DOI: 10.7860/JCDR/2022/51598.16057

Original Article

Microbiology Section

Microbiological Evaluation of Patients Admitted with Acute Respiratory Illness during First Wave of COVID-19 Pandemic in New Delhi, India

GITALI BHAGAWATI[1], SANIA PAUL[2], SARITA RANI JAISWAL[3], ASHUTOSH BHARDWAJ[4], REKHA SAJI KUMAR[5], MANSI[6], ANITA BHATIA[7], SUPARNO CHAKRABARTI[8]

ABSTRACT

Introduction: The Coronavirus Disease-2019 (COVID-19) is associated with damage of cells of both innate and adaptive immunity, which results in immune system's impairment leading to secondary infections. Microbiological evaluation helps in diagnostic as well as antimicrobial stewardship leading to accurate treatment of COVID-19 infected patients.

Aim: To evaluate superadded bacterial and fungal infections in COVID-19 infected patients and to evaluate bacterial and fungal infections in COVID-19 non infected patients admitted with Acute Respiratory Illness (ARI).

Materials and Methods: This retrospective study was carried out in a tertiary care hospital in Delhi, India, over a period of eight months (May to December 2020). Respiratory samples, received from indoor patients with history of ARI, were processed for COVID-19 (TrueNat based real time polymerase chain reaction) as well as for bacterial and fungal cultures following Standard Operating Procedures (SOP). Identification and susceptibility pattern was evaluated by Vitek2 compact system (bioMérieux, Inc. Durham, North Carolina/USA). Quality control strains used were American Type Culture Collection (ATCC) *Staphylococcus aureus* 29213, *Escherichia coli* 25922 and *Candida parapsilosis* ATCC 22019. Minimum Inhibitory Concentration (MIC) levels were standardised as per Clinical and Laboratory Standards Institute (CLSI) guideline 2020. All statistical analysis was done by Chi-square test using Software Statistical Package for the Social Sciences (SPSS) version 22.0.

Results: Total patients admitted with the history of ARI were 542; COVID-19 Positive Group (CPG) included 115 (21.22%) while COVID-19 Negative Group (CNG) included 427 (78.78%). Growth in bacterial and fungal cultures in CPG was 59.13% (68/115) while in CNG; it was 47.78% (204/427). Among the bacterial isolates, most common isolate was *Klebsiella pneumoniae* {CPG: 41.93% (26/62); CNG: 36.72% (76/207)}, followed by *Pseudomonas aeruginosa* {CPG: 33.87% (21/62); CNG: 31.88% (66/207)}. Fungal isolates in CPG was 19.48% (15/77) (p-value 0.0445). On comparing Antimicrobial Susceptibility (AST) pattern of Enterobacterales in both CPG (n=36) and CNG (n=102), no statistically significant difference was observed. Co-morbid conditions were found mostly in CNG 89% (140/158) with ARI while only 11% (18/158) was found in CPG.

Conclusion: Secondary respiratory infections are quite common amongst COVID-19 positive patients. However, growth in culture, type of isolates, Antimicrobial Resistance (AMR) was almost similar with COVID-19 non infected patients admitted with ARI. Co-morbidity had the similar impact as COVID-19 infection with respect to co-infections.

Keywords: Co-infection, Coronavirus disease-2019 infection, Respiratory tract infection, Superinfection, TrueNat real time polymerase chain reaction

INTRODUCTION

The coronavirus pandemic is a biggest global health threat that we have faced after the Second World War with 42,32,949 confirmed cases and 1,02,896 deaths till date [1]. The COVID-19 illness has demonstrated variability in severity, from asymptomatic or mildly symptomatic to Acute Respiratory Distress Syndrome (ARDS) and Multi-Organ Failure (MOF) [2]. This disease is associated with damage of B cells, T cells and Natural Killer (NK) cells, which leads to the immune system's impairment leading to secondary infections [3]. Secondary infections can be superinfections or co-infections. Superinfection is defined as an infection following a previous infection especially when caused by microorganisms that are resistant or have become resistant to the antibiotics used earlier, while a co-infection is one occurring concurrently with the initial infection, the difference being purely temporal [4-6].

These secondary infections can raise the difficulties of diagnosis, treatment, prognosis of COVID-19 and even increase the morbidity and mortality [7]. Therefore, simultaneous evaluation of co-infections in COVID-19 infected patients is necessary so that one can provide a better patient treatment [8]. There are many published reports of respiratory co-infections and superinfections in COVID-19 patients especially in the hospitalised patients [3,8-10].

