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A study on efficacy of multiplex PCR assay (rapid test) in diagnosis and treatment of bacterial pneumonia in patients with COVID-19

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Abstract---Introduction: an inflammatory condition of the tissues within one or maybe both lungs which is most typically, but sometimes not, triggered by infection. Bacterial Pneumonia comes in a variety of forms. COVID-19 primarily is largely a respiratory illness, including symptomatology ranging from a cold or flu sickness to acute respiratory distress syndrome (ARDS). COVID-19 infestation will activate both innate and adaptive immune responses, along with localised antibody production, macrophage and monocyte migration, cytokine production, as well as priming of adaptive T- and B-cells during an attempt to settle underpinning inflammatory response. Secondary bacterial pneumonia has become one of the potential aspects related to COVID-19. Secondary bacterial infections have been found to be strongly related to higher risks and mortality in COVID-19 individuals throughout recent research. Quantitative PCR assays may be used to determine overall patterns of viral growth, evaluate the effectiveness of therapy, as well as distinguish between latent and active transmission among viruses that survive in designated cell types, especially in addition to evaluating infection rate at such a given instant of time. Aims and Objectives: To find out the efficacy of Rapid Test as compared to Standard Procedure (bacterial culture and identification; and assessment of AMR gene), in effective diagnosis of bacterial pneumonia in COVID-19 patients. Materials and Methods: The study is of prospective design which was conducted between October 2021 and April 2022. The study has considered 85 patients with COVID-19 who are diagnosed by RT-PCR assay. The patients were tested with Standard Procedure and Rapid Test and was analyzed for efficacy in diagnosis of bacterial pneumonia. Result: the rapid test kit is significantly effective in diagnosing the genes involved in bacterial pneumonia ($P < 0.005$) and it is also as effective as Standard Procedure in diagnosing the causative organism ($P > 0.005$). Conclusion: It was found that the Rapid Test is as effective as Standard Procedure in diagnosing bacterial pneumonia associated genes which will help in diagnosing the bacterial pneumonia more effectively and prevent further mortality

Keywords---rapid test, bacterial culture, COVID-19, bacterial pneumonia

Introduction

Bacteria, viruses, fungus, as well as parasites, are among the different causative organisms of pneumonia. In clinical terms, this is an inflammatory condition of the tissues within one or maybe both lungs which is most typically, but sometimes not, triggered by infection. Bacterial Pneumonia comes in a variety of forms. CAP: Acute lung tissue disease in an individual who contracted it in the environment or even within two days of arrival at the hospital. HAP: Acute lung tissue inflammation which occurs following two days of hospitalisation in some kind of a non-intubated person. VAP is really a sort of community-acquired pneumonia of the lungs that generally occurs 2 days or more following ventilation system intubation. HCAP: An acute illness of lung tissue obtained from a health institution including a nursing facility, dialysis centre, health centre, or an individual who has just been hospitalised. *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, Group A streptococci, *Moraxella catarrhalis*, anaerobes, and aerobic gram-negative bacteria are among the microorganisms that cause typical pneumonia. *Legionella*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Chlamydia psittaci* are the most common pathogens responsible for atypical pneumonia [Sattar SBA et al., 2021(1) & Chong et al., 2021].

COVID-19 primarily is largely a respiratory illness, including symptomatology ranging from a cold or flu sickness to acute respiratory distress syndrome (ARDS). Secondary micro - organisms illnesses have indeed been linked to viral-related respiratory diseases pertaining to the very same coronavirus category like SARS-CoV as well as Middle East respiratory syndrome coronavirus (MERS-CoV). Additional infectious as well as non-infectious consequences, including pneumothorax, myocarditis, and sometimes even gadget secondary infections, were also reported among hospitalised COVID-19 individuals with chronic COVID-19 disease. COVID-19 infestation will activate both innate and adaptive immune responses, along with localised antibody production, macrophage and monocyte migration, cytokine production, as well as priming of adaptive T- and B-cells during an attempt to settle underpinning inflammatory response [vallian court et al., 2020]

