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BACTERIAL OR FUNGAL COINFECTION IN COVID-19 PATIENTS

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ABSTRACT
Bacteria or fungal secondary infection might be a key element that promoted severe disease and mortality in COVID-19 patients. Methods: COVID-19 patients with suspected secondary infections were included in the study and lower respiratory tract samples, blood, catheters and urine samples were collected according to clinical necessity and culture were performed. Automated blood culture and bacterial identification methods were conducted. For fungus, KOH mount and fungal culture were performed. Results: A total of 227 COVID-19 positive patients were included, 49 (21.58%) had secondary infections. From respiratory, blood and urine samples, 55.5%, 9% and 25% of microorganisms were isolated respectively. Respiratory samples comprise *Candida albicans* (25%) followed by *Acinetobacter baumannii* (16.7%), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E. coli* (12.5% each), *mucor* (8.3%), *Staphylococcus aureus*, *Enterococcus sp*., and *Cryptococcus laurenti* (4.2% each). From blood, *Acinetobacter baumannii* (38.4%) were isolated, followed by *Pseudomonas aeruginosa* (23.1%), *Klebsiella pneumoniae*, *Staphylococcus aureus*, Staphylococcus hominis, CONS, *Enterococcus faecalis* (7.6% each). In urinary secondary infections, *E. coli* (33.3%), *Klebsiella pneumoniae*, Pseudomonas aeruginosa, *Candida albicans* (16.6% each), *Enterococcus faecium* and *Cryptococcus laurenti* (8.3% each) were isolated. The majority of isolated bacteria were extreme drug resistant. Conclusion: A high rate of secondary infections with resistant pathogens in COVID-19 patients highlights the importance of regular monitoring and management including antimicrobial stewardship programs based on culture reports.

KEYWORDS: COVID-19, co-infection.

INTRODUCTION
Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a causative agent of COVID-19 disease and it is associated with high morbidity and mortality. It has impelled healthcare systems to a near of collapse and incapacitated economic activities, globally.[1, 2] The route of transmission is through respiratory droplets from symptomatic as well as asymptomatic patients. In COVID-19 patients mortality was high worldwide; the cause of death was not only COVID-19 other co-infections also play a crucial role in morbidity and mortality. COVID-19 is a primary causative agent, while other bacteria and fungi were also associated with complications of patient's health which should be rule out for proper treatment.[3]

Some studies reported that the prevalence of co-infection was variable; most of the patients who had co-infection were non-survivor.[4] The pathogens of respiratory co-infection could be many, either common or rare, including bacteria, viruses, fungus, etc. and bacteria were considered one of the most commonly isolated pathogens.[5,6] In the present study, bacterial or fungal isolates associated with COVID-19 were studied and its antimicrobial resistance pattern was also determined.

MATERIAL AND METHODS
This was a retrospective observational study of COVID-19 positive patients admitted in Government Institute of Medical Sciences, Greater Noida from January 2021 to June 2021. All adult patients with positive COVID-19 who were suspected of secondary infection were included in the study. Patients with bacterial or fungal infections not related to COVID-19 were excluded, and patients whose samples were not sent to the laboratory were also excluded.

Microbiological data was collected from Microbiology Laboratory which included the patient's demographics, bacterial or fungal isolates and antibiotic resistant pattern of isolated bacteria. Clinical details of patients were recorded from Medical Records Department.

Patients were suspected for secondary infection when patient developed clinical symptoms, i.e. continuous high grade fever, respiratory distress, respiratory rates ≥30 per minute. Respiratory failure where patients is on invasive ventilation, signs of shock, difficulty in urination, low urine output.

A total of 227 clinical samples including blood, urine, and respiratory samples that included sputum, bronchoalveolar lavage fluid, and endotracheal aspirate from patients, were recorded during the study period. All clinical specimens were processed in the biosafety cabinets, using recommended personal protective equipment. All samples were discarded as per the biomedical waste management guidelines of India.

