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Original Research Article

Microbiological profile of COVID-19 patients admitted in a tertiary care hospital Mathura, Uttar Pradesh, India

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ABSTRACT

Background: The pandemic due to severe acute respiratory syndrome corona virus-2 (SARS-CoV-2) has drawn worldwide worst effect with diagnostic challenge. Every investigation has its own importance for diagnosis, care, treatment and for management of corona virus disease-2019 (COVID-19) patients. Here this prospective study aimed to investigate the microbiological profile, prevalence of co-infection, and antibiotic susceptibility pattern of patients with confirmed SARS-CoV-2.
Methods: A total of 336 samples were processed in COVID laboratory, Department of Microbiology. An array of serological investigations was done by rapid card screening test. C-Reactive protein (CRP) was analyzed by nephelometer. Blood culture was done by automated system and urine culture on Cystine-Lactose-Electrolyte-Deficient (CLED) Agar. Antibiotic susceptibility tests were done by Kirby Bauer disc diffusion method.
Results: Out of 336 samples tested 76%were male and 24%were female. All samples tested were negative for HIV, HBsAg, HCV, syphilis, malarial parasite. CRP and Typhi -dot with IgM and IgG antibody were positive in 89.28% and 11.42% respectively. About 27% of COVID-19 patients showed bacterial and fungal co-infections. The most prevalent organisms were MR-CoNS (26%), *K. pneumoniae* (19%) and less prevalent were *P. aeruginosa* (6%) and *A. baumannii* (4%). *C. albicans* (11%) was the only isolated fungi. All gram positive isolates were 100% sensitive to Linezolid and vancomycin, among gram negative isolates, 100% were sensitive to colistin and polymyxin B.
Conclusions: Microbiological investigation for presence of other co-infecting agents among patients with COVID-19 infection should be considered, and prompt treatment should be carried out accordingly.

Keywords: COVID-19, Co-infection, SARS-CoV-2, CRP

INTRODUCTION

The corona virus disease 2019 (COVID-19) was first recognized in Wuhan, China, in December 2019. It rapidly spread across mainland China and became a global threat. As of Oct 10, 2020, the causative pathogen, namely severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) previously known as 2019 novel Corona virus, has infected 37,119,547 people and caused 1,072,825 deaths globally.[1,2] First case of COVID-19 infection was reported in Kerala, India, on January 27, 2020 who presented to the Emergency Department in General Hospital, Thrissur, Kerala, with a one-day history of dry cough and sore throat.[3] As of December 31, 2020, SARS-CoV-2 has infected 10,286,023 people and caused 148,968 deaths in India.[1] Advances in Modern medicine facilitated the early recognition of SARS CoV-2, and identification of potential treatments, such as lopinavir/ritonavir, chloroquine/hydroxychloroquine, and remdesivir.[4]

It is well established that seasonal viral respiratory tract infections have been linked to increased risk of bacterial coinfection and current evidence suggest that the innate

immune response against SARS-COV-2 can similarly compromise host defence against bacteria. Variable incidence of concurrent bacterial infection in COVID-19 has been reported.[5-9]

Identification of co-infections is very complicated. The organism itself might be carried by the patient before the viral infection, might be part of an underlying chronic infection, or might be picked up nosocomially increasing chances of hospital and ventilator acquired infections. Hence, initial diagnosis of co-infection is required, preferably using methods capable of detecting a broad range of potential pathogens and antimicrobial resistances, with subsequent monitoring for infection development. However, this should be an important concern for clinicians in the management of COVID-19 infections and should not neglect the possibility of other infections among COVID-19 patients. In India, such studies on the microbiological profile of COVID-19 patients is lacking, therefore we conducted a study which may play an important role for treating the COVID-19 patients with other infections and will give a clear idea to clinicians for better management of the patients which will reduce the morbidity and mortality. Therefore, this study aimed to investigate the microbiological profile, prevalence of co-infections and antibiotic susceptibility pattern of microbial agents in hospitalized patients of COVID-19.