As the world continues to respond to COVID-19, there is a larger hidden threat of AMR lurking behind, one that is already killing hundreds of thousands of people globally (about 700000 deaths annually) [11]. Moreover, AMR amongst the pathogens causing secondary infections is also a hidden threat lurking behind COVID-19 [11]. However, this should be noted that in the pre-COVID era, the rise in Multidrug-Resistant Organisms (MDROs), related to AMR, were undetected, undiagnosed, and increasingly untreatable threatening the health of people globally projecting death of 10 million people per year by 2050. During COVID-19 pandemic, antibiotics were rampantly used which again exacerbated the prevailing AMR as shown by United States (US) multicentre study reporting 72% of COVID-19 patients received antibiotics without indication [12].

This study was to analyse superadded bacterial and Yeast and Yeast Like Fungus (YYLF) infections apart from COVID-19 infection in patients admitted with ARI. Here, authors also compared the microbiological isolates and their AST of two groups of patients: COVID-19 infected patients and COVID-19 non infected patients.

MATERIALS AND METHODS

The retrospective study was carried out amongst hospitalised patients at a tertiary care hospital in Delhi, India, over a period

of eight months (May to December 2020), during the 1st wave of COVID-19 in India. Data was retrieved from the Microbiology and Molecular Department of the hospital from the sample request forms, Laboratory Information Management System (LIS) and WHONET 5.6 software. Analysis of the study was done from the collected data during the declined phase of 1st wave of COVID-19 infection in India over a period of three months (January to March 2021).

To rule out secondary infections, non duplicate respiratory samples were subjected to culture in microbiology laboratory. Respiratory samples comprised of sputum, Bronchoalveolar Lavage (BAL) and Endotracheal Aspirate (ET). Sample size was based on duration of study period including 1st wave of COVID-19 in India, from May to December 2020. Microbiological isolates were reviewed along with their susceptibility pattern.

Inclusion criteria:

a. Sputum: Samples showing Bartlett's score more than 1 [13].

b. BAL: Colony count ≥104 CFU/mL in quantitative culture [14,15].

c. ET: Colony count ≥10^5 CFU/mL in quantitative ET culture [15].

Exclusion criteria:

a. Salivary sample with Bartlett's score less than 1 [13].

b. >1% bronchial cells in BAL fluid smears [15].

c. >10 squamous cells in the lower field magnification in ET smears [15].

Identification and Susceptibility Testing

All the samples were inoculated on routine culture media like Blood Agar (BA), Macconkey Agar (MA) and Chocolate Agar (CA). YYLF easily grows in routine culture media used for bacterial culture. However, for pure growth to check further identification and antifungal susceptibility pattern colonies were inoculated on two Sabouraud's Dextrose Agar (SDA) slants; one was incubated at room temperature and other at 37°C for 24-48 hours. Identification of bacteria was done by gram stain, motility test and other biochemical tests as per standard protocol [16]. For identification YYLFs, gram stain, Lactophenol Cotton Blue (LPCB) test, germ tube test were performed [17]. The final identification and antifungal susceptibility tests were performed by Identification cards and AST cards respectively using Vitek 2 Compact System 8.01 (bioMérieux, Inc. Durham, North Carolina/USA). Control strains used were: *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Candida parapsilosis* ATCC 22019.

The TrueNat based RT-Rt PCR for Detection of COVID-19 [18,19]

All COVID-19 tests were done by Truenat Rt RT-PCR test. Samples taken were oropharyngeal or nasopharyngeal swab collected using standard nylon flocked swab. Swab is inserted into the Viral Transport Medium (VTM) were provided from the same company (Molbio diagnostics Pvt., Ltd., Goa, India). Samples were transported immediately to the molecular laboratory maintaining proper temperature and processed as per manufacturer's guideline. (Truenat Beta CoV Chip-based RT-PCR test for Beta Coronavirus, Molbio diagnostics Pvt., Ltd., Goa, India).

The target sequence for this assay is *E* gene of Sarbecovirus and human RNaseP (serves as internal positive control). Confirmatory gene used was *RdRP* gene or *ORF1a* gene.

STATISTICAL ANALYSIS

All statistical analysis was done by Chi-square test using Software Statistical Package for the Social Sciences (SPSS) version 22.0. The outcome was determined to be significantly different if the observed p-value was <0.05.