The samples were processed as per standard microbiological methods. Reparatory and urine samples were inoculated in Blood agar and MacConkey agar and CLED agar, respectively. All the culture media were overnight incubated at 37 C. Blood samples were directly inoculated in Blood culture bottles and installed in Bactech Blood culture system for microbial growth.

The identification of bacteria/fungi was done by Vitek2. Antimicrobial susceptibility test (AST) of the clinical isolates was determined by the Gram-negative, Gram-positive, and yeast Vitek2 AST cards (N235, N280, N281, P628& YST08) (bioMerieux, France), as per manufacturer's instructions. Descriptive statistics were summarized using frequencies and percentages, or medians.

RESULT

with co-infection was 49.84 years with SD 19.62. Here, 34.7% (17/49) of patients with secondary infection were more than 60 years of age and 65.3 % (32/49) COVID-19 positive male patients had secondary infections [Table 1].

The highest and lowest rates of secondary infections were found in patients aged more than 60 years, and below 20 years, respectively. In total, 24 patients (48.9%) had positive respiratory culture; 13 patients (26.53%) had positive blood culture, and 12 patients (24.48%) had positive urinary tract infection. [Fig 1] In addition, 3 patients (6.1%) had polymicrobial cultures.

From 24 patients with positive respiratory culture, 13 gram negative bacilli (54.2%), 9 fungi (37.5%) and 2 gram positive cocci (8.3%) were isolated. The most common identified organism was *Candida albicans* (6,25%) followed by *Acinetobacter baumannii* (4,16.7%), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E. coli* (3,12.5% each), mucor (2, 8.3%), *Staphylococcus aureus*, *Enterococcus sp*., and *Cryptococcus laurenti* (1, 4.2% each).[Fig 2] All patients were admitted to ICUs and most patients were intubated (95%).

Among 129 Bacteraemia-suspected patients, 13 pathogens were isolated. Predominantly, *Acinetobacter baumannii* (5, 38.4%) were isolated, followed by *Pseudomonas aeruginosa* (3,23.1%), *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus hominis*, CONS, *Enterococcus faecalis* (1,7.6% each).[Fig2]

In urinary secondary infections, 12 pathogens from 12 patients were illustrated, including gram-negative bacteria; *E. coli* (4,33.3%), *Klebsiella pneumoniae* (2,16.6%), *Pseudomonas aeruginosa* (2,16.6%) and Gram Positive cocci, *Enterococcus faecium* (1,8.3%) and Fungi, *Candida albicans* (2,16.6%) and *Cryptococcus laurenti* (1,8.3%). [Fig 2]

The AMR profiles of pathogens isolated from the clinical samples of COVID-19 patients are given in Table 2.

Table 1: Age and Gender wise distribution.

Age	Total no. of patients n(%)	Patients with Secondary Infection n(%)
<20	12(5.3%)	2 (4.1%)
21-40	53 (23.3%)	16 (32.7%)
41-60	102 (44.9%)	14 (28.6%)
>60	60 (26.4%)	17 (34.7%)
Gender		
Male	141 (62.1%)	32 (65.3%)
Female	86 (37.9%)	17 (34.7%)