METHODS

This prospective study was conducted among 336 COVID-19 patients admitted in K.D Medical Hospital and Research Center, a tertiary care hospital in the period from May - December 2020 following consecutive sampling technique. This study was carried out after taking approval from the institutional ethics committee. We included hospitalized COVID-19 patients of different age groups including children (confirmed by RT-PCR test). Pregnant women and Non-COVID Patients were excluded from the study. Blood samples were collected from 336 COVID-19 confirmed patients referred by the clinicians for microbiological investigations were included in this study. Appropriate safety measures were followed during collection of samples. These samples were processed in COVID laboratory, Department of Microbiology. The investigations include viral serology: HIV Tri-Dot, HCV Tri-Dot, Dengue Day 1 Test (J. Mitra and Co. Pvt. Ltd.), HBsAg (Diagnostic Enterprises) and other serological investigations like Typhoid IgG/IgM Rapid Test (CTK Biotech), Syphilis Ab Rapid test (CTK Biotech) and Advantage MAL CARD (J. Mitra and Co. Pvt. Ltd.) for Malaria parasite. Widal test (Beacon diagnostics Pvt Ltd.) was done using tube method. Tests for CRP was analyzed by nephelometer (Mispa i2). Details of the number of samples and investigations done were presented in Table 1. All the samples were tested following the manufacturer's instruction.

Blood and urine culture was done for 101 and 97 patients with suspected bacteremia and urinary tract infection respectively. Following all the aseptic precautions, blood samples were collected and inoculated directly into BD BACTECTM FX 40 (Becton, Dickinson Maryland USA) automated blood culture bottles and were monitored for bacterial growth. Positive blood cultures were subcultured on Blood agar and MacConkey agar (Hi-Media) and incubated at 37˚C for 24-48 hrs. Initial identification of the bacterial isolates was done by Gram staining, colony morphology and further identified by biochemical test. CLED Agar (Hi-Media) was used for urine culture. Antimicrobial susceptibility patterns were identified using Kirby Bauer disc diffusion method on Mueller Hinton agar. Antibiotic discs procured from Hi-Media were used and the susceptibility patterns of the isolates were interpreted following Clinical and laboratory standard Institute (CLSI) guidelines 2020.[10] The data obtained was entered in Epicollect5 and analyzed using JASP software. The resistance and sensitivity pattern of the isolates were compared using the paired t-test. P value of <0.05 was considered as statistically significant at 95% level of confidence interval.

RESULTS

Among 336 patients with COVID-19 infection majority, 76% (256/336) of the included cases were males and 24% (80/336) were females. All the samples investigated were negative for HIV, HBsAg, HCV, Syphilis and Malaria parasite. Only one case of Dengue-SARS-CoV-2 coinfection was reported. Majority, 89.28% (300/336) of the samples were positive for CRP. Typhi -dot with IgM and IgG antibody for Salmonella typhi was positive in 11.42% of patients. Details of the investigation and the percentage positivity are presented in Table 1.

Table 1: Serological investigations done for covid-19 positive patients.

Investigation	Total	Positive	%
Widal	32	14	43.75
Typhi dot	35	4	11.42
Dengue	29	1	3.44
CRP	336	300	89.28
MP	60	0	0
Syphilis	37	0	0
HIV	140	0	0
HCV	140	0	0
HBSAG	140	0	0

CRP- C-Reactive Protein, MP- Malaria parasite, HIV- Human Immunodeficiency Virus; HCV- Hepatitis C Virus, HBs Ag - Hepatitis B Virus Surface Antigen.

Out of 198 samples investigated for culture and sensitivity, 21% (42/101) of blood samples and 6% (12/97) of urine samples were positive.

Table 2: Organisms isolated from blood and urine culture of COVID-19 positive patients.