RESULTS

Total patients suspected of ARI were 542; out of which 115 (21.22%) were found to be positive for COVID-19 by TrueNat based RT-PCR CPG, while rest 427 (78.78%) were found to be CNG. Being a known cancer care hospital, cancer treatment was also going on simultaneously irrespective of COVID-19 status of the patients. Since, COVID-19 infects irrespective of immunocompromised status, control group was taken from the cancer patients.

In both CPG and CNG, males predominated over females, 88 (76.52%) and 317 (74.24%) respectively. Overall, age group of the study varied from 4-91 years with a mean±Standard Deviation (SD) age of 59.70±14.73 years, median age was 62. However, age group range of CPG was 20-91 years with a mean±SD age of 61.30±14.75 years, median age 63 and that of CNG ranged from 4-88 years with a mean±SD age of 59.24±14.74 years, median age 61. No statistically significant variation was found on comparing both the groups with respect to age.

Respiratory culture results were divided into three categories:

a. Significant Growth (SG)

b. Insignificant Growth (IG)

c. No growth (NG)

In case of culture with SG, the growth was further processed for final identification and antimicrobial/antifungal susceptibility testing. No further processing was done in case of IG or NG.

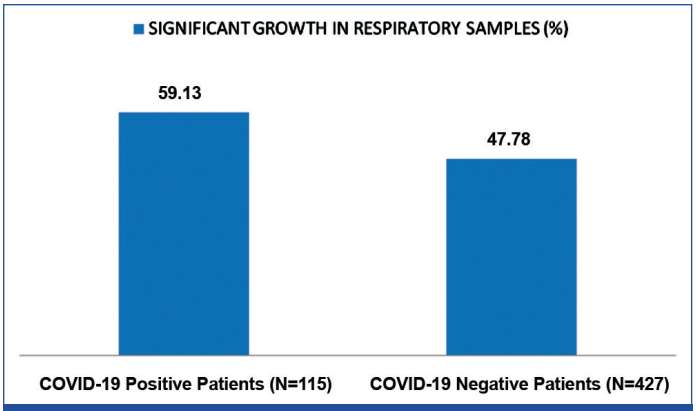
In CPG, SG in sputum and ET cultures was 43/79 (54.43%) and 25/34 (73.53%) respectively. No SG was seen in BAL. In CPG, the number of cultures with SG in overall respiratory samples (Sputum, BAL and ET) was found to found to be 68/115 (59.13%). This depicts rate of secondary infection in SARS-Cov-2 patients was 59.13% [Table/Fig-1,2].

On comparing significant growth (SG) in sputum, BAL and ET cultures in both CPG and CNG, no statistically significant difference (p-value >0.05) was observed [Table/Fig-1,2]. Number of respiratory cultures with SG in CPG was 68. The SG in culture was either of single isolate or of double isolates.

Sample	Growth type	All patients (n=542)	CPG (n=115)		CNG (n=427)		p-value
		Total No.	No.	%	No.	%	
Sputum	SG	167	43	54.43	124	42.47	0.0740
	NCF	204	36	45.57	168	57.53	
	NG	0	0	0.00	0	0.00	
	Total	371	79	100.00	292	100.00	
BAL	SG	3	0	0	3	17.65	1
	NCF	11	2	100	9	52.94	
	NG	5	0	0	5	29.41	
	Total	19	2	100	17	100.00	
ET	SG	102	25	73.53	77	65.25	0.4135
	NCF	32	6	17.65	26	22.03	
	NG	18	3	8.82	15	12.71	
	Total	152	34	100.00	118	100.00	

[Table/Fig-1]: Comparative analysis of outcome of respiratory samples in both COVID-19 Positive Groups (CPG) and COVID-19 Negative Groups (CNG).
BAL: Bronchoalveolar lavage; ET: Endotracheal aspirate; SG: Significant growth; NCF: Normal commensal flora; NG: No growth

Number of total isolates (bacterial and or fungal) in CPG was 77 while that in CNG it was 232. Cultures with single isolate in CPG were 59/68 (86.76%) while the rest showed growth of double isolates 9/68 (13.23%). Cultures with double isolates in CNG were 34/204 (16.67%). No statistically significant difference was observed in both CPG and CNG with respect to number of isolates in cultures [Table/Fig-3].



[Table/Fig-2]: Distribution of significant growth in respiratory isolates in both COVID-19 Positive Groups (CPG) and COVID-19 Negative Groups (CNG).

