Int.J.Curr.Microbiol.App.Sci (2025) *14*(11): 275-290

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 14 Number 11 (2025)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2025.1411.028

Spectrum of Pathogens Causing Pneumonia by Using Multiplex PCR, Culture and Clinical History of Patients in a Tertiary Care Hospital

P. P. Vismaya[1*], Swathy Viswanath[1], M. Savitha[1], Vipin Viswanath[2], Reshmi Gopalakrishnan[3], M. P. Anjali[1], M. M. Athulya[1], Gayathri Meyana[1] and Irfana[1]

[1]Department of Microbiology, MIMS College of Allied Health Sciences Malappuram, Kerala University of Health Sciences, Kerala 673633, India
[2]Department of Molecular Biology and Transplant Immunology, Aster MIMS Hospital, Calicut, India
[3]Department of Microbiology Aster MIMS Hospital, Calicut, India
**Corresponding author*

Keywords

Pneumonia, Multiplex PCR, Culture, Clinical history, Clinical parameters

Article Info

Received:
24 September2025
Accepted:
30 October 2025
Available Online:
10 November 2025

A B S T R A C T

The incidence of various pneumonia cases (viral, bacterial and fungal) is increases as shown in studies. The study aims to identify a broad spectrum of pathogens responsible for pneumonia by employing multiplex PCR, culture methods, and clinical history analysis in patients at a tertiary care hospital. This approach seeks to assess the comparative effectiveness of multiplex PCR against traditional culture techniques. The anticipated outcomes include improved diagnostic accuracy, informed treatment strategies, and enhanced understanding of pneumonia etiology within the studied population. To correlate the findings from Multiplex PCR with the patient's clinical history and to detect the pathogens responsible for pneumonia using both Multiplex PCR and culture methods. And analyze the relationship between clinical history and laboratory parameters such as Complete Blood Count (CBC), C - reactive Protein (CRP), Erythrocyte Sedimentation Rate (ESR), and Procalcitonin (PCT). Samples such as Sputum, Broncho alveolar lavage (BAL), Bronchial wash and nasopharyngeal swabs from pneumonia patients are examined using standard bacteriological methods and Multiplex PCR assays to identify the causative organisms. The VITEK®2 Compact automatic system, manual methods and Multiplex PCR assays and parameters like CBC, CRP, ESR and PCT used for detecting the organisms causing pneumonia. Fungal pathogens are detected by SDA slant culture and LPCB techniques. During the specified period, a total of 1676 respiratory samples were processed in the hospital, with 341cases (20.34%) are positive for pneumonia. The most isolated organism were viruses (58.06%), followed by bacteria (36.65%) and then fungus (5.27%). Most prevalent viral organism isolated were Influenzae virus A H1N1, bacterial organism were *Klebsiella pneumoniae*. *Aspergillus species* were the most prevalent fungal organism. *Klebsiella pneumoniae* were the most prevalent co infecting pathogens in viral pneumonia cases. CRE be the most prevalent MDR organism. Viral infections were frequently associated with higher lymphocyte counts and elevated CRP and ESR levels, while bacterial infections showed moderate neutrophilia and a significant rise in procalcitonin (PCT), distinguishing them from viral and fungal causes. Fungal infections presented with the lowest neutrophil levels but elevated inflammatory markers such as CRP and ESR. Viral, bacterial and fungal pathogens shoes seasonal variations. This study identifies the various organisms causing pneumonia by multiplex PCR, culture and clinical history. Accurate detection is essential for effective treatment, as it enables precise diagnosis and personalized care. By identifying the causative organisms can give the proper and accurate treatment for the pneumonial infection.

Introduction

The World Health Organization (WHO) emphasizes, pneumonia is defined as an acute respiratory infection affecting the lung tissue, including the bronchi, bronchioles, and alveoli. It's the main reason of infectious diseases related deaths, lung consolidation due to the presence of typical inflammatory exudates. It can be

caused by variety of organisms including viruses, bacteria, and fungi. In children, viral infections are the major contributor, with pathogens - Respiratory syncytial virus, Rhinovirus (RV), Influenza virus (IV), Parainfluenza viruses (PIV), and Adenovirus (ADV) being frequent agents [1]

Bacterial pneumonia substantially contributes to the illness and death rates associated with pneumonia [2]

Classification of pneumonia include; Anatomical classification (based on lung involvement) are bronchopneumonia, lobar pneumonia, interstitial pneumonia and Clinical classification (based on context and patient population) are Community - acquired pneumonia (CAP), Hospital - acquired pneumonia, Ventilator - associated pneumonia, Healthcare - associated pneumonia (HCAP),Pneumonia in elderly patients and Pneumonia in immuno compromised individuals.[3]

Pneumonia is typically attributed to specific bacteria, including: *Streptococcus pneumonia, Haemophilus influenza, Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa*. The main reason for fungal pneumonia are *Pneumocystis, Cryptococcus* and *Aspergillus species*.[4] These fungi are ubiquitous in the environment, found in the air, soil, and healthcare settings such as hospital

Identifying the precise cause of pneumonia can be difficult due to the overlapping symptoms shared by different organisms, both viruses and bacteria. To overcome this, using multiplex real - time PCR assays can be beneficial for rapid and accurate identification of the underlying cause.[5] Multiplex PCR assays enable the identification of multiple viral and bacterial pathogens in a single reaction, delivering results with high sensitivity and specificity within a few hours.[6] And also culture, CBC, ESR, PROCALCITONNIN and CRP are done.

Aim of the study to detect the spectrum of microorganisms behind pneumonia by using multiplex PCR, culture and clinical history of patients in tertiary care hospital.

Materials and Methods

Specimen

Sputum
Bronchio alveolar lavage
Bronchial wash
Nasopharyngeal swabs

Specimen processing

Patient clinical history was collected (suspecting pneumonia). Specimens were obtained in appropriate, sterile containers using standard precautions, properly labeled, and transported to the microbiology and molecular biology laboratories Aster MIMS Calicut. Bacterial cultures were inoculated onto suitable media and incubated at **37 °C** for 48 hours. Bacterial identification was carried out using standard microbiological techniques, including both manual methods and the Vitek 2 Compact system. Viral identification was performed by multiplex PCR assays.

Results and Discussion

The study was conducted in the Department of Microbiology and Molecular diagnostic laboratory during the time period of January 2024 to December 2024**.** Total 1676 respiratory samples were taken, out of which 341 were confirmed pneumonia cases (20.34%).

Total incidence of pneumonia

Total incidence of infectious agents among pneumonia patients

Total incidence of agents causing pneumonia are 341.Most isolated species are viruses 58.06% (n=198), and second most one are bacteria 36.65% (n=125) and then funguses 5.27% (n=18).

Distribution of pathogens in pneumonia patients

Total 341 pneumonia causing organisms were isolated. Out of which 198 were virus, 125 were bacteria and 18 fungus. Out of total virus isolated 30.79% are Influenza A H1N1 viruses (n=105), 9.09% are SARS COVID 2(31), Influenza A H3N2 with a rate of 6.15% (n=21), Influenza B with a rate of 4.98% (n=17), RSV with a rate of 4.69% (n=16). A total of 125 bacteria are isolated, out of which most isolated species are *Klebsiella pneumoniae* with a rate of 19.35% (n=66), followed by *Pseudomonas aeruginosa* with a rate of 7.33% (n=25), *Acinetobacter baumannii* with a rate of5.27% (n=180). Out of 18 funguses most isolated fungus are *Aspergillus*

species with a rate of 3.81% (n=15) and then *Penicillum species* with a rate of 1.46% (n=3).

Multi drug resistant organisms

Among 125 bacterial isolates 31 were identified as multidrug - resistant (24.8%). The most common MDR organism are: Carbapenem resistant Enterobacteriaceae (CRE) 54.83%, Methicillin resistant *Staphylococcus aureus* (MRSA) 19.35%, Carbapenem resistant *Acinetobacter baumannii* (CRAB) 16.12% and Carbapenem resistant *Pseudomonas aeruginosa* (CRPA) 9.67%.