Isolated organism	No. of isolate from blood culture	%	No. of isolate from urine culture	%	Total	%
MRSA	5	13.5	1	5.8	6	11
MR-CoNS	14	37.8	0	0	14	26
Streptococcus pneumoniae	4	10.8	0	0	4	7
CoNS	4	10.8	0	0	4	7
Acinetobacter	2	5.4	0	0	2	4
Escherichia coli	1	2.7	4	23.5	5	9
Klebsiella pneumoniae	4	10.8	6	35.2	10	19
Pseudomonas aeruginosa	3	8.1	0	0	3	6
Candida albicans	0	0	6	35.2	6	11
Total	37	68.5	17	31.5	54	100

MRSA- Methicillin resistant Staphyloccus aureus, MR-CoNS-Methicillin resistant Coagulase Negative staphylococcus, CoNS-Coagulase Negative staphylococcus

Table 3: Antimicrobial susceptibility pattern of bacteria isolated from COVID-19 positive cases.

Name of the antibiotics	Frequency of sensitive isolates, n (%)							
	CoNS (n=4)	**MR-CoNS (n=14)**	**Strepto-coccus n=4**	**MRSA n=6**	**Acinetobacter (n=2)**	***Escherichia coli* (n=5)**	**Klebsiella n=10**	**Pseudo-monas n=3**
Azithromycin	4 (100)	0	2 (50)	2 (33)	-	-	-	-
Erythromycin	1 (25)	0	0	0	-	-	-	-
Clindamycin	2 (50)	4/ (28)	1 (25)	0	-	-	-	-
Moxifloxacin	4 (100)	12 (86)	2 (50)	5 (83)	-	-	-	-
Cefoxitin	4 (100)	0	-	0	-	-	-	-
Chloramphenic	2 (50)	6 (42)	1 (25)	0	-	3 (60)	0	-
Cotrimoxazole	1 (25)	8 (57)	0	0	1 (50)	0	2 (20)	-
Linezolid	4 (100)	12 (86)	3 (75)	6 (100)		-	-	
Teicoplanin	2 (50)	11 (83)	3 (75)	6 (100)	-	-	-	-
Nitrofurantoin	-	-	-	-	-	4 (80)	8 (80)	-
Levofloxacin	2 (50)	9 (66)	2 (50)	4 (66)	1 (50)	3 (60)	6 (60)	2 (66)
Imipenem	-	-	4 (100)	-	2 (100)	4 (80)	8 (80)	1 (33)
Meropenem	-	-	4 (100)	-	2 (100)	4 (80)	8 (80)	2 (66)
Amoxicillin		0	1 (25)	0	-	-	-	-
Vancomycin	4 (100)	14 (100)	4 (100)	6 (100)	-	-	-	-
Cefepime	0	-	2 (50)	-	-	0	6 (60)	2 (66)
Tobramycin	-	-	-	-	1 (50)	3 (60)	6 (60)	-
Amikacin	-	-	-	-	1 (50)	3 (60)	6 (60)	-
Ceftazidime	-	-	-	-	1 (50)	2 (40)	2 (20)	3 (100)
PIT	-	-	-	-	0	4 (80)	6 (60)	100
CAC	-	-	-	-	0	3 (60)	0	2 (66)
Aztreonam	-	-	-	-	-	2 (40)	4 (40)	-
Colistin	-	-	-	-	100	100	100	100
Polymixin-b	-	-	-	-	100	100	100	100
Fosfomycin	-	-	-	-	-	100	100	-
Ceftrixone	--	-	-	-	100	3 (60)	3 (25)	-
ELO	-	-	-	-	100	100	100	100
Ofloxacin	-	-	-	-		0	0	100
Ciprofloxacin	1 (25)	8 (57)	3 (75)	3 (50)	100	4 (80)	2 (20)	2 (66)
Tetracycline	0	-	2 (50)		1 (50)	4 (80)	6 (60)	-

CoNS-Coagulase Negative staphylococcus, MR-CoNS-Methicillin resistant Coagulase Negative staphylococcus, MRSA- Methicillin resistant Staphyloccus aureus, PIT-Piperacillin Tazobactam, CAC-Ceftazidime clavulanate, ELO-Ceftriaxone sulbactam disodium edetate.