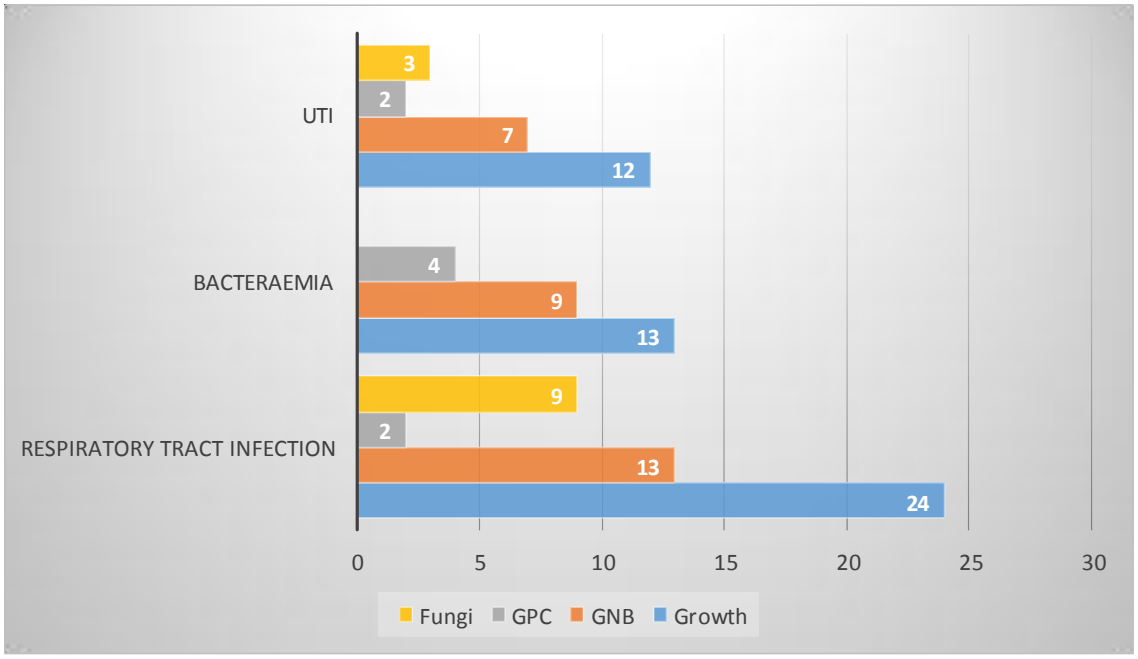

Fig 1: Distribution of secondary infection among COVID-19 infected patients.

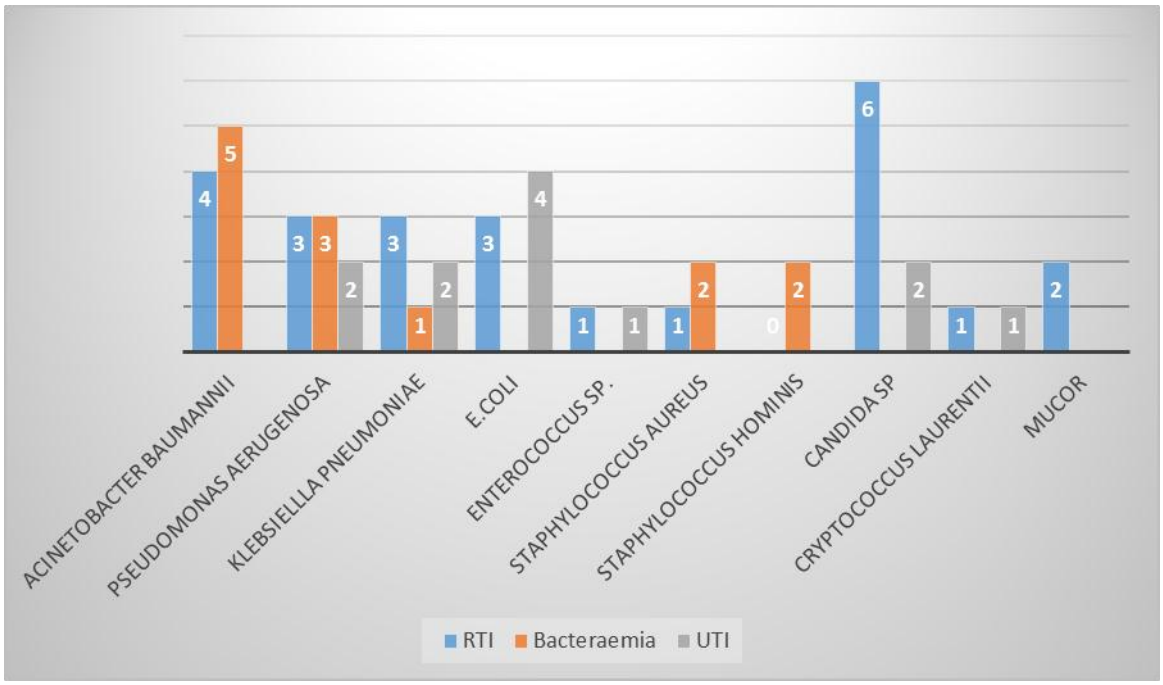

Fig 2: Distribution of pathogen.