Distribution of neutrophil and lymphocyte counts in patients with pneumonia

Out of 341 pneumonia patients 97.56% had neutrophilia (total=246), 2.43% with neutropenia (total=246), 62.5%patients with lymphocytosis (total=192) and 24.34% with lymphocytopenia (total=192).

Bacterial co - infections with viral pneumonia

In this viral pneumonia associated with bacterial co - infections. Among the isolated species *Klebsiella pneumonia* were the predominant. Followed by *Pseudomonas aeruginosa* and *Acinetobacter baumannii* and Streptococcus pneumonia.

Elevated ESR level in pneumonia

In viral, bacterial and fungal pneumonia had elevated ESR level. Virus with a rate of 78.53%, bacteria with a rate of 17.07% and fungal with a rate of 4.39%.

Elevated CRP level in pneumonia

In this elevated serum C - reactive protein (CRP) levels were observed across patients with viral (58.73%), bacterial (36.5%), and fungal (4.76%) pneumonia patients.

Elevated procalcitonin level in pneumonia

PCT level only significant in bacterial pneumonia. PCT level high in bacterial pneumonia with a rate of 13.48%. No values are obtained in the viral and fungal pneumonia cases.

Gender Wise Distribution

In viral pneumonia out of 198 patients, with a rate of 52.02% are male and 47.97% are female. In bacterial pneumonia out of 125 patients, with a rate of 65.6% are male and 34.4% are female. Out of 18 fungal pneumonia cases equal distribution in male and female with a rate of 50%.

Age Wise Distribution

According to age wise distribution of infections all infections (viral, bacterial, fungal) are peak at age of 60 to 80 age group. Additionally viral infections increased in 1 to10 age group (i.e., children's). Most predominant infections are viral and bacterial than fungus.

Month Wise Distribution

In this study viral infections peak at May, June and July. Bacterial infection are peak at June, August and November. Fungal infections are peak at May.

The study highlights the distribution of pathogens in pneumonia patients, with viruses being the most prevalent, followed by bacteria and fungi. Total 1,676 respiratory samples examined, 341 organisms were found to cause pneumonia, constituting 20.34% of the total. According to Vijaydeep Siddharth *et al.,* total 365 patients were tested, of which 29.58% (n=108) were found to be positive cases [130]

Among the 341 pneumonia patients included in this study, 198 (58.23%) had viral infections, including Influenza A H1N1 (30.79%) most prevalent. 125 (36.65%) had bacterial infections, most prevalent bacteria was *Klebsiella pneumonia* (19.35%) and 18 (5.27%) had fungal infections Aspergillus species (3.81%) being the most predominant fungus. Similar study was done by Jamila Nambafu *et al.,* according to their study 48% had viral infections, including influenza A (24%).[131]Similar study of Srujana Mohanty *et al.,* a total of 18,731 samples were processed 456 (2.43%) were culture positive for Aspergillus species [132]. According to Naif A Jalal *et al.,* study Out of 61,027 isolates found during the study period, 9014 (14.7%) were *K.pneumonia*[133]

In the present study, SARS - CoV - 2 was identified in 31 cases (9.09%), Influenza A H3N2 in 21 cases (6.15%),

Influenza B in 17 cases (4.98%), Respiratory Syncytial Virus (RSV) in 16 cases (4.69%), and Adenovirus, Human Metapneumovirus A & B, and Human Parainfluenza Virus 3a were each detected in 2 cases (0.58%). Additionally, Human Parainfluenza Viruses 1& 2 and Cytomegalovirus (CMV) were each identified in 1 case (0.29%).

The study of Hirawati Deval *et al.,* shows Parainfluenza Virus (PIV) 105 cases (11.13%),Adenovirus (HAdV) 82 cases (8.7%), Respiratory Syncytial Virus B (RSV - B) 68 cases (7.21%), Influenza A 46 cases (4.9%), including H1N1 (29) and H3N2 (14), SARS - CoV - 228 cases (3%), HumanMetapneumovirus (hMPV) 13cases (1.4%), *RSV* - A 4 cases (0.42%), *Influenza B* (Victoria lineage) 1 case (0.10%).[134] The present study, 125 bacterial pathogens were isolated from patients with pneumonia. The most prevalent bacteria identified was *Klebsiella pneumoniae*, detected in 66 cases (19.35%). Other bacterial pathogens identified included *Pseudomonas aeruginosa*: 25cases (7.33%), *Acinetobacter baumannii*: 18cases (5.27%), *Staphylococcus aureus*: 7cases (2.05%), *MRSA*: 6cases (1.75%) *Haemophilusinfluenzae*: 2 cases (0.58%), *Streptococcus pneumoniae*: 2cases (0.58%).

Naif A Jalal *et al.,* study states that out of 61,027 isolates found during the study period, 9014 (14.7%) were *K. pneumonia* [135]. Weijian Yang *et al.,* study mentioned a total of 1489 strains among these accounted for *Acinetobacter baumannii* was 461 strains (30.96%*), Pseudomonas aeruginosa* was 242 strains (16.25%).[136]

In this study Out of 125 bacterial isolates, 31 were classified as multidrug - resistant (24.8%). The distribution of MDR organisms was as follows: Carbapenem - resistant Enterobacteriaceae (CRE) accounted for 54.83%, Methicillin - resistant *Staphylococcus aureus* (MRSA) 19.35%, Carbapenem - resistant *Acinetobacter baumannii* (CRAB) 16.12%, and Carbapenem - resistant *Pseudomonas aeruginosa* (CRPA) 9.67%. Similar study was done by *Zhao - Ya Fan et al.,* their study mentioned a t total of 7,841 pathogen strains were identified, of which 2,127 (27.13%) were classified as multidrug - resistant organisms (MDROs). The distribution of these MDROs was as follows: CRAB accounted for 63.74% (958 strains), MRSA 46.37% (460 strains), CRPA 24.87% (334 strains), and CRE 13.14% (370 strains) [137]

In the present study out of 341 pneumonia patients 51.31% had neutrophilia (n=175), 4.39% with neutropenia (n=15), 48.68%patients with lymphocytosis (n=83) and 24.34% with lymphocytopenia (n=166). According *to* Prashant Yadav *et al.,* study, the findings revealed that 97% of the patients had neutrophil counts exceeding 70%, with a mean differential leukocyte count (DLC) of 85.46 ± 6.25%.[138]According to Susanne E. Doeleman *et al.,* studies involving 149patients hospitalized for community - acquired pneumonia (CAP) found that 30.2% (n=45) had lymphocyte counts below normal range[139]

In the present study *Klebsiella pneumonia* was the leading bacterial pathogen found in viral pneumonia cases with co - infections, followed by *Pseudomonas aeruginosa*, *Acinetobacter baumannii,* and *Streptococcus pneumoniae*. According to Mitra Kar *et al.,* study states that most commonly isolated bacterial microorganisms were *Klebsiella pneumoniae* and *Acinetobacter baumannii* followed by *Pseudomonas aeruginosa*[140]

In the present study elevated CRP and ESR levels were observed in all types of pneumonia—viral, bacterial, and fungal—while a rise in PCT was specific to bacterial infections. This result matched findings of Meili *et al.,* [141] and Esposito *et al.,* [142] who stated that PCT but not CRP was found to be specific for bacterial infection.

According to El Wakeel *et al.,* study an elevated ESR than both the viral pneumonia and bacterial pneumonia groups [143]

This present study observed a greater prevalence of viral and bacterial pneumonia among males than females, whereas fungal pneumonia cases were equally distributed between genders. According to Aliya Naheed *et al.,* study found that a higher proportion of hospitalized females had very severe pneumonia compared to males.[144] In the study of Rafat *et al.,* [145] in positive cases there was no significant difference in the prevalence of fungal elements isolated from symptomatic patients hospitalized in pulmonary units between the genders.