The respiratory system is indeed not pristine, so it is regularly subjected to microorganisms from the surroundings. Bacterial pneumonia is caused by that the infiltration and spread of the germs listed earlier through into pulmonary parenchyma at the alveoli level. The clinical syndrome of pneumonia is caused by the body's natural immune activation towards it. The major cause of bacterial pneumonia's symptomatology is indeed an inflammatory condition. The subjective effects are caused by cytokines, which are generated as consequence to that of immune activation; for instance, IL-1 (interleukin-1) and TNF (tumour necrosis factor) lead to infection. Chemokine-like IL-8 (interleukin-8), as well as colony-stimulating factors such as G-CSF (granulocyte colony-stimulating factor), enhance cellular responses and neutrophil growth, accordingly, leading to

leukocytosis but also exudate discharges in serological labs. Such cytokines cause this same alveolar-capillary barrier to drain there at sites of infection, resulting in decreased responsiveness as well as breathlessness. This same lung parenchyma is indeed not entirely solidified inside this Congestion phase, and also the alveolar lobes include serous secretions, microorganisms, limited neutrophils, and macrophages on microscopy. This region gets solidified, hard, and very liver-like during the red hepatization phase. Fibrin and serous exudate, infections, neutrophils, and macrophages could all be seen under the microscope. The alveolar membranes become thicker and also the capillaries become clogged. Because of suppurative as well as exudate-filled alveolar lobes, this lobe still seems to be hepatic like in consistency although grey in appearance. In a resolution, it begins to resolve within the week when lymphatic drainage or vigorous coughing flushes accumulated sputum [vallian court et al., 2020].

Secondary bacterial pneumonia has become one of the potential aspects related to COVID-19. Secondary bacterial infections have been found to be strongly related to higher risks and mortality in COVID-19 individuals throughout recent research. During the previous epidemic of the severe acute respiratory syndrome coronavirus (SARS-CoV), the extensive use of antibiotics resulted in a rise in the incidence of multidrug-resistant bacteria. Secondary lung invasions are quite common among COVID-19 individuals who are extremely unwell and hospitalised. *Pseudomonas aeruginosa*, *Klebsiella* species, *Staphylococcus aureus*, *Escherichia coli*, and *Stenotrophomonas maltophilia* are by far the most prevalent bacteria found within respiratory system samples. Antibiotic-resistant bacteria are now on the rise, but our ability to remove these is dwindling. This makes humans increasingly susceptible to bacterial diseases, as well as weakening humans in viral outbreaks. According to research, the occurrence of ground-glass opacities (GGO) with a peripheral as well as subpleural dispersion is by far the most common CT characteristic of COVID-19 pneumonia. Nearly the bulk of COVID-19 sufferers describes an invasion of numerous lobes, especially the lower lobes. Such GGO zones may well be formed by combining alongside localised compaction areas and/or overlaid intralobular reticulations, culminating in some kind of a bizarre pavement arrangement [watzinger et al.,].

The most common approach in diagnostic techniques is real-time polymerase chain reaction (PCR). A board of 23 TaqMan-based real-time Pcr - based methods has indeed been developed for the simultaneous determination as well as tracking of 16 viral agents and virus families, along with human polyomaviruses BK and JC, human herpesviruses 6, 7, and 8, human adenoviruses, herpes simplex viruses 1 and 2, varicella-zoster virus, cytomegalovirus, Epstein-Barr virus, parvovirus B19, influenza A and B Viruses. These offered testing methods do have a wide field of view plus great responsiveness, repeatability, but also accuracy. Furthermore, these tests enable exact virological load determination in a wide spectrum of clinical samples. This capability to employ standard PCR settings with all tests allows again for sequencing and identification of several pathogens at the same time, reducing diagnostic time. These quantitative PCR tests provided effective diagnostic methods for prompt commencement of correct medication as well as fast evaluation of the efficiency of the antiviral treatment protocol in viral diseases about which particular alternatives exist. The use of PCR methods for

viral identification and quantification has the benefits of excellent sensitivity and repeatability, as well as a very dynamic bandwidth [watzinger et al.,].

Quantitative PCR assays may be used to determine overall patterns of viral growth, evaluate the effectiveness of therapy, as well as distinguish between latent and active transmission among viruses that survive in designated cell types, especially in addition to evaluating infection rate at such a given instant of time. Furthermore, using successive quantitative PCR assays for virus tracking assists uncover false-positive findings produced through unintentional contamination of specimens having remnants containing viral nucleic acids or PCR products from a technological perspective [watzinger et al.,].

Materials and Methods

The study is of prospective design which was conducted between October 2021 and April 2022. The study has considered 85 patients with COVID-19 who are diagnosed by RT-PCR assay. The samples from the patients' nasopharyngeal swab was obtained. These samples were obtained at the time of suspicion. The patients who were included in this current study had fever of more than 38°C, purulent secretion from trachea, white blood cell count of more than 10×10^9 /liter, chest X-ray shows progressive infiltrates and reduction in blood gas exchange.