When CPG and CNG groups were compared as per Gram Negative (GN), Gram Positive (GP) and YYLF isolates, no such statistically significant difference was observed with respect to GN and GP. However, as per YYLF infection, CPG group isolation rate 15/77 (19.48) which was found to be statistically significant with p-value 0.0445 [Table/Fig-4].

Cultures with growth	COVID-19 negative group	%	COVID-19 positive group	%	p-value
Single isolate	59	86.76	170	83.33	0.57
Double isolate	9	13.24	34	16.67	0.57
Total cultures with significant growth	68	100	204	100	

[Table/Fig-3]: Distribution of cultures with growth of single/double isolates in COVID-19 positive and COVID-19 negative groups.

Overall bacterial isolate (Gram positive and gram negative) in CPG was 62 (80.52%) and in CNG it was 207 (89.22%) [Table/Fig-4]. Among the bacterial isolates, most common isolate in CPG was *Klebsiella pneumoniae* 26/62 (41.93%), followed by *Pseudomonas aeruginosa* 21/62 (33.87%) and *Escherichia coli* 9/62 (14.52%). Similarly, in CNG also, *Klebsiella pneumoniae* 76/207 (36.72%) was the predominant isolate followed by *Pseudomonas aeruginosa* 66/207 (31.88%) and *Escherichia coli* 20/207 (9.66%) [Table/Fig-5].

Isolates	COVID-19 positive group	%	COVID-19 negative group	%	p-value
Gram negative isolates (246)	60	77.92	186	80.17	0.625
Gram positive isolates (23)	2	2.60	21	9.05	0.0781
Yeast and yeast like fungus isolates (40)	15	19.48	25	10.78	**0.0445**
Total (309)	77	100	232	100	

[Table/Fig-4]: Comparative analysis of co-infection by gram negative, gram positive and Yeast And Yeast Like Fungus (YYLF) in COVID-19 positive and COVID-19 negative patients.

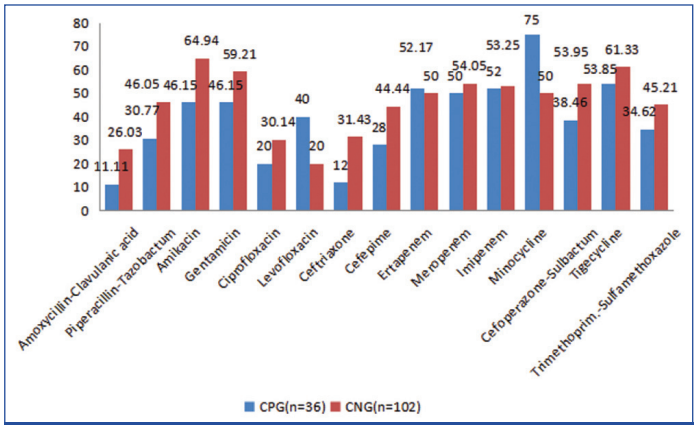
Bacterial isolates	CPG isolates (n=62)	CPG isolates (%)	CNG isolates (n=207)	CNG isolates (%)
Klebsiella pneumoniae (102)	26	41.93	76	36.72
Pseudomonas aeruginosa (87)	21	33.87	66	31.88
Escherichia coli (29)	9	14.52	20	9.66
Streptococcus pneumoniae (14)	2	3.23	12	5.80
Acinetobacter baumannii (11)	2	3.23	9	4.35
Stenotrophomonas maltophilia (6)	1	1.61	5	2.42
Staphylococcus aureus (6)	0	0	6	2.90
Serratia marcescens (3)	0	0	3	1.45
Enterococcus spp. (3)	0	0	3	1.45
Enterobacter cloacae (2)	1	1.61	1	0.48
Chryseobacterium indologenes (2)	0	0	2	0.97
Citrobacter koseri (1)	0	0	1	0.48
Proteus mirabilis (1)	0	0	1	0.48
Sphingomonas paucimobilis (1)	0	0	1	0.48
Burkholderia cepacia (1)	0	0	1	0.48
Total bacterial isolates (269)	62	100.00	207	100.00

[Table/Fig-5]: Distribution of bacterial isolates in both COVID-19 positive and COVID-19 negative patients.

Out of YYLFs, *C. albicans* was the predominating isolate in both CPG and CNG, 8/15 (53.33%) and 14/25 (56%), respectively. This was followed by *C. tropicalis*, 6/15 (40%) in CPG and 5/25 (20%) in CNG [Table/Fig-6]. On comparing AST pattern of Enterobacterales in both CPG (n=36) and CNG (n=102), no statistically significant difference was observed [Table/Fig-7].