Table.1 Total incidence of pneumonia

Total samples	Pneumonia patients	%
1676	341	20.34

Table.2 Total incidence of infectious agents among pneumonia patients

Infectious agents	Total No	%
Virus	198	58.06
Bacteria	125	36.65
Fungus	18	5.27
Total	341	

Table.3 Distribution of pathogens in pneumonia patients

Pathogens	Total No	%
Influenza virus A H1N1	105	30.79
SARSCOVID2	31	9.09
Influenza virus A H3N2	21	6.15
Influenza B	17	4.98
Respiratory Syncytial Virus	16	4.69
Adenovirus	2	0.58
Humanmetapneumo virus A&B	2	0.58
Humanparainfluenzae3a	2	0.58
Humanparainfluenzae1&2	1	0.29
CMV	1	0.29
Klebsiella pneumoniae	66	19.35
Pseudomonas aeruginosa	25	7.33
Acinetobacter baumannii	18	5.27
Staphylococcus aureus	7	2.05
MRSA	6	1.75
Haemophilus influenzae	2	0.58
Streptococcus pneumoniae	2	0.58
Aspergillus species	13	3.81
Penicillium species	5	1.46

Table.4 Multidrug resistant organisms

	Number(n)	Rate (%)
CRAB	5	16.12
CRE	17	54.83
CRPA	3	9.67
MRSA	6	19.35

CRAB - Carbapenem resistant *Acinetobacter baumannii*
CRE - Carbapenem resistant Enterobacteriaceae
CRPA - Carbapenem resistant *Pseudomonas aeruginosa*
MRSA - Methicillin resistant *Staphylococcus aureus*

Table.5 Distribution of neutrophil and lymphocyte counts in patients with pneumonia

	Neutrophils (n=341)		Lymphocytes (n=341)	
	Neutrophilia	Neutropenia	Lymphocytosis	Lymphocytopenia
Total Cases (N)	240	6	120	**72**
%	**97.56**	**2.43**	**62.5**	**37.5**

Table.6 Bacterial co - infections with viral pneumonia

Bacterial isolates	Number
Acinetobacter baumannii	**3**
Escherichia coli	**1**
Haemophilus influenzae	**1**
Klebsiella pneumoniae	**4**
Pseudomonas aeruginosa	**3**
Streptococcus pneumoniae	**2**
Staphylococcus aureus	**1**

Table.7 Elevated ESR level in pneumonia

Pathogen	Total No of Elevated ESR cases (n=205)	%
Virus (n=198)	161	**78.53**
Bacteria (n=125)	35	**17.07**
Fungus (n=18)	**9**	**4.39**

Table.8 Elevated CRP level in pneumonia

Pathogen	Total No of Elevated CRP	%
Virus(n=198)	185	**58.73**
Bacteria(n=125)	115	**36.50**
Fungus(n=18)	**15**	**4.76**

Table.9 Elevated Procalcitonin level in pneumonia

Pathogens	Elevated PCT level	%
Virus	0	**0**
Bacteria	46	**13.48**
Fungus	**0**	**0**

Table.10 Gender wise distribution

	Virus	%	Bacteria	%	Fungus	%
Male	103	52.02	82	65.6	9	**50**
Female	**95**	**47.97**	**43**	**34.4**	**9**	**50**

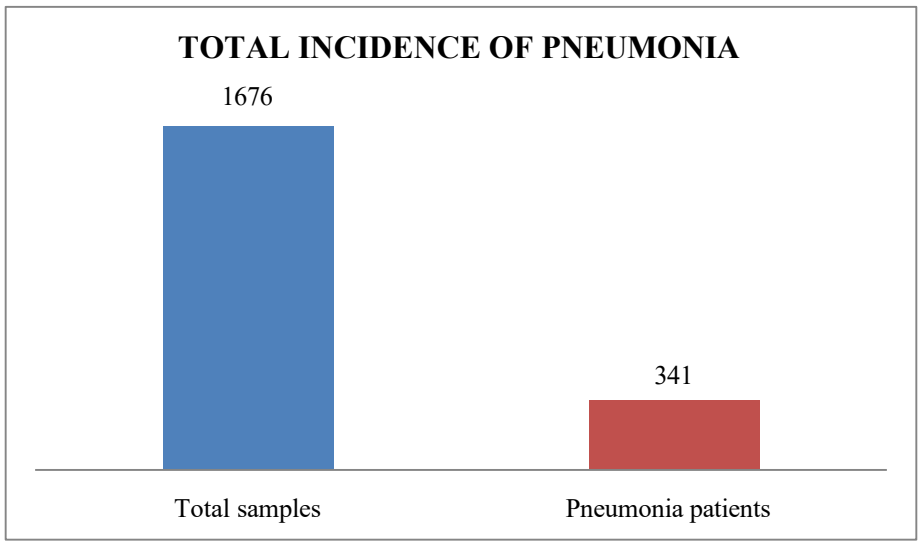
Table.11 Age wise distribution

Age	Virus	Bacteria	Fungus
1 - 10	25	-	**1**
11 - 20	4	4	**-**
21 - 30	15	5	**-**
3140	21	8	**-**
41 - 50	21	15	**6**
51 - 60	30	18	**3**
61 - 70	26	38	**5**
71 - 80	38	34	**3**
81 - 90	16	4	**-**
91 - 100	**2**	-	**-**

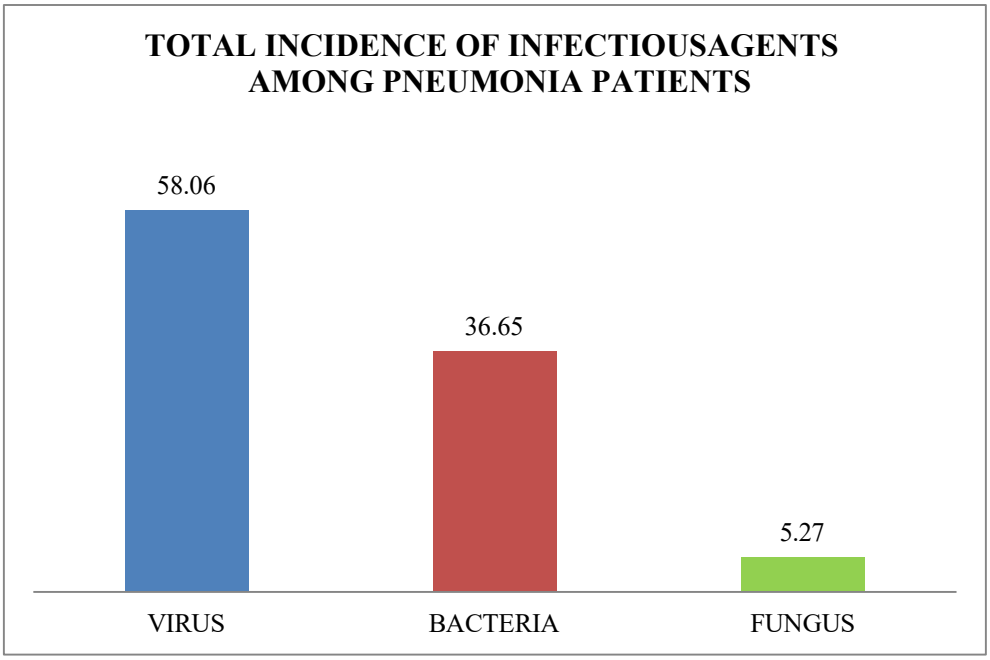
Table.12 Month wise distribution

Month	Virus	Bacteria	Fungus
January	11	11	**2**
February	15	10	**3**
March	21	13	**3**
April	17	10	**1**
May	15	5	**3**
June	31	13	**1**
July	29	9	**1**
August	13	14	**1**
September	12	8	**0**
October	11	7	**1**
November	10	13	**1**
December	**13**	**9**	**1**