The current study has recorded the demographic characteristics of the sample and the severity of the disease, laboratory results and the outcome of the disease. Lower Respiratory Tract samples were obtained and observed under microscope with gram staining. All the plates were observed after incubating the same in air which is 5% CO₂ enriched. Then after incubation, it was examined for growth after 24 to 48 hours. For considering bacterial quantification, growth of bacteria above 1×10^4 CFU/ml for a sample of Broncho alveolar lavage (BAL) fluid or in case of Endotracheal Aspirate (ETA), the level of reference considered was 1×10^5 CFU/ml.

The bacterial quantification was assessed by mass spectrometry and automatic genomic sequencer were used for genome sequencing. The same diagnosis was also done using Rapid Test kit. Required antibiotic therapy was given to each patient and was recorded accordingly. According to the organism (type and load), the anti-biotic therapy was prescribed and each case was evaluated on the basis of result from culture test and rapid test.

Data analysis was done in SPSS 25 and analysis tests that were employed for this were Mann-Whitney U test or Fisher's Exact Test. The significance was considered to be 0.005 ($P < 0.005$).

Results

The patients were considered from Indian population and their ages were found to be 31.25 ± 9.33 years old. The male and female ratio was 2.42:1. The current study has tested 85 patients who had confirmed COVID-19. The result has shown that 48 patients revealed positive by both the methods (culture and rapid test), 3 patients' samples came to be positive by culture method while negative by rapid

test and 34 patients' samples revealed to be negative by both methods. Table 1 reveals the findings of rapid test and standard procedure (culture method) for Lower Respiratory Tract (LRT) samples obtained from COVID-19 patients with bacterial pneumonia.

Table 1: The findings of cases detected by Rapid Test and Standard Procedure based on causative organism and genes

Causative organism	Percentage of cases detected by Standard Procedure out of total cases detected by Rapid Test (%)	No. Of Positive by Rapid Test and /No. Of positives by Standard Procedures* only	Significance (p-value)
Acinetobacter calcoaceticus-baumannii complex	85.7	7/6	> 0.005
Escherichia coli	100	8/8	> 0.005
Enterobacter cloacae	83.3	6/5	> 0.005
Klebsiella pneumoniae	0.5	2/1	> 0.005
Staphylococcus aureus	100	25/25	> 0.005
Antimicrobial Resistance Genes (AMR genes)			
KPC	84	25/21	<0.005
CTX-M	81.8	11/09	<0.005
mecA/-C	66.67	12/8	<0.005

* Standard Procedure includes bacterial culture and identification and assessment of AMR gene

The study found out that rapid tests successfully detected all the cases of pneumonia and their respective causative organism but Standard Procedures which includes culture and identification of the sample and also assessing the AMR gene, has detected 83.3% of cases of Enterobacter cloacae and 0.5% of Klebsiella pneumoniae cases detected by rapid test. Similarly, 84% of the gene KPC detected by rapid test, was also detected by Standard Procedure and the same for CTX-M and mecA/-C, was 81.8% and 66.67%, respectively. All the patients were treated empirically with antibiotics. The antibiotics that were used are given below.

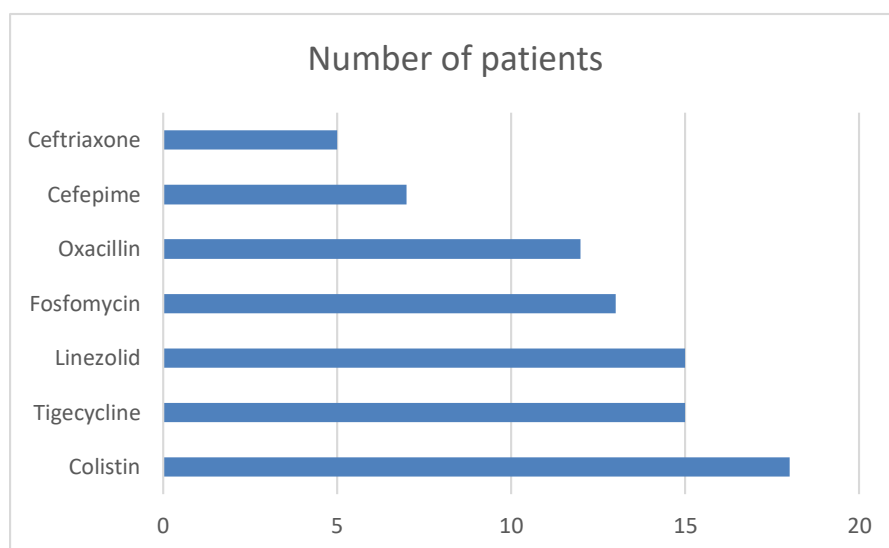


Figure 1: The number of patients who were given anti-biotics

The study found that the anti-biotics were given to the patients because they were diagnosed with bacterial pneumonia in addition to COVID-19. It was found that 13.36% of the patients did not survive which cannot be attributed to bacterial pneumonia alone as they all had COVID-19. Figure 2 shows the number of patients who did not survive with respect to the anti-biotic that was prescribed for them.