Table 2: Antimicrobial resistance profile of clinical isolates causing secondary infections in COVID-19 patients.

	Acinetobacter	Klebsiella	Pseudomonas	Aeromonas	E.coli	Enterococcus spp.	S. aureus
Amikacin	100	60	66.66	0	100	NA	NA
Amoxicillin/clavulanic acid	100	80	66.66	100	100	NA	NA
Ampicillin	100	60	83.33	100	100	NA	NA
Cefepime	100	100	66.66	100	100	NA	NA
Cefeperazone/Sulbactum	100	100	66.66	100	100	NA	NA
Ceftazidime	100	100	83.33	100	100	NA	NA
Imipenem	100	60	50	100	50	NA	NA
Ciprofloxacin	100	100	83.33	100	100	100	50
Levofloxacin	100	100	83.33	100	100	75	50

Meropenem	100	60	50	100	50	NA	NA
Nitrofurantoin	100	100	16.66	100	25	75	NA
Piperacilline/Tazobactam	100	60	83.33	100	50	NA	NA
Tigecycline	100	100	100	100	100	NA	NA
Cotrimoxazole	100	80	100	100	100	NA	50
Colistin	0	0	0	0	0	NA	NA
Polymyxin	0	0	0	0	0	NA	NA
Chloramphenicole	0	0	0	0	0	75	50
Penicillin	NA	NA	NA	NA	NA	100	100
Clindamycin	NA	NA	NA	NA	NA	50	50
Vancomycin	NA	NA	NA	NA	NA	50	50

DISCUSSION

In the study total 227 COVID-19 positive patients were included, 21.58% had secondary infection. We reported three types of secondary infections in COVID-19 patients.

The pathogenesis of secondary infection in COVID-19 cases depend on virulence of pathogens, dysregulation of immune responses, and distributed microbiota during viral pneumoniae. Virus pneumonia and secondary infection acted as mutually reinforcing factors to promote the progression of COVID-19. Severe SARS-CoV-2 infection caused multiple damages in the lungs, which can largely decrease the oxygen and carbon dioxide diffusion capacities. The disruption of surfactant and the sloughing of cells into the airways may provide access and a rich source of nutrients, promoting rapid bacterial growth.[7] Both impact of microbiome change and bacteria virulence factor can alter the immune responses to SARS-CoV-2, resulting in the rebound of viral titre[8] and high mortality in severe and critical patients.

Respiratory infections were the most common secondary infection followed by blood and urinary tract infection in the present study. Yang et al and Zhou at al. reported 13.5% and 31% cases of pneumoniae in SARS-CoV-2 patients.[9,10] In the present study also 48.9% respiratory infections were found and *Candida, Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterococcus faecium, Staphylococcus aureus* were isolated.

Two meta analyses published so far from COVID-19 patients, documented rates of bacterial co-infections as 3.5% and 7%, respectively.[11,12] A multicentre study conducted by Sonam Vijay et.al found 3.6% of secondary infection in COVID-19 pateints while in our study its high.[13] Out of 227 patients, 49 (21.58%) had secondary infection. Overall, 49 (21.58%) pathogens were isolated from 227 clinical samples, among which, 24, 13, and 12 were isolated from respiratory, blood and urine samples respectively.