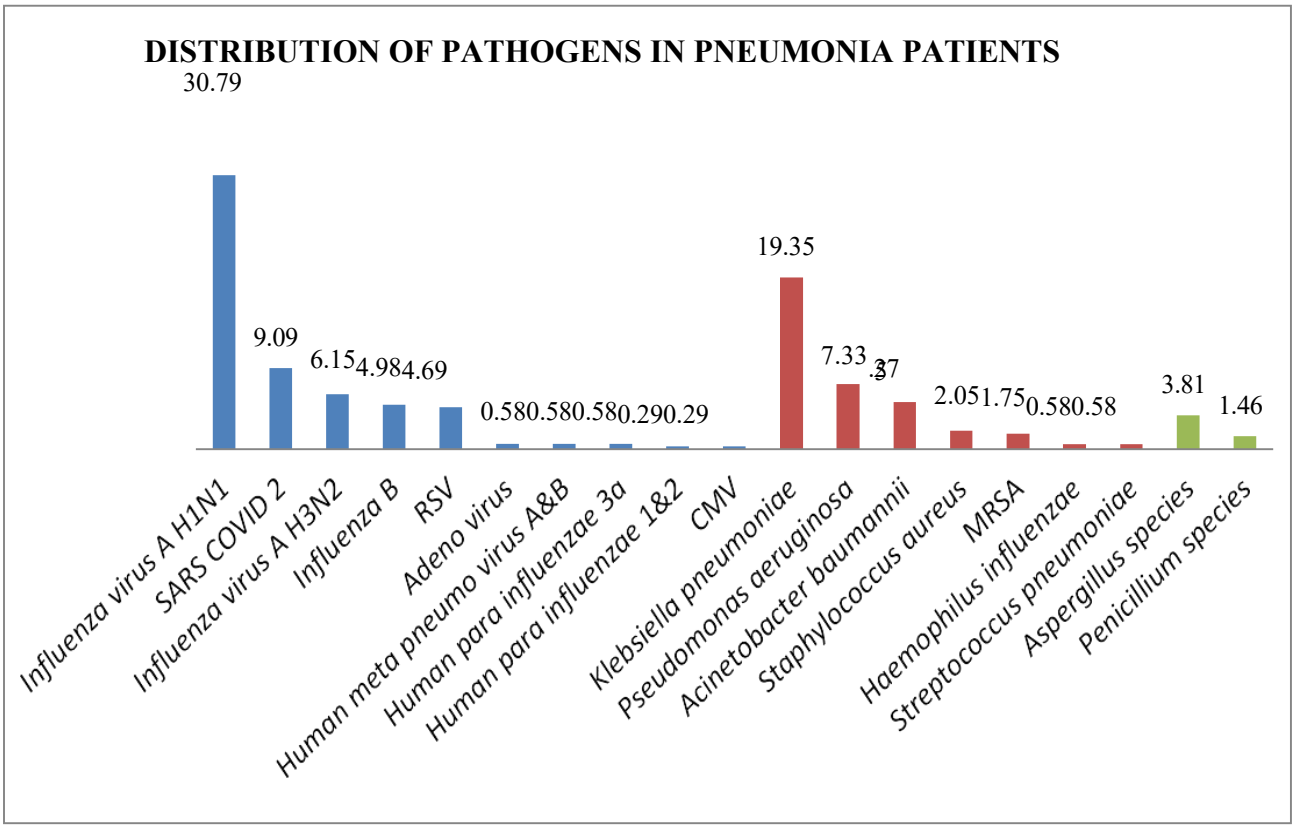
Graph.1 Total incidence of pneumonia



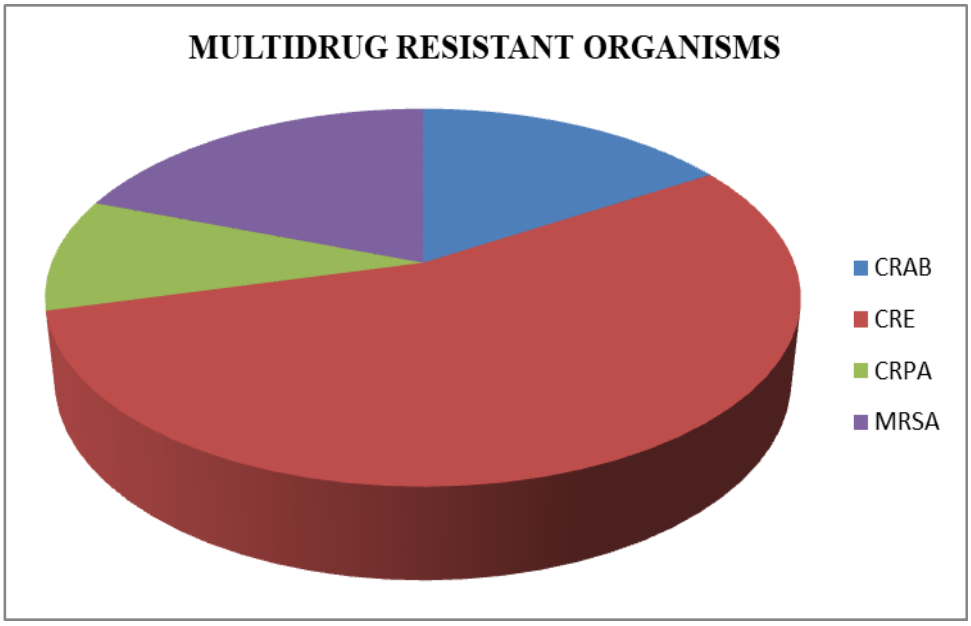
Graph.2 Total incidence of infectious agents among pneumonia patients



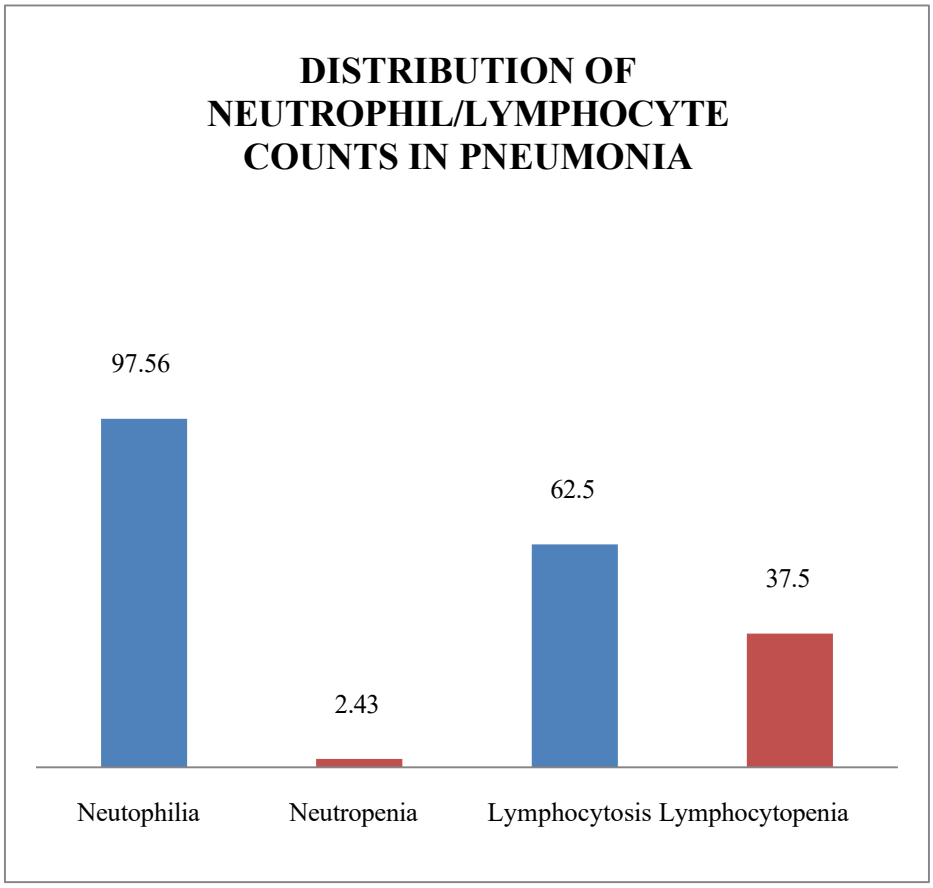
Graph.3 Distribution of pathogens in pneumonia patient