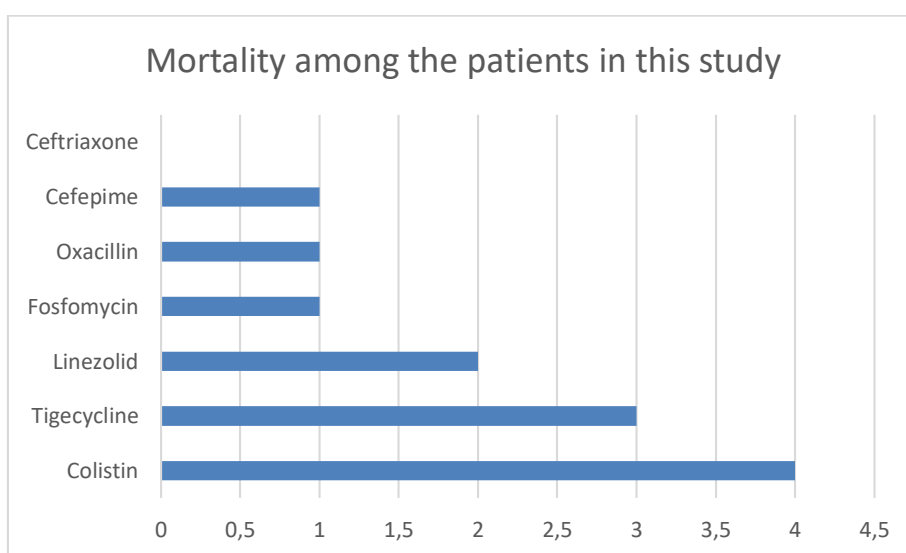


Figure 2: The mortality of the patients in this study with respect to the anti-biotic prescribed

Discussion

Mucus samples, nasopharyngeal swabs, as well as nasopharyngeal aspirates involving more than 200 adult patients having community-acquired pneumonia plus more than 100 controls, have been subjected to respiratory culture as well as multiplex PCR detecting *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*. With said samples obtained, all culture and multiplex PCR worked well enough and seem to really be helpful diagnostic methods. The survey's objective was to determine the diagnostic performance of respiratory culture as well as a single-run multiplex PCR (mPCR) for individual proteins of *Streptococcus pneumoniae* (Lyta), *Haemophilus influenzae* (16S rRNA, with PCR for P6), *Mycoplasma pneumoniae* (P1), and *Chlamydia pneumoniae* (ompA) (12) implemented to respiratory specimens in CAP individuals. Lung culture and mPCR administered towards phlegm, NpSs, and NpAs can indeed be utilised to generate preliminary determinations of both the aetiology of CAP in adult populations having predictably reduced levels of respiratory pathogens transmission, judging by the current findings. Antibiotic treatment and management utilising narrow-spectrum antibiotics could be guided by such probable diagnosis [stralin et al., 2006]

This research evaluated *Pneumocystis jiroveci* PCR with traditional staining among HIV-uninfected people with weakened immune systems. *Pneumocystis jiroveci* PCR shows greater sensitivity over standard staining however it cannot differentiate colonisation versus contamination. Individuals with blood cancers accounted for 65% of the total; 15.1 % received bone marrow transplants; 8.8 % had tumour growth; 5.7 % had kidney transplants, and 4.4 % have been on immunosuppressive drugs for widespread illnesses. In more than 350 cases, BAL was used as part of the testing approach, while induced sputum (IS) was used in over 95 cases. Traditional pneumocystis pneumonia (PCP) staining showed positive in 8.7% of individuals, with much more than 30 having a positive PCR. PCR was indeed positive in 33 individuals, 22 of which had comprehensive follow-up and 13 of which were identified as presumptive or conclusive PCP. Prognostic and predictive scores reached 49.05 % and 97.07 %, correspondingly, for PCR, which would have been 85.02 % responsive and 89.02 % precise. On IS, the responsiveness, as well as accuracy, were both % [stralin et al., 2006].