Fungal isolates	CPG isolates (n=15)	CPG isolates (%)	CNG isolates (n=25)	CNG isolates (%)
Candida albicans (22)	8	53.33	14	56.00
Candida tropicalis (11)	6	40.00	5	20.00
Candida duobushaemulonii (2)	0	0	2	8.00
Candida parapsilosis (1)	0	0	1	4.00
Candida auris (2)	1	6.67	1	4.00
Candida famata (1)	0	0	1	4.00
Candida glabrata (1)	0	0	1	4.00

[Table/Fig-6]: Distribution of Yeast and Yeast Like Fungus (YYLF) in both COVID-19 positive and COVID-19 negative patients.



[Table/Fig-7]: Antimicrobial Susceptibility (AST) pattern of Enterobacterales in both COVID-19 Positive Groups (CPG) and COVID-19 Negative Groups (CNG). Enterobacterales include *Klebsiella pneumoniae* (CPG 26; CNG 76), *Escherichia coli* (CPG 9; CNG 20), *Serratia marcescens* (CPG 0; CNG 3), *Enterobacter cloacae* (CPG 1; CNG 1), *Citrobacter koseri* (CPG 0; CNG 1) and *Proteus mirabilis* (CPG 0; CNG 1).

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infects people irrespective of the immune status of the host. In present study, co-morbidity was mostly associated with CNG 140/158 (89%)with ARI while only 18/158 (11%) was found in CPG. Type of cancer was not found to be statistically significant with respect to COVID-19 infection, except the lung cancer (p-value=0.003) [Table/Fig-8].

Co-morbid condition (n=158)	COVID-19 positive group (n=18)	Percentage (%)	COVID-19 negative group (n=140)	Percentage (%)	p-value
Oropharyngeal cancer (46)	4	22.22	42	30.00	0.5910
Lung cancer (11)	5	27.78	6	4.29	**0.0033**
Gastrointestinal cancer (12)	0	0	12	8.57	0.3628
Genitourinary cancer (5)	0	0	5	3.57	1

Chronic kidney disease (20)	4	22.22	16	11.43	0.2497
Hypertension (8)	0	0	8	5.71	0.5988
Transplant patients (2)	0	0	2	1.43	1
Haematopoietic disorder (8)	1	5.56	7	5.00	1
Microvascular coronary disease (5)	0	0	5	3.57	1
Diabetes mellitus (12)	2	11.11	10	7.14	0.6294
Cavitary lung disease (20)	0	0	20	14.29	0.131
Breast cancer (4)	0	0	4	2.86	1
Cancer of unknown origin (5)	2	11.11	3	2.14	0.0998

[Table/Fig-8]: Distribution of co-morbid conditions amongst the COVID-19 positive and COVID-negative patients admitted with acute respiratory symptoms.

Among expired patients of COVID-19, 10/16 (62.5%) had SG in respiratory culture. However, on comparing mortality, no statistically significant variation was observed in CPG and CNG with SG in respiratory samples [Table/Fig-9].

Expired patients	Covid-19 positive group (n=16)	%	Covid-19 negative group (n=115)	%	p-value
Expired with SG in RS	10	62.5	73	63.48	1
Expired patients without SG in RS	6	37.5	42	36.52	
Total	16	100	115	100.00	

[Table/Fig-9]: Distribution of expired patients with and without significant growth (SG) in Respiratory Samples (RS).

