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Research Article

# STUDY OF MICROBIOLOGICAL PROFILE OBTAINED FROM LUNG SWABS OF CONFIRMED COVID-19 CASES AT AUTOPSY

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**Abstract-** Background: In December 2019, SARS-CoV-2, a novel Coronavirus was identified in Wuhan, China. In a short span of time, this virus spread across the globe infecting millions, and causing Coronavirus disease (COVID) which ranged from minimal symptoms to severe pneumonia. Superinfection is a usual complication viral infection; thus, post-mortem examination of confirmed COVID-19 positive cases are valuable tool for studying the microbiological profile of the same. Methods: Between November 2020 and April 2021, serial post-mortem examinations were conducted at Department of Forensic Medicine, B.J.Medical College, Ahmedabad. The autopsies were conducted in patients with confirmed severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) infection who died at Civil Hospital Ahmedabad. Post-mortem lung swabs from 30 confirmed COVID-19 cases were sent to the Bacteriology laboratory of B.J. Medical College, Ahmedabad. We are reporting the identification of microorganisms isolated from the aforementioned specimens. The identification was carried out by routine microbiological methods namely, gram stain, culture and biochemical reactions. Results: Of the 30 samples, 20 were from males and 10 from females with a mean age of 65 years. 25 out of the 30 cases had one or more co-morbidities. The CRP levels were found to be elevated in 21 cases. Lymphopenia was seen in 19 of the cases. Microorganisms were isolated from all specimen and *E. coli* (56.7%) was found to be the most common isolate. Conclusion: This study has highlighted the need for continuous research with regard to epidemiology, clinical spectrum and microbiological profile of superinfections, that may enable better patient management.

**Keywords**- *COVID-19, Autopsy, Superinfections*

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## Introduction

Coronaviruses (CoVs) are single-stranded, enveloped RNA viruses that cause respiratory illnesses among human and animals [1,2]. These ubiquitous viruses cause variably severe diseases that range from the common cold to severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). Several cases of pneumonia reported in Wuhan, China in late 2019 led to identification of a novel coronavirus, later designated as SARS coronavirus-2 (SARS-CoV-2), the etiologic agent of COVID-19 [3,4]. Since that time, SARS-CoV-2 has spread rapidly across the world causing more than 10 million infections and more than half a million deaths by early summer 2020 [5]. Coronaviruses gain entry into cells via the angiotensin-converting enzyme 2 (ACE2), which is expressed in multiple tissue types [6]. The virus disproportionately affects older patients with co-morbidities such as hypertension, diabetes, obesity, and cardiovascular disease [4]. Regardless of its viral origin, a usual practice by clinicians is to initiate antibiotic therapy because fever and radiological proof of infiltrates are regarded as hallmarks of bacterial community- acquired pneumonia, which requires treatment by antibiotics [7]. The definite incidence of bacterial superinfections in COVID-19 is not known so far: it seems to be lower than in severe influenza cases [8,9]. A few reports, mainly from China, reported secondary infections as 5-27% of severe acute respiratory syndrome (SARS)-CoV-2 infected adults in several hospitals, which included 50-100% of those who died [4, 10-15]. These infections are predominantly seen in patients with severe disease who are critically ill and receiving mechanical ventilation. The reported incidence of super added infection in intensive care unit (ICU) cases ranges between 13.5 and 44% in patients with COVID-19, the most common type being Ventilator-associated pneumonia (VAP) due to bacterial or fungal causes, followed by bacteremia with sepsis and urinary tract infections (UTIs) [11,15,16].

An association between COVID-19 and superinfection can be possibly attributed to major lung damage caused by viral replication which results in cytokine storm and complex inflammatory processes. In order to optimize patient care and antimicrobial stewardship, more data on superinfections is imperative for understanding COVID-19 and its complications. Post-mortem examinations are underutilized but play a major role in our understanding of disease pathogenesis and manifestations. The purpose of this study is to identify the microbiological profile obtained from lung swabs of deceased patients who were confirmed cases of COVID-19 and to assess the impact of co-morbidities on clinical outcome.

## Methods

Swabs from Lungs were sent for Bacterial culture. Sterile swabs were used to collect the samples during autopsy and were sent to the Bacteriology Laboratory, B.J.Medical College, Ahmedabad. The swab was used to inoculate culture media ( blood agar and MacConkey agar). Following 18-24 hours incubation at 35+2◦C, the culture plates were examined for growth. In plates where growth was seen, Gram stain was performed. A loop-wire was used to lift colonies from the culture plate and was used to prepare a smear on a clean glass slide which was then air-dried and heat-fixed. The slide was then stained by the Gram method and then examined microscopically first at 40X and then at 100X. The observations were noted down. Based on Gram stain, appropriate biochemical reactions were performed to identify the organism.