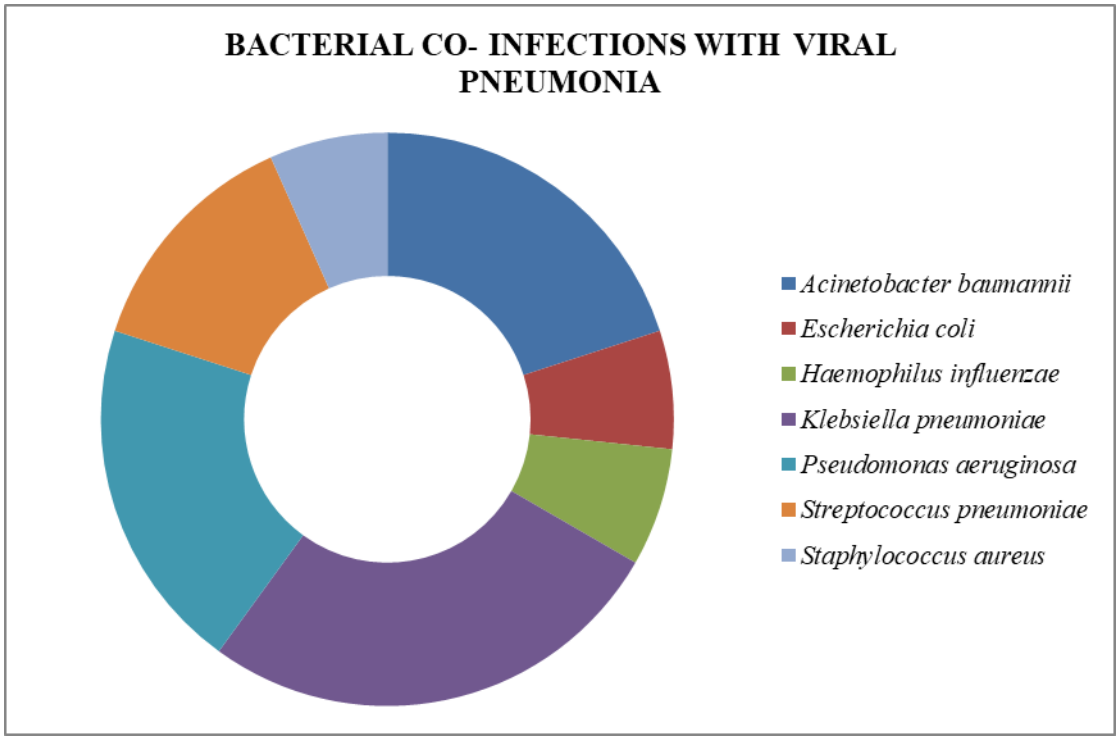
Graph.4 Multidrug resistant organisms



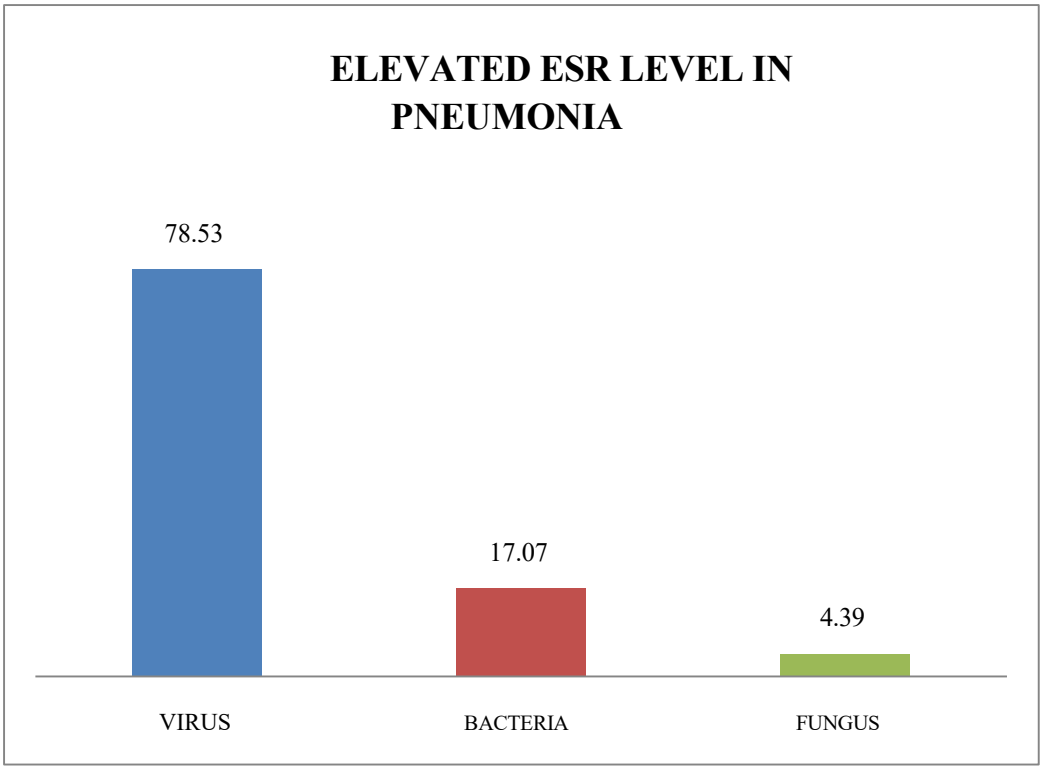
Graph.5 Distribution of neutrophil and lymphocyte counts in patients with pneumonia



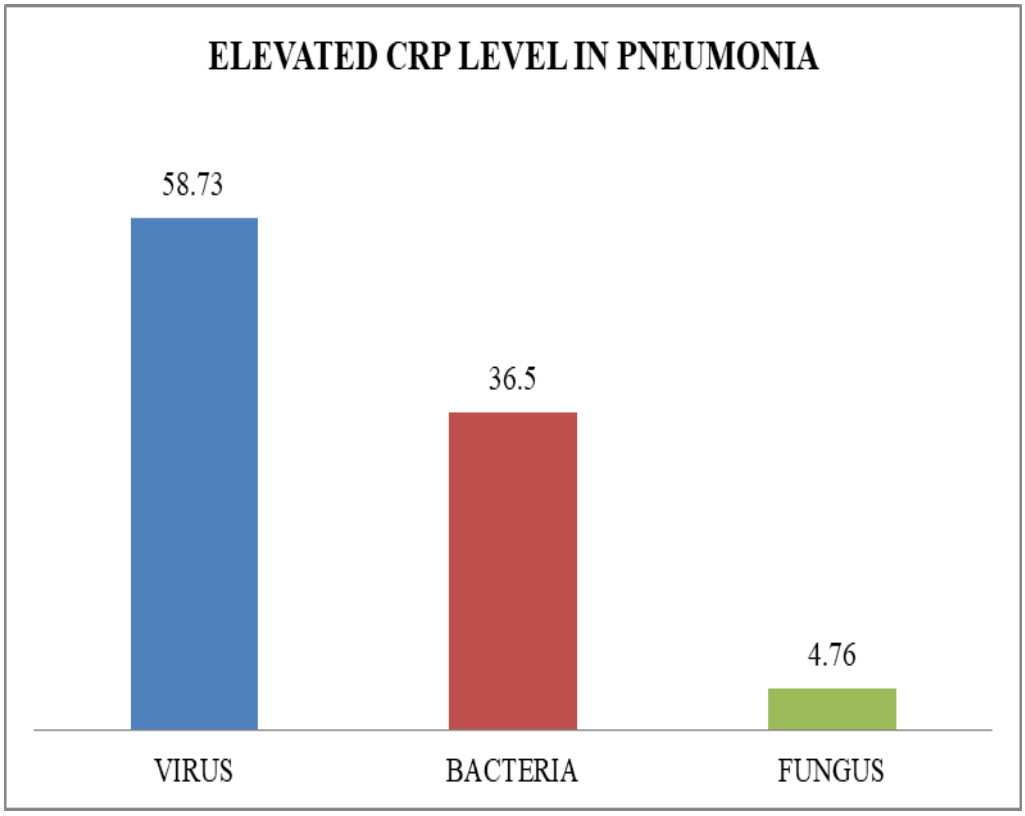
Graph.6 Bacterial co - infections with viral pneumonia