Respiratory and Blood infection were the most common sites of secondary infection in COVID-19 patients.[13] Gram negative pathogens were predominant in respiratory infections, with a significant proportion of fungi and Gram-positive pathogens. All the patients were hospitalized and prescribed steroids that reduce the immune system of the pateints. They have undergone invasive procedures and sometimes have a prolonged hospital stay, rendering them vulnerable to HAIs.[13]

Among Bacteraemia suspected patients, 13(10.1%) pathogens were isolated. Predominantly *Acinetobacter baumannii* were isolated followed by *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus hominis, CONS, Enterococcus faecalis.* Predominance of Gram-negative pathogens could be due to the invasive devices including central venous catheter during hospitalization of the patients.[13]

In urinary secondary infections, 12 pathogens from 12 patients were illustrated, including gram-negative bacteria, Gram positive and fungi. In the present study out of 49 patients with secondary infection, 3 patients were also reported to have multiple coinfection of bacterial and fungus. The presence of multiple infection conditions could have also contributed to the higher mortality seen in these patients.

Here, we have found Extreme drug resistant (XDR) Gram-negative pathogens (*A. baumannii*, *K. pneumoniae*, *P. aerugenosa*) were the predominant pathogens causing secondary infections in COVID-19 infected. XDR *A. baumannii* and *K. pneumoniae* constituted more than 60% of the isolates. They were sensitive to only polymyxin.

High isolation rates along with reduced susceptibility of Acinetobacter to drugs like piperacillin-tazobactam, cefoperazone sulbactam and carbapenems are alarming and need urgent containment measures through appropriate infection control and antimicrobial stewardship interventions.

CONCLUSION

Our study presents the proportion of secondary infections in COVID-19 patients. Patients tend to have secondary infections after receiving invasive respiratory ventilations. There is no doubt that the co-infection could be a significant promoter to the final mortality of COVID-19 patients. Health sectors should keep monitoring the situation, as the more we learn about this novel virus and its associated infections for better

treatment and health care. Extreme drug resistant against higher drugs also a alarming situation that need strict antimicrobial stewardship program from optimal selection of empiric treatment and de-escalation, based on culture reports.

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Declaration of interest
All authors report no conflict of interest.

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REFERENCES

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med, 2020; 382(8): 727–33.
2. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet, 2020; 395(10229): 1054–62.
3. Surbhi Khurana et.al. Profile of co-infections & secondary infections in COVID-19 patients at a dedicated COVID-19 facility of a tertiary care Indian hospital: Implication on antimicrobial resistance. Indian Journal of Medical Microbiology, 2021; 39: 147–153.
4. Lai CC, Wang CY, Hsueh PR: Co-infections among patients with COVID-19: The need for combination therapy with non-anti-SARS-CoV-2 agents? J Microbiol Immunol Infect, 2020.
5. Li ZT, Chen ZM, Chen LD, Zhan YQ, Li SQ, Cheng J, et al: Coinfection with SARS-CoV-2 and other respiratory pathogens in COVID-19 patients in Guangzhou, China. J Med Virol, 2020.
6. He S, Liu W, Jiang M, Huang P, Xiang Z, Deng D, et al. (2021) Clinical characteristics of COVID-19 patients with clinically diagnosed bacterial co-infection: A multi-center study. PLoS ONE, 16(4): e0249668.
7. Ghoneim HE, Thomas PG, McCullers JA.. Depletion of alveolar macrophages during influenza infection facilitates bacterial superinfections. *J Immunol*, 2013 Aug 1; 191(3): 1250–1259.
8. Haocheng Zhang et al. Risks and features of secondary infections in severe and critical ill COVID-19 patients. Emerg Microbes Infect, 2020; 9(1): 1958–1964.
9. Yang X, Yu Y, Xu J, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Respir Med*, 2020; 8(5): 475–481.
10. Zhou F, Yu T, Du R, et al. . Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan. China: a Retrospective Cohort Study. *The Lancet*, 2020; 395(10229): 1054–1062.
11. Langford BJ, So M, Raybardhan S, et al. Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. Clin Microbiol Infect, 2020; 26(12): 1622–1629.
12. Lansbury L, Lim B, Baskaran V, Shen lim W. Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect, 2020; 81(2): 266–275.
13. Sonam Vijay, Nitin Bansal et al. Secondary Infections in Hospitalized COVID-19 Patients: Indian Experience. Infection and Drug Resistance, 2021; 14: 1893–1903.