylococcus aureus (23.63%) and Acinetobacter baumannii (21.81%). These findings are in line with other global and Indian studies. For instance, Njouom from Cameroon also reported influenza as the predominant virus (28.2%), followed by rhinovirus (17.8%).[20] In India, Koul et al detected viruses in 19.7% of adult COPD patients, primarily influenza and rhinovirus, while Mohan et al reported a 13.1% viral detection rate in similar patients.[21,22] The relatively higher detection rate in our study may be attributed to broader sampling, inclusion of immunocompromised individuals, and the use of highly sensitive multiplex PCR.

Interestingly, we observed the highest prevalence of viral infections in the 56–65 year age group (28.57%), followed by the 46–55 and 66–75 year age groups. This contrasts with pediatric studies, where children under five years tend to dominate the burden. However, studies such as those by Prasetyo et al and Njouom et al confirm a substantial prevalence of respiratory viral infections in adults, particularly between 41 and 70 years of age.[20,23]

Seasonal variation was evident in our study, with the majority of viral cases detected during winter (77.35%) and rainy seasons (72.54%), a pattern consistent with other research indicating influenza peaks in colder months and rhinovirus being more prevalent in warmer seasons.[24]

Gender distribution in our study revealed a female preponderance (78.72%) in viral detection, which contrasts with several studies that reported either male dominance or no significant difference.[25] However, Mohan et al from AIIMS also observed a higher detection rate among females.[22] Mixed infections were not uncommon. We observed viral-viral coinfection in 4.44% and virus-bacteria coinfection in 11.85% of cases, comparable to other studies that reported co-infection rates ranging from 11% to 19.8%.[26,27] Such coinfections complicate clinical presentations and may influence outcomes and treatment decisions. Notably, in three AIDS patients, we detected influenza B and dual infections involving H1N1, adenovirus, and RSV, highlighting the vulnerability of immunocompromised populations to multiple pathogens. Influenza A (H1N1) swine lineage has replaced many seasonal strains since 2009 and remains the dominant circulating virus in many regions.[28] Rhinovirus, although traditionally associated with upper RTIs, is increasingly recognized as a cause of lower RTIs and exacerbations of asthma and COPD.[29] We also noted a moderate prevalence of RSV (7.4%), mainly in older adults, aligning with its established role in elderly and immunocompromised patients.[30] Other viruses such as parainfluenza (2.96%), human metapneumovirus (2.22%), adenovirus (2.22%), and coronaviruses (4.44%) were detected at lower frequencies, consistent with global observations. Human bocavirus was detected in 1.48% of our patients, one of whom had a triple viral infection. Notably, bocavirus is rarely reported in Indian adults with respiratory illness, making our observation particularly significant.[31] Bacterial pathogens detected were both community- and hospital-acquired. Acinetobacter baumannii showed high carbapenem resistance (66.66%), and Klebsiella pneumoniae exhibited both carbapenem resistance and ESBL production in 50% of isolates. These findings underscore the growing challenge of antimicrobial resistance in respiratory infections. Interestingly, S. aureus and S. pneumoniae were detected primarily by PCR rather than culture, suggesting low bacterial loads and potential colonization rather than active infection, which warrants clinical correlation before initiating antibiotic therapy.

CONCLUSION

This study demonstrates the utility of multiplex real-time PCR in the comprehensive detection of respiratory pathogens among adults with ARTIs. A high prevalence of viral infections, particularly

H1N1 and rhinovirus, was observed, along with bacterial pathogens like Streptococcus pneumoniae and Acinetobacter baumannii. The superior sensitivity of PCR over conventional culture methods enabled the identification of coinfections and low-abundance pathogens that are often missed by traditional diagnostics. The findings highlight the importance of accurate and early pathogen identification for guiding appropriate antimicrobial or antiviral therapy, reducing unnecessary antibiotic use, and supporting antimicrobial stewardship. Seasonal variation and demographic distribution patterns provide additional insights for infection control and surveillance. Integrating multiplex PCR into routine diagnostics can significantly enhance clinical outcomes, especially in adult populations where ARTIs are under-recognized. This study underscores the need for broader implementation of molecular diagnostics in tertiary care settings in India and similar regions.

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