DISCUSSION

During the 1st wave of SARS-Cov-2 pandemic in India, a total 542 patients were admitted with ARI in our hospital that underwent COVID-19 test by TrueNat based RT-PCR. Out of these patients, 115/542 (21.22%) were laboratory confirmed COVID-19 positive patients. In present study, median age of SARS-Cov-2 infected patients was 63 (20-91 year) which was almost similar to the finding of Zhang JJ et al., 57 years and Contou D et al., 61 years [20,21]. Present study found 76.52% males in CPG which was similar the finding of Contou D et al., 73% but dissimilar to the finding of Zhang JJ et al., 56% [20,21]. However, when CPG and CNG groups were compared with respect to age and sex, no statistically significant difference was observed. Significant growth in respiratory samples in CPG, 68/115 (59.13%) was considered as the secondary infection rate in that group. Secondary infection rate in SARS-Cov-2 positive patients varied in literature; range varied from, 28% by Contou D et al., 34.8% by Hughes S et al., and 11% by Huttner B et al., [21-23]. Low rate of isolation of secondary infections in previous literatures were because of many reasons. First, the scare associated with the pandemic at its onset along with the high workload, high levels of stress amongst healthcare workers for which secondary infections might not get the importance. Secondly, the guidelines were particularly made for COVID-19 sample collections but not for respiratory sample collection for microbiological evaluation under proper safety precaution to avoid droplet infection. Third, adequate microbiological set ups were not available in most of the hospitals worldwide for proper evaluation. Forth, shortage of Personal Protective Equipment (PPEs) at the onset of the pandemic made it difficult for biosample collection for better diagnostic evaluation. As time passed with the pandemic, acceptance level of COVID-19 had increased, availability of PPEs were increased and patients were properly evaluated for rest of the superadded infections in those hospitals where adequate facilities were available. The same reasons were applicable to the mortality rate amongst CPG; out of 16 deaths, 10 had co-infections (62.5%) [Table/Fig 9]. On contrary, Huttner B et al., mentioned that in Italy 16,654 patients who died of COVID-19 had superadded infections in only 11% [23].

In present study, gram negative isolates predominated (77.92%) over YYLFs (19.48%) and gram positives (2.60%) isolates in CPG. Enterobacterales isolated among CPG in present study was 36/77 (46.75%) which was similar to finding of Hughes S et al., 32% while dissimilar to finding of Contou D et al., 16% [21,22]. Amongst Enterobacterales, *Klebsiella pneumoniae* 26/77 (33.77%) was the most common respiratory tract isolate in COVID positive patients which was in concordance with Chen X et al., *Pseudomonas aeruginosa* was also second most common isolate, 21/77 (27.27%) in Covid positive patients which is in concordance to other findings of Hughes S et al., 36% but dissimilar to the finding of Contou D et al., 6%. No *S. aureus* was isolated from respiratory samples in present study which was in contrast to other findings; Hughes S et al., 31% and Sharifipour E et al., 10% [22,24]. Isolation rate of *Acinetobacter baumannii*, one of the most common hospital acquired respiratory isolate was 2/77 (2.6%) in present study. This was in contrast to some literatures; Sharifipour E et al., 90%, Wang Z et al., 20% [24,25] while similar to other literatures; Contou D et al., 3% and Lansbury L et al., 7.40% [21,26]. Bacterial isolates and related AMR in both the groups had no statistically significant difference (p-value >0.05). This implies that AMR was a global crisis which was prevailing before the onset of COVID-19 pandemic. Antibiotic use in inappropriate dose and duration at inappropriate indication with inadequate infection control practices increased the burden of AMR in pre-COVID era itself. That is why, in September 2016 (years prior to the emergence of the COVID-19 pandemic), the World Health Organisation (WHO) committed itself to fighting AMR, which had become a problem of global public health importance [27]. Various COVID-19 management protocols itself had heightened the concern of AMR over the prevailing crisis.

In CPG, YYLF was the second most common respiratory isolate (19.48%) which was almost similar to the finding of other literature; Hughes S et al., 21.81% [22]. On comparing with CNG, YYLFs were found to be statistically significant amongst CPG with p-value 0.0445. *C. albicans* predominated amongst all YYLF (53%, 8/15) which was in concordance with one study, Salehi M et al., 70% [28]. COVID-19 infection is associated with over-expression of inflammatory cytokines, and impaired cell-mediated immune response with decreased CD4+T and CD8+T cell counts, indicating its susceptibility to fungal co-infection [29]. Other reasons for YYLF infection include use of steroid, monoclonal antibodies, anti-virals, anti-malarials and use of broad spectrum antibiotics amongst moderate to severe forms of COVID-19 infected patients in the hospitalised group. In present study, co-morbidity in CPG was less (11%) as compared to CNG (89%) as because majority of our patients in CNG were immunocompromised patients with cancer or chronic kidney diseases. In CPG, present study finding was in contrast to the findings various literatures [20,30].

Limitation(s)

Since, the study was done retrospectively, samples processed for YYLFs were only included in this study. Other fungal isolates were excluded.

CONCLUSION(S)

Secondary respiratory infections are common in SARS-Cov-2 infected patients. Bacterial flora of COVID-19 infected patients is not different than that of rest of the patients with respiratory illness. However, YYLF infections in COVID-19 infected patients were found to be more because of the association of the viral strain itself and its management with steroid, broad spectrum antibiotics, monoclonal antibodies etc. AMR is a raising global concern in pre-COVID-19 era; which was

exacerbated by the COVID-19 pandemic. This novel virus seems to stay with human being infecting in the form outbreak, endemic or epidemic in future. Therefore, microbiological evaluation with AST of the isolates with respect to antimicrobial stewardship is the utmost need of the hour to preserve some antibiotics as well as anti-fungals for near future.