## Results

Patient demographics and clinical information are summarized in [Table-1]. The ratio of males to females was 2:1, with an average age of 65 years (range = 40–100 years).

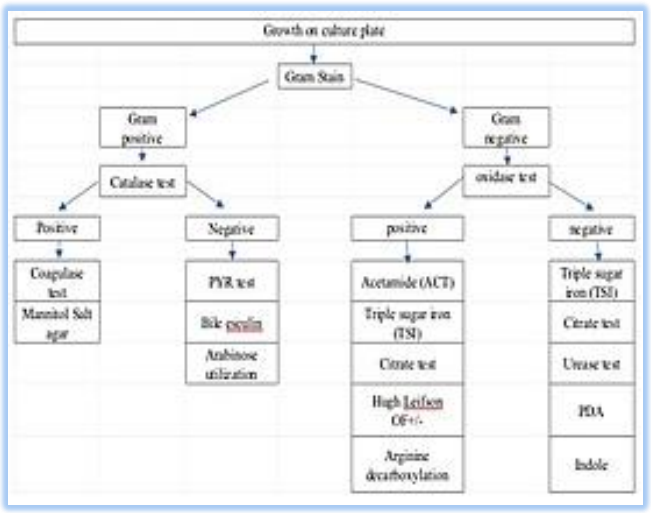



Fig-1 Standard operating procedure for bacterial identification

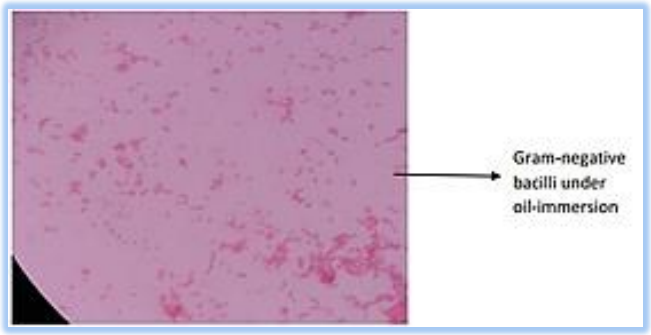
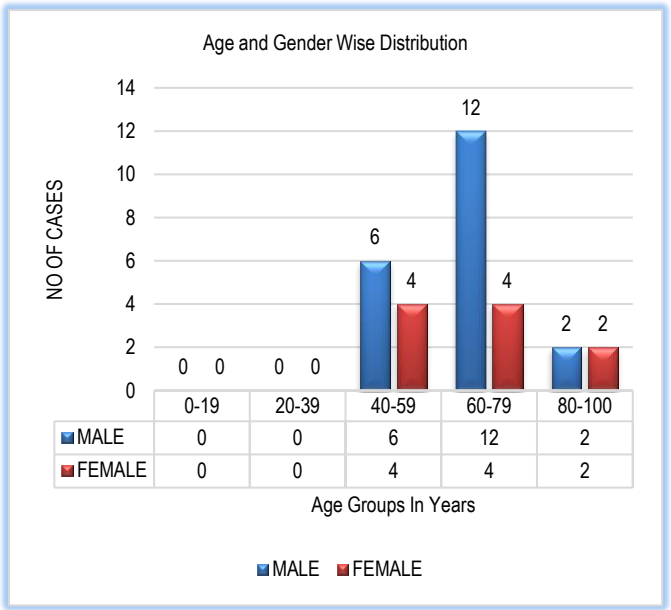