To successfully use antibiotic treatment for lower respiratory tract infections (LRTIs) within a reasonable timeframe, categorization of pathological changes plus definitive cultures are required. Proper judicious utilisation of narrow-spectrum antibiotics for successful patient therapy is hampered by evidence-based antibiotic treatment lacking microbiological confirmation. To reduce non-essential or unnecessary antibiotic usage as well as speed individual recuperation following LRTI-induced harm, consistent and efficient screening approaches which may be easily implemented by clinics are required. unique multiplex real-time polymerase chain reaction (mRT-PCR) test has excellent sensitivity as well as statistical efficiency to identify 4 pathogenic bacteria that cause pneumonia: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Moraxella catarrhalis*. Limit of detection (LOD), selectivity, and reproducibility was used to assess the analytical performance of mRT-PCR against target microorganisms. more than 200 clinical samples involving pneumonia individuals have been

handled for such "respiratory bacteria four" (RB4) mRT-PCR test utilising an automated nucleic acid extraction equipment, and also the findings have been actually linked to standards using bacterial culture and/or Sanger sequencing. Using sputum samples, the RB4 mRT-PCR test found all intended microorganisms. The test had % agreement with reference-positive samples, whereas reference-negative samples revealed further bacterial infections. Consequently, the RB4 mRT-PCR test had a higher response rate and better results than the benchmark tests. This RB4 mRT-PCR test is indeed an elevated, dependable instrument in selection which surpasses existing established procedures. The above technology aids patient care by drastically lowering the overall usage of antibiotics that aren't needed [Lim, H.J et al., 2021].

For both the quick pathogenesis diagnosis of pneumonia, molecular detection methods including polymerase chain reaction (PCR) are highly promising. Again for the identification of *Mycoplasma pneumoniae*, *Legionella* species, as well as *Chlamydia pneumoniae*, PCR seems to have the capability to be more effective than traditional techniques. While evaluating pulmonary specimens with pneumococcal pneumonia in people, PCR contributes nothing whatsoever to current medical tests and is therefore unable to discriminate pneumococcal colonisation from illness. Whilst PCR is likely to become more reactive over traditional microscopy-based approaches for identifying *Pneumocystis carinii* pneumonia, its applicability is unknown since *P. carinii* can sometimes be found without symptomatology. Among immunocompromised individuals, PCR can help diagnose viral pneumonia. More studies are needed to further identify the importance of PCR than other procedures in detecting pneumonia, as well as to create conventional PCR tests which could be used in normal laboratory testing [Murdoch, D.R et al., 2003].

Researchers created 2 groups of stacked gene sequences to see whether the polymerase chain reaction (PCR) could identify *Streptococcus pneumoniae* in circulation. The first one identified the pneumolysin gene's 559-bp and 649-bp sections, whereas the next one identified the autolysin gene's 445-bp and 553-bp areas. The nucleotide sequences have been found throughout all 19 pneumococcal serotypes studied, though not in DNA across 39 non-pneumococcal bacteria and fungal strains. Utilising pure pneumococcal Genetic material, overall responsiveness has been tested. Researchers found 10 fg of *S. pneumoniae* DNA, which is 4.3 genomic counterparts. Blood samples were drawn from 15 individuals experiencing pneumococcal bacteremia confirmed by culture and analysed by PCR. Six of the eight buffy coat portions analysed exhibited PCR sensitivity using pneumolysin sequences, while five of the eight generated the anticipated results employing autolysin primers. The above mixture of accuracy and precision might enable PCR-based identification of *S. pneumoniae* within the circulation, an extremely impressive substitute for clinical specimens for conclusive confirmation [Murdoch, D.R et al., 2003].

Using specimens taken from more than 100 persons engaged in an investigation, standard monitoring systems plus multiplex real-time PCR for identifying atypical bacteria as well as pulmonary viruses have been used. Well over 50 patients had their microbial diagnostics established using traditional methods, while more than 75 individuals had their illnesses established using real-time PCR. Real-time

PCR results might be obtained in as little as 6 hours. For said identification of atypical infections as well as viruses, PCR technology was much more efficient. Using traditional techniques, respiratory infectious agents and combined illnesses have been discovered in much more than 20% of total patients, while by real-time PCR, they have been identified in far more than 80% of treated participants [Templeton, K.E et al., 2005].

Conclusion

The current study has shown that the rapid test kit is significantly effective in diagnosing the genes involved in bacterial pneumonia ($P < 0.005$) and it is also as effective as Standard Procedure in diagnosing the causative organism ($P > 0.005$). The study has highlighted an essential point about the diagnosis of pneumonia in COVID-19 patients. If Rapid Test can be used effectively, then, it will be very easy and quick for diagnosis of pneumonia, specially among the patients of COVID-19 for whom prompt action must be required. As the current study found out, the Rapid Test is as effective as Standard Procedure in diagnosing bacterial pneumonia associated genes which will help in diagnosing the bacterial pneumonia more effectively and prevent further mortality.

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