REFERENCES

[1] WHO Coronavirus (COVID-19) Dashboard (https://covid19.who.int). Accecced date: 18th January, 2021.
[2] Choubey A, Sagar D, Cawley P, Miller K. Retrospective review analysis of COVID-19 patients co-infected with *Mycoplasma pneumoniae*. Lung, India. 2021;38(7):22-26.
[3] Chen X, Liao B, Cheng L, Peng X, Xu X, Li Y, et al. The microbial coinfection in COVID-19. Appl Microbiol Biotechnol. 2020;104(18):7777-85.
[4] Antibiotic resistance threats in the United States, 2013. US Department of Health and Human Services. Centers for Disease Control and Prevention [https://www. cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf]. [Accecced date: 20th January, 2021].
[5] Kwon WJ, Li G, Zheng M, Kaur H, Magbual N, Dalai S. Superinfections and coinfections in COVID-19 – separating the signal from the noise. Medpage Today. 2020;28. [https://www.medpagetoday.com/infectiousdisease/covid19/861].
[6] Feldman C, Anderson R. The role of co-infections and secondary infections in patients with COVID-19. Pneumonia. 2021;13(1):5.
[7] Ruuskanen O, Lahti E, Jennings LC, Murdoch DR. Viral pneumonia. Lancet. 2011;377(9773):1264-75.
[8] Roh KH, Kim YK, Kim SW, Kang ER, Yang YJ, Jung SK, et al. Coinfections with respiratory pathogens among COVID-19 patients in Korea. Can J Infect Dis Med Microbiol. 2021;2012:6651045.
[9] Khurana S, Singh P, Sharad N, Kiro VV, Rastogi N, Lathwal A, et al. Profile of co-infections and secondary infections in COVID-19 patients at a dedicated COVID-19 facility of a tertiary care Indian hospital: Implication on antimicrobial resistance. Indian J Med Microbiol. 2020;39(2):147-53. [published online ahead of print, 2020 Nov 16] Doi:10.1016/j.ijmmb.2020.10.014.
[10] Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. Lancet. 2020;395(10223):507-13.
[11] Adebisi AY, Alaran AJ, Okereke M, Oke GI, Amos OA, Olaoye OC, et al. COVID-19 and antimicrobial resistance: A review. Infect Dis (Auckl). 2021;14:11786337211033870. [https://doi.org/10.1177/11786337211033870].
[12] Strathdee SA, Davies SC, Marcelin JR. Confronting antimicrobial resistance beyond the COVID-19 pandemic and the 2020 US election. The Lancet. 2020;396:10257.
[13] Wong LK, Barry AL, Horgan SM. Comparison of six criteria for judging the acceptability of sputum specimens. J Clin Microbiol. 1982;16(4):627-31.
[14] Zaccard CR, Schell RF, Spiegel CA. Efficacy of bilateral bronchoalveolar lavage for diagnosis of ventilator associated pneumonia. J Clin Microbiol. 2009;47(9):2918-24.
[15] de Amorim Correa R, Luna CM, dos Anjos JCFV, Barbosa EA, de Rezende CJ, Rezende AP, et al. Quantitative culture of endotracheal aspirate and BAL fluid samples in the management of patients with ventilator associated pneumonia: A randomized clinical trail. J Bras Pneumologia. 2014;40(6):643-51.
[16] LJR Milne. Fungi. Mackie & McCartney Practical medical Microbiology. J.G. Colle, A.G. Fraser, B.P. Marmion, A. Simmons Elsevier 14th Edition 2008: 695-717.
[17] Chander J. Fungal Culture Media. Textbook of Medical Mycology. 3rd Edition. 2009: 514-536.
[18] Gupta N, Rana S, Singh H. Innovative point of care molecular diagnostic test for COVID-19 in India. Lancet Microbe. 2020;1(7):e277.
[19] Truenat SARS-Cov-2 Chip based Real Time PCR Test for COVID-19. [http://www.molbiodiagnostics.com]. [Accecced date: 15th July, 2020].
[20] Zhang JJ, Cao YY, Dong X, Wang BC, Liao MY, Lin J et al. Distinct characteristics of COVID-19 patients with initial rRT-PCR-positive and rRT-PCR-negative results for SARS-CoV-2. Allergy. 2020;75(7):1809-12.
[21] Contou D, Claudinon A, Pajot O, Micaëlo M, Longuet Flandre P, Dubert M, et al. Bacterial and viral co-infections in patients with severe SARS-CoV-2 pneumonia admitted to a French ICU. Ann Intensive Care. 2020;10:119.
[22] Hughes S, Troise O, Donaldson H, Mughal N, Moore LSP. Bacterial and fungal coinfection among hospitalised patients with COVID-19: A retrospective cohort study in a UK secondary-care setting. Clin Microbiol Infect. 2020;26(10):1395-99.
[23] Huttner B, Catho G, Pano-Pardo JR, Pulcini C, Schouten J. COVID-19: Don't neglect antimicrobial stewardship principles! Clin Microbiol Infect. 2020;26(7):808-10.
[24] Sharifipour E, Shams S, Esmkhani M, Khodadadi J, Fotouhi-Ardakani R, Koohpaei A, et al. Evaluation of bacterial co-infections of the respiratory tract in COVID-19 patients admitted to ICU. BMC Infect Dis. 2020;20:646.
[25] Wang Z, Yang B, Li Q, Wen L, Zhang R. Clinical features of 69 cases with Coronavirus Disease 2019 in Wuhan, China. Clin Infect Dis. 2020: ciaa272.
[26] Lansbury L, Lim B, Baskaran V, Lim WS. Co-infections in people with COVID-19: A systematic review and meta-analysis. J Infect. 2020;81(2):266-75.
[27] WHO United Nations High-Level Meeting on Antimicrobial Resistance. [https://www.who.int/antimicrobial-resistance/events/UNGA-meeting-amrsept2016/en/]. [Accecced date: 20th December, 2020].
[28] Salehi M, Ahmadikia K, Mahmoudi S, Kalantari S, JamalimoghadamSiahkali S, Izadi A, et al. Oropharyngeal candidiasis in hospitalised COVID-19 Patients from Iran: Species identification and antifungal susceptibility pattern. Mycoses. 2020;63:771-78.
[29] Song G, Liang G, Liu W. Fungal co-infections associated with global COVID-19 pandemic: A clinical and diagnostic perspective from China. Mycopathologia. 2020;31:01-08.
[30] Yang, X, Yu Y, Xu J, Shu H, Xia J, Liu H, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: A single-centered, retrospective, observational study. The Lancet Respiratory Medicine. 2020;8(5):475-81.