The common organisms isolated were Gram Positive cocci 14% (28/198) followed by Gram Negative Bacilli 10% (20/198) and Fungus (Candida albicans) 3% (6/198). The details of the isolate grown in blood and urine were presented in Table 2. Most of the gram-positive isolates were sensitive to Vancomycin, Moxifloxacin and Linezolid, whereas among the gram-negative organisms most of the isolates were sensitive to Imipenem, Meropenem, Fosfomycin, Piperacillin Tazobactam and Ceftriaxone sulbactam disodium edetate. The antibiotic susceptibility pattern of various isolates are presented in Table 3.

DISCUSSION

Knowledge of the microbiological profile of COVID- 19 infection would help in better management of infected patients. However, the diagnosis of COVID-19 infection may be challenging due to the diversity of clinical picture and radiological finding in the suspected cases. In this study we focus on the microbiological profile of COVID-19 patients and the current study shows that males are frequently infected than females which are similar to other studies Females have often less susceptibility to viral infections which could be attributed to sex hormones.[11-14] No viral co-infection especially HIV, HCV, HBs Ag was recorded in our study. In contrast a case of co-infection by SARS-Co-V-2 and HIV has been reported from Uganda.[15] Only one case of dengue – SARS-CoV-2 co-infection was found in this study. Similar type of co-infection studies has already been reported from Asian Countries such as Pakistan, Singapore, Thailand and Bangladesh.[16,17] In tropical areas where arboviruses and COVID 19 may coexist, clinical diagnosis is difficult, and patients should be tested for both viruses.[18]

Serum CRP level is a known diagnostic marker for inflammation and infection.[19] In our study 89% of COVID-19 positive patients had elevated levels of CRP and 11.42% of patients were positive by Typhi -dot with IgM and IgG antibody for *Salmonella typhi*. This may confuse the clinicians, and hence further evaluation is needed by confirmatory tests at large level.

Bacterial and fungal co-infections are very common in viral pneumonia especially in critically ill patients.[20] 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.[21] In our study we report 21% bacteremia and 6% bacteriuria from COVID 19 patients admitted in ICU. In a similar study conducted in Germany among 118 patients, only 5 patients had true bacteremia producing a blood culture diagnostic yield of 4.2% and no multidrug-resistant gram-negative pathogen or methicillin-resistant *Staphylococcus aureus* were reported.[22] A retrospective study from Wuhan reports *Acinetobacter baumanii* and *Klebsiella pneumoniae* as the common bacterial agent of co-infection while *Aspergillus flavus*, *Candida glabrata* and *Candida albicans* were the most commonly isolated fungus.[23] In our study MR-CONS (26%) and *K. Pneumoniae* (19%) was the most commonly isolated bacterial agent and *Candida albicans* (11%) was the only isolated fungus of co-infection. Another study from Italy reported 11% co-infection with other bacteria and fungi.[24]

The emergence of drug resistance among microorganisms is a global threat and demands strict action. Another study reported the extensive and excessive use of empirical antibiotics among 90% of the patients during this COVID pandemic which leads to antibiotic resistance.[25] In our study antimicrobial susceptibility testing was carried out to determine the susceptibility pattern of bacterial pathogens, which helps the physician to select the appropriate antibiotic for prompt treatment. The main strength of our study is the inclusion of microbiological investigations among COVID-19 patients for better management. But the limitation of this study is that microbiological investigation was not carried out for all patients. This is a single centre study, the agents of co-infection and their antibiotic susceptibility pattern cannot be generalized to other geographical settings. Furthermore, this study lacks information of other laboratory parameters and imaging studies that distinguish co-infection from COVID-19.

CONCLUSION

This study has revealed the presence of other co-infecting organisms among patients with COVID-19. The emergence of bacterial drug resistance among patients with co-infection can be avoided by proper treatment with antibiotics based on antibiotic susceptibility testing. Further, this study emphasizes the need for standardization of microbiological investigation for COVID-19 patients.

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