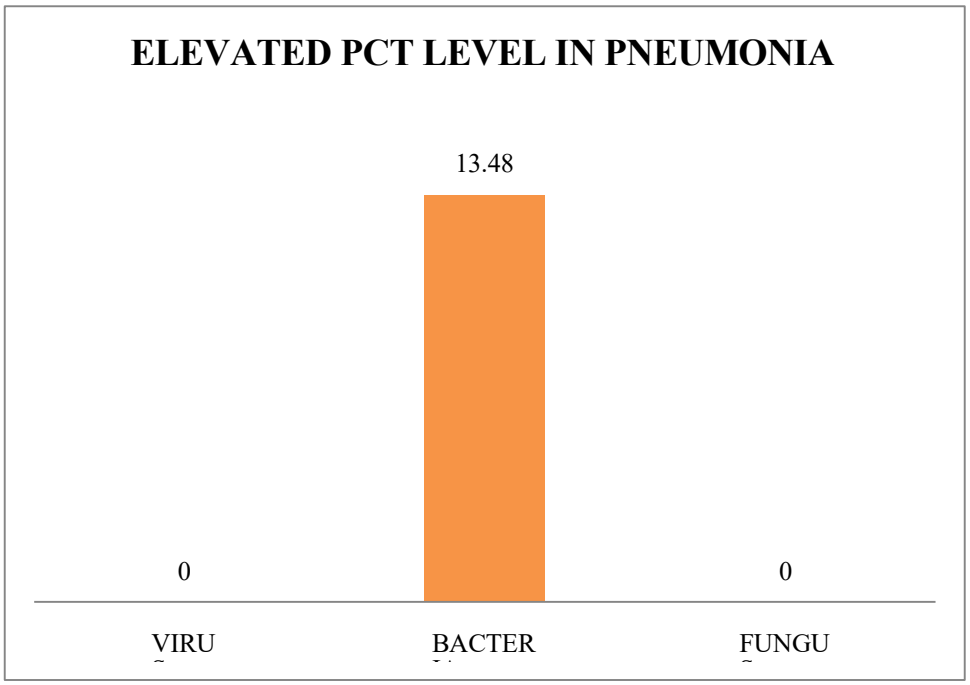
Graph.7 Elevated ESR level in pneumonia



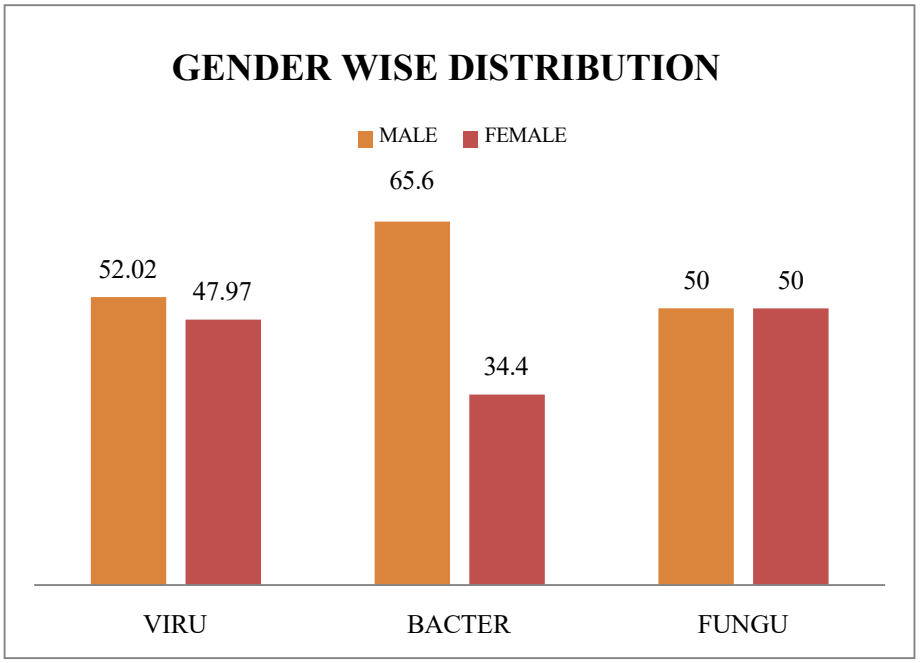
Graph.8 Elevated CRP level in pneumonia



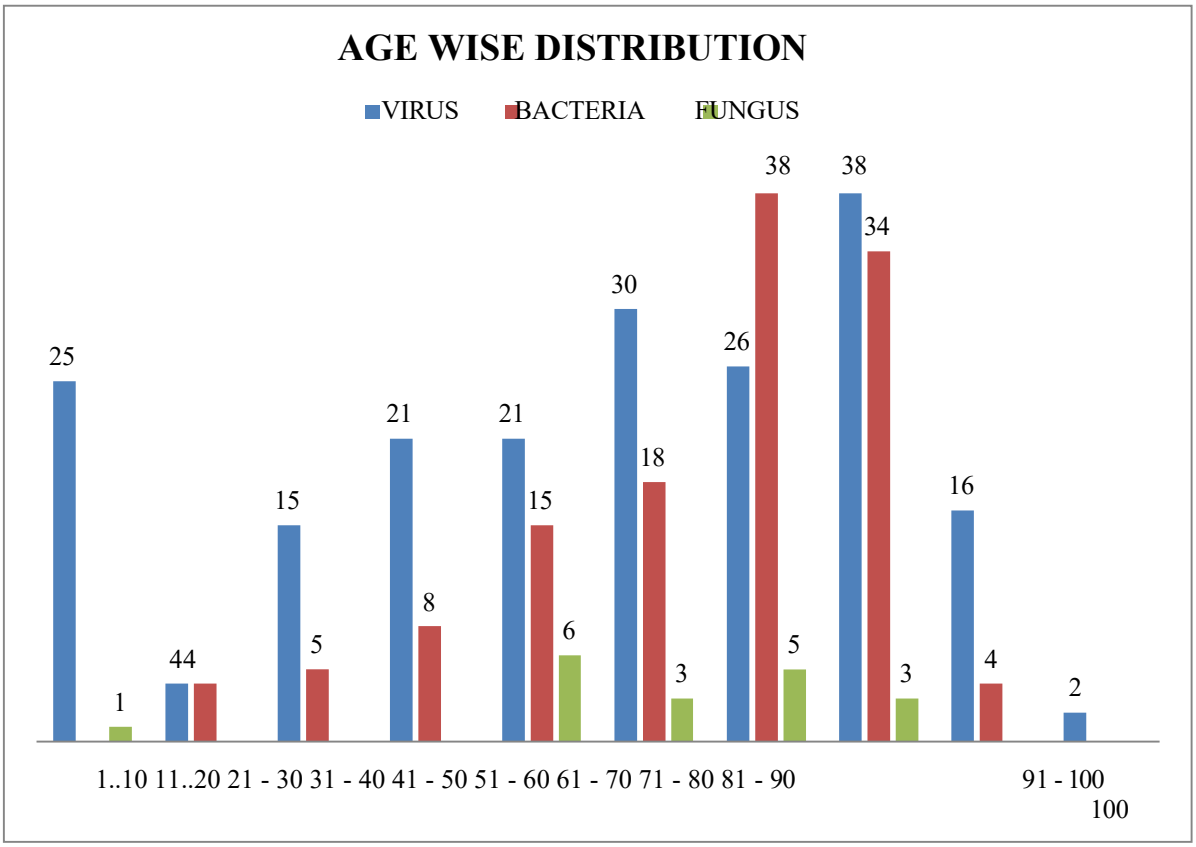
Graph.9 Elevated PCT level in pneumonia



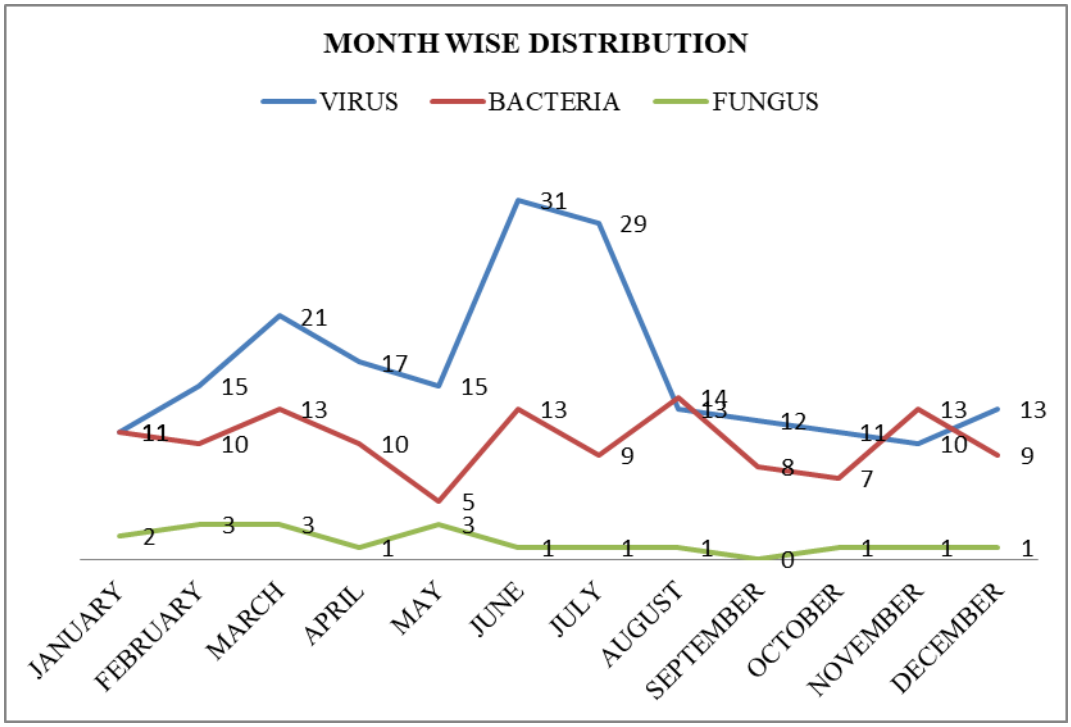
Graph.10 Gender wise distribution



Graph.11 Age wise distribution



Graph.12 Month wise distribution



In the present study observed that the incidence of all types of infections—viral, bacterial, and fungal—was highest among individuals aged 60 to 80 years. Additionally, viral infections were more prevalent in children aged 1 to 10 years. Overall, viral and bacterial infections were more common than fungal infections. Jing Chen *et al.,* study mentioned in the group of patients ≥65 years old, viral infection was the majority, while in the group of patients <65 years old, viruses, atypical pathogens and bacteria were all the main pathogens in CAP.[146]

In the present study, viral infections had their highest incidence in May, June, and July; bacterial infections peaked in June, August, and November; and fungal infections were most prevalent in May.

According to the study of Mansour Almuqbil *et al.,* the incidence of viral infections was observed to increase in October, peak in January, and gradually decline thereafter.[147] Moriyama *et al*., study similarly found that virus - induced illnesses are most prevalent between December and February.[148]

In conclusion, the study was conducted in the Department of Microbiology Laboratory and Transplant immunology and Molecular diagnostic laboratory, Aster MIMS Calicut. This study comprehensively evaluated the spectrum of pathogens causing pneumonia by integrating clinical history with diagnostic findings from multiplex PCR and conventional culture methods. Out of 1676 respiratory samples analyzed, 341 (20.34%) yielded causative organisms. Viral pathogens were the predominant etiological agents (58.06%), followed by bacterial (36.65%) and fungal (5.27%) infections. Among the viruses, Influenza A H1N1 was the most frequently detected, while *Klebsiella pneumoniae* emerged as the leading bacterial pathogen, with a significant proportion exhibiting multidrug resistance. Aspergillus species were the most common fungal isolates.

Clinical correlation with laboratory parameters revealed that neutrophilia was common in pneumonia patients, and elevated inflammatory markers such as CRP and ESR were prevalent across all infection types. Notably, procalcitonin levels were significantly elevated only in bacterial pneumonia, reinforcing its utility as a biomarker for bacterial infections.