PARTICULARS OF CONTRIBUTORS:

1. Consultant and Head, Department of Microbiology and Infection Control, Dharamshila Narayana Superspeciality Hospital, Narayana Health, New Delhi, India.
2. Senior Resident, Department of Microbiology, Dharamshila Narayana Superspeciality Hospital, New Delhi, India.
3. Program Director- Haploidentical BMT and Consultant- BMT and Haematology, Department of BMT, Dharamshila Narayana Superspeciality Hospital, Narayana Health, New Delhi, India.
4. Clinical Lead and Senior Consultant, Department of Critical Care Medicine, Dharamshila Narayana Superspeciality Hospital, Narayana Health, New Delhi, India.
5. Laboratory Technologist, Department of Microbiology, Dharamshila Narayana Superspeciality Hospital, Narayana Health, New Delhi, India.
6. Laboratory Technician, Department of Microbiology, Dharamshila Narayana Superspeciality Hospital, Narayana Health, New Delhi, India.
7. Infection Control Nurse, Department of Infection Control, Dharamshila Narayana Superspeciality Hospital, Narayana Health, New Delhi, India.
8. Head, Department of Blood and Marrow Transplantation, Dharamshila Narayana Superspeciality Hospital, New Delhi, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Gitali Bhagawati,
Consultant and Head, Department of Microbiology and Infection Control,
Dharamshila Narayana Superspeciality Hospital, Narayana Health, Vasundhara Enclave,
Near Ashok Nagar Metro Station, New Delhi, India.
E-mail: gitali.bhagawati.dr@narayanahealth.org

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Jul 28, 2021
- Manual Googling: Jan 14, 2022
- iThenticate Software: Jan 27, 2022 (13%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? NA
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Jul 27, 2021**
Date of Peer Review: **Sep 02, 2021**
Date of Acceptance: **Jan 17, 2022**
Date of Publishing: **Mar 01, 2022**