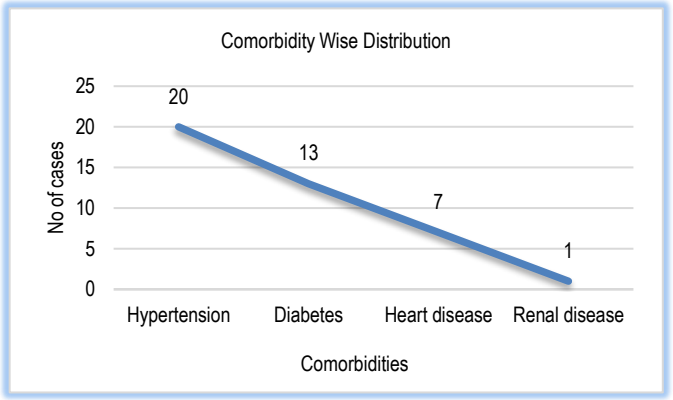

Fig-2 Gram negative bacilli

Fig-3 Colonies of *E.coli* (Left) and *K.pneumoniae* (right) on MacConkey agar

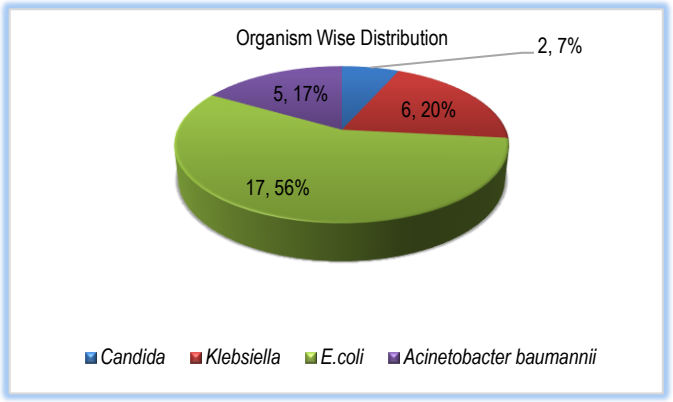



Highest number of cases were found in age group of 60-79 years in which 12 were male patients and 4 were female patients. Most (80%) were admitted to the hospital Triage area while 10% were admitted to covid ward and 10% patients visited medicine opd. Average length of stay was 5 days (range = 0–14 days).

A substantial number of patients had multiple comorbidities, particularly diabetes mellitus (43.33%), hypertension (66.67%), heart disease (23.33%) and renal disease (3.33%). Hypertension was the most common cardiac comorbidity followed by ischemic heart disease.




Out of total 30 patients IL-6, CRP values of 21 patients were available and all 21 patients had elevated CRP level which averaged 12.37 mg/dl. Out of 30 samples, hemograms of 24 cases were available, out of which 19 showed lymphopaenia.




The most common organism isolated was *Escherichia coli* (56.7%) followed by *Klebsiella pneumoniae* (20%), *Acinetobacter baumanni* (16.6%) and *Candida* species (6.7%).

**Discussion**

For better management of COVID-19 infected patients, knowledge of microbiological profile in the context of superinfections is essential. In this study, we have focused on the microbiological profile of 30 confirmed COVID-19 cases at autopsy. Our study shows a male preponderance, similar to other studies. Females have been found to be less susceptible to viral infections and the cause has been attributed to sex hormones [17-20]. Serum CRP level is a known diagnostic marker for infection and inflammation [21].

In our study, 70% of the cases showed raised CRP levels. Bacterial and fungal co-infections are very common in viral pneumonia especially in critically ill patients [22]. However, it is certain that the infection rate of bacterial and fungal co infection with SARS-CoV-2 is proportional to severity of the disease [23]. In our study, out of the 30 samples, 28 bacterial isolates were obtained and 2 isolates were of *Candida* species. Out of the 28 bacterial isolates, *E.coli* (56.7%) was found to be the most common, followed by *K.pneumoniae* (20%), *A.baumanii* (16.6%) and *Candida* spp. (5.6%). Rani A *et al.*[28] found CONS to be the most common cause of superinfections in COVID-19 patients followed by *K.pneumoniae* and *Candida* species. A. Fernandez-Rodriguez *et al*. [29]. found *S.aureus* to be the most common among Gram-positive cocci and among Gram-negative bacilli, *Acinetobacter baumanii* was the most common followed by *K.pneumoniae*.

Bacterial superinfections have been found to significantly increase the mortality and morbidity in critically ill patients [24]. Two studies from China reported secondary bacterial infection in 47.6 and 42.8% cases, respectively [25]. The elevated risk for bacterial superinfection may be attributed to lymphopenia in more than 80% reported COVID-19 patients. Direct infection of T cells and depletion has been studied in SARS and the impaired lymphocyte defense resulting in bacterial secondary infections [26,27].

**Conclusion**

This study has exposed the presence of other co-infecting organisms among patients with COVID-19. Further, this study has highlighted the positive co-relation between elevated inflammatory markers and lymphopenia to the severity of infection. Superinfections constitute a major risk factor for adverse outcomes in hospitalised COVID-19 patients. Patient outcome can be improved by rapid diagnosis of superinfections especially in those with co-morbidities.

**Application of research:** Epidemiological, clinical and microbiological data on nosocomial superinfections are required to guide and update antimicrobial stewardship and result in appropriate antibiotic prescribing practices.

**Research Category:** COVID-19

**Abbreviations:** PYR: Pyrrolidonyl aminopeptidase
PDA: phenylene diamine, OF: Oxidation-fermentation

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**Author Contributions:** All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

**Study area / Sample Collection:** Bacteriology Laboratory, B.J.Medical College, Ahmedabad

**Strain name:** *E.coli, K.pneumoniae*

**Conflict of Interest:** None declared

**Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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