Gender - wise, male predominance was noted in bacterial and viral pneumonia cases, while fungal

infections showed equal gender distribution. Age - wise, the highest infection rates were observed in the 60–80 age group, with an additional peak in viral infections among children aged 1–10years. Seasonal trends showed that viral infections peaked during May to July, bacterial infections during June, August, and November, and fungal infections in May.

The findings emphasize the utility of multiplex PCR as a sensitive and rapid diagnostic tool for pathogen detection in pneumonia, facilitating timely and targeted therapeutic interventions. Moreover, the integration of molecular diagnostics with clinical and laboratory parameters enhances the understanding of pneumonia etiology, epidemiology, and aids in the appropriate management of infected patients.

Acknowledgement

I express my sincere gratitude to my honorable guide, Mrs. Swathy Viswanath M., Associate Professor, Department of Microbiology, MIMS COAHS, Malappuram, for her dedicated guidance, personal involvement, critical evaluation, and timely advice throughout the study. I extend my heartfelt thanks to Mrs. Savitha Anoop, Professor, Department of Microbiology, MIMS COAHS, Malappuram, for her unwavering support, insightful guidance, and constructive feedback during the course of my research. I am deeply thankful to Dr. Vipin Viswanath, Head of Laboratory Medicine, Aster MIMS Hospital, Calicut, and Technical Head, Aster Labs Kerala Cluster, for his invaluable support, guidance, and mentorship. I also express my sincere appreciation to Dr. Reshmi Gopalakrishnan, Consultant Microbiologist, Department of Microbiology, Aster MIMS, Calicut, for providing me the opportunity to carry out this work under her supervision; without her support, this research would not have been possible.

Author Contributions

P. P. Vismaya: Investigation, formal analysis, writing—original draft. M. Swathy Viswanath: Validation, methodology, writing—reviewing. M. Savitha:—Formal analysis, writing—review and editing. Vipin Viswanath: Investigation, writing—reviewing. Reshmi Gopalakrishnan: Resources, investigation writing—reviewing. M. P. Anjali: Validation, formal analysis, writing—reviewing. M. M. Athulya: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript. M. Gayathri: Validation, methodology, writing—reviewing. Irfana:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

1. Almuqbil M, Al - Asmi R, AlRamly S, Hijazi N, Alotaibi H, AlMubarak A, AlAnezi K, Al - Rowaili M, Al - Yamani M, Duwaidi BS, Alshammari DR, Alabdulsalam AM, Almutairi JA, Alasmari FM, Asdaq SMB. Parental COVID - 19 vaccine hesitancy for children and its influencing factors: A Riyadh - based cross - sectional study. Vaccines (Basel). 2023 Feb 23; 11(3): 518. https://doi.org/10.3390/vaccines11030518.
2. Bruijnesteijnvan Coppenraet LES, Swanink CMA, van Z wet AA, *et al.,* Comparison of two commercial molecular assays for simultaneous detection of respiratory viruses in clinical samples using two automatic electrophoresis detection systems. J Virol Methods. 2010; 169(1): 188–192. https://doi.org/10.1016/j.jviromet.2010.07.032.
3. Cabello C, Manjarrez M, Olvera R, Villalba J, Valle L, Paramo I. Frequency of viruses associated with acute respiratory infections in children younger than five years of age at a locality of Mexico City. Mem Inst Oswaldo Cruz. 2006; 101(1): 21–24. https://doi.org/10.1590/s0074-02762006000100005.
4. Chandy S, Manoharan A, Hameed A, Jones LK, Nachiyar GS, Ramya MS, SudhakarA, SumanthA, Balasubramanian S. Astudyonpediatric respiratorytract infections in hospitalized children

from Chennai. *Clin Epidemiol Glob Health*. 2022; 15: 101067. Available from: https://doi.org/10.1016/j.cegh.2022.101067
5. Chen J, Li X, Wang W, Jia Y, Lin F, Xu J. The prevalence of respiratory pathogens in adults with community - acquired pneumonia in an outpatient cohort. Infect Drug Resist.
6. Deval H, Kumar N, Srivastava M, Potdar V, Mehta A, Verma A, Singh R, Kavathekar A, Kant R, Murhekar M. Human Metapneumovirus (hMPV): An associated etiology of Severe Acute Respiratory Infection in Children of Eastern Uttar Pradesh, India. *Access Microbiol*. 2024 Aug 1; 6(8): 000829. https://doi.org/10.1099/acmi.0.000829.v3.
7. Doeleman SE, Reijnders TDY, Joosten SCM, Schuurman AR, van Engelen TSR, Verhoeff J, Léopold V, Brands X, Haak BW, Prins JM, Kanglie MMNP, van den Berk IAH, Faber DR, Douma RA, StokerJ, Saris A, Garcia Vallejo JJ, Wiersinga WJ, vander Poll T. Lymphopenia is associated with broad host response aberrations in community - acquired pneumonia. JInfect.2024 Apr; 88(4): e106131. https://doi.org/10.1016/j.jinf.2024.106131.
8. El Wakeel MA, Nassar MS, El Batal WH, Amer AF, Darwish MK, Aziz AA. Evaluation of procalcitonin as a biomarker for bacterial and nonbacterial community - acquired pneumonia in children. J Arab Soc Med Res. 2017; 12(2): 68–72. https://doi.org/10.4103/jasmr.jasmr_19_17.
9. Esposito S, Principi N. Pneumococcal vaccines and the prevention of community - acquired pneumonia. Pulm Pharmacol Ther. 2015; 32: 124–9. https://doi.org/10.1016/j.pupt.2014.02.003.
10. Fan ZY, You SJ, Li LB, Bian J, Tan FL. Multidrug - resistant organism infections of inpatients in a hospital in Eastern China from 2015 to 2021. Infect Drug Resist. 2023; 16: 4387–95. https://doi.org/10.2147/IDR.S412491.
11. Hu FP, Guo Y, Zhu DM, *et al.,* CHINET surveillance of antimicrobial resistance among the bacterial isolates in 2021. *Chin J Infect Chemother*. 2021; 25(5): 20
12. Jalal NA, Al - Ghamdi AM, Momenah AM, Ashgar SS, Bantun F, Bahwerth FS, Hariri SH, Johargy AK, Barhameen AA, Al - Said HM, Faidah H. Prevalence and Antibiogram Pattern of Klebsiella pneumoniae in a Tertiary Care Hospital in Makkah, Saudi Arabia: An 11 - Year Experience. Antibiotics (Basel). 2023 Jan 12; 12(1): 164. https://doi.org/10.3390/antibiotics12010164.
13. Kalil AC, Metersky ML, Klompas M, *et al.,* Management of adults with hospital - acquired and ventilator - associated pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. Clin Infect Dis.2016; 63(5): e61–e111. https://doi.org/10.1093/cid/ciw353.
14. Kar M, Siddiqui T, Dubey A, Hashim Z, Sahu C, Ghoshal U. Respiratory bacterial co - infections and their antibiotic resistance pattern in COVID - 19 patients at a tertiary care centre in India. Access Microbiol. 2023 Jun 22; 5(6): acmi000514.v3. https://doi.org/10.1099/acmi.0.000514.v3
15. Malhotra B, Swamy MA, Janardhan Reddy PV, Gupta ML. Viruses causing severe acute respiratory infections (SARI) in children ≤5 years of age at a tertiary care hospital in Rajasthan, India. *Indian J Med Res*. 2016; 144(6): 877–85
16. Meili M, Kutz A, Briel M, Christ - Crain M, Bucher HC, Mueller B, Schuetz P. Infection biomarkers in primary care patients with acute respiratory tract infections— comparison of procalcitonin and C - reactive protein. BMC Pulm Med. 2016; 16: 43. https://doi.org/10.1186/s12890-016-0206-4
17. Moriyama M, Hugentobler WJ, Iwasaki A. Seasonality of respiratory viral infections. Annu Rev Virol. 2020 Sep; 7(1): 83–101. https://doi.org/10.1146/annurev-virology-012420-022445.
18. Naheed A, Saha SK, Breiman RF, Khatun F, Brooks WA, El Arifeen S, Sack D, Luby SP; Pneumococcal Study Group. Multihospital surveillance of pneumonia burden among children aged <5 years hospitalized for pneumoniain Bangladesh. ClinInfect Dis. 2009 Mar; 48 Suppl 2: S82–9. https://doi.org/10.1086/596485.
19. Olofsson S, Brittain - Long R, Andersson LM, Westin J, Lindh M. PCR for detection of respiratoryviruses: seasonal variations of virus infections. Expert Rev Anti Infect Ther. 2011; 9(8): 615–626. https://doi.org/10.1586/eri.11.75.
20. Rafat Z, Hashemi SJ, Ashrafi K, Nikokar I, Jafari A, Rahimi Foroushani A, RoohiB, Borjian Boroujeni Z, Najar - Shahri N. Fungal isolates of the respiratory tract in symptomatic patients hospitalized in pulmonary units: a mycological and molecular epidemiologic study. J Multidiscip Healthc. 2020 Jul 22; 13: 661–9. https://doi.org/10.2147/JMDH.S252371.
21. Ruuskanen O, Lahti E, Jennings LC, Murdoch DR. Viralpneumonia. Lancet. 2011; 377(9773): 1264–

1275. https://doi.org/10.1016/s0140-6736(10)61459-6. PubMed + 2PubMed + 2EuropePMC + 2
22. Waghmode R, Jadhav S, Nema V. The Burden of Respiratory Viruses and Their Prevalence in Different Geographical Regions of India: 1970–2020. *Front Microbiol*. 2021; 12: 723850. Available from: https://doi.org/10.3389/fmicb.2021.723850
23. Yadav P, Gupta AK, Gautam AK, Kumar A, Priyadarshi S, Srivastav DK. Clinico – bacteriological profile of community – acquired pneumonia patients at a tertiary care center of North India. Indian J Respir Care. 2022 Apr–Jun; 11(2): 117–123. https://doi.org/10.4103/ijrc.ijrc_145_21
24. Yang W, Yao H, Xi C, Ye X, Chen Q, Zhang J, Yu J, Hu J. Prevalence and Clinical Characteristics of Bacterial Pneumonia in Neurosurgical Emergency Center Patients: A Retrospective Study Spanning 13 Years at a Tertiary Center. Microorganisms. 2023 Aug 2; 11(8): 1992. https://doi.org/10.3390/microorganisms11081992.

How to cite this article:

Vismaya P. P., M. Swathy Viswanath, M. Savitha, Vipin Viswanath, Reshmi Gopalakrishnan, M. P. Anjali, M. M. Athulya, Gayathri Meyana and Irfana. 2025. Spectrum of Pathogens Causing Pneumonia by Using Multiplex PCR, Culture and Clinical History of Patients in a Tertiary Care Hospital. *Int.J.Curr.Microbiol.App.Sci.* 14(11): 275-290.
doi: https://doi.org/10.20546/ijcmas.2025.1411.028