

Bacterial co-infection in Covid-19 patients visiting a tertiary care hospital in Maharashtra

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Abstract

Background and objectives: Several patients with SARS-CoV-2 infection presents with bacterial co-infection. The aim of the present study was to determine the bacteria responsible for co-infection in Covid-19 infected patients visiting a tertiary care hospital of Maharashtra, India.

Material and methods: A cross sectional study was conducted for 3 months at tertiary care center. Covid-19 patients attending the hospital were included in the study. All the specimens were collected either at the time of admission at outdoor or within 24-48 hours of admission. All the specimens were processed for culture and antibiotic susceptibility testing as per institutional policy and standard methods.

Results: Total 200 samples were collected out of which 98 (49%) patients were diagnosed with bacterial co-infection. Majority of cases with bacterial co-infection were above 21 years of age. Culture was positive in 80%, 66.7%, 49.2% and 38.8% of tracheal aspirate, pus, blood and urine samples respectively. Out of 98 cases of bacterial co-infection, 62.2% and 37.8% had infection with Gram negative and positive bacteria respectively. Most common organism isolated was *Klebsiella pneumoniae* (20.4%) followed by *Enterococcus species* (14.3%). Over 70% of *Klebsiella pneumoniae* isolates were resistant to aminoglycosides, cephalosporins, fluroquinolones and carbapenems while 100% *Acinetobacter* was resistant to all antimicrobials tested. Among 57 Of the Gram negative isolates, 5 and 24 isolates were positive for ESBL carbapenemase respectively.

Conclusion: The study revealed that bacterial co-infection was present in considerable proportion of Covid-19 patients and the organisms responsible were resistant to several antimicrobial agents.

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Introduction

The novel coronavirus first emerged in Wuhan, China, in December 2019 and has led to a global pandemic and as of May, 2022, about 500 million cases and more than 6 million deaths have been recorded around the world [1]. People with underlying morbidities are more susceptible to complications [2]. However, healthy individuals experience a mild flu-like illness or may be

asymptomatic, recuperating from the infection even without any particular intervention [3]. Multiple studies have reported a correlation between SARS-CoV-2 infection and bacterial co-infections/superinfections [4-8]. About 20% of patients with SARS-CoV-2 are presented with co-infection, while 41% of superinfections were found among the ICU patients [7]. Therefore, antimicrobial agents are frequently used in cases of

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Covid-19 disease. The availability of bacterial and antimicrobial resistance profiles is important for rational prescription of antibiotics to treat Covid-19 patients effectively. However, data regarding types of bacteria causing co-infections and their antimicrobial resistance profiles are lacking. So, the present study was undertaken to determine the bacteria responsible for co-infection in Covid-19 patients visiting a tertiary care hospital in Maharashtra, India.

Material and methods

This cross sectional study was conducted for 3 months from January to March 2022 at a tertiary care hospital of western Maharashtra. The study was approved by the institutional ethical committee prior to the initiation of the study (Ethical approval letter No. I.E.S.C./31/2022).

Study population and sample collection: Covid-19 patient attending the outpatient department or admitted in the hospital were included in the study. Covid-19 was defined if a case was positive for SARS-CoV-2 either by RT-PCR or rapid antigen test or both. Detailed history regarding age, sex, associated conditions, and antibiotic, steroid, or antiviral therapies was taken from the enrolled patients. All the specimens were collected within 24-48 hours of admission. None of the sample was collected after 48 hours of hospital admission to exclude patient with hospital acquired infection in our current study sample. Samples from patients attending the outpatient were collected at the time of visit or admission of the patients. Samples for the culture were collected aseptically only from those who had suspected co-infection(s).

Sample processing: All the collected specimens were processed for culture as per institutional policy and standard methods. Specimens were cultured on blood agar, MacConkey agar, Cystine-Lactose-Electrolytes-Deficient (CLED) agar by streaking methods and incubated at 37°C for 18-24 hours. Blood specimen was collected in automated BacT/Alert blood culture bottle and incubated at 37°C for up to 7 days. Positive sample was sub-cultured on blood agar, MacConkey agar and incubate for 18 -24 hours. Suspected colonies were identified by Gram stain, motility, catalase, oxidase,

coagulase and other standard biochemical tests [9]. All bacterial isolates were tested for antibiotic susceptibility using Kirby Bauer disc diffusion method on Mueller-Hinton agar plates. Isolated colonies were inoculated by lawn culture and antibiotic discs were placed on the surface and the plates were kept for incubation for 18-24 hours. The zone of inhibition was interpreted following the CLSI 2022 guidelines [10]. Automated Vitek 2C was used for identification of organism and antibiotic susceptibility testing as and when required. Extended spectrum Beta lactamase (ESBL) and carbapenemase production were detected by double disc diffusion method and modified Hodge test respectively [11,12].

Results

Total 200 samples were collected and out of which 98 (49%) specimens yielded bacterial growth. Out of 200 cases, Gram negative and positive bacteria were isolated from 61 (30.5%) and 37 (18.5%) cases respectively and 86 (43%) had infection in single site and in 12 (6%) cases bacteria were isolated from more than one anatomical sites.

The rate of culture positivity was 45.9% and 55.4% in male and female patients respectively while the rates ranged from 55.7% - 37.7% in samples collected from outdoor, indoor and ICU. Majority of cases belonged to 21 years to above 60 years of age. Culture was positive in 80%, 66.7%, 49.2% and 38.8% of tracheal aspirate, pus, blood and urine samples respectively. Only 30 (15%) patients had comorbidities and of them 6 (20%) had co-infection (Table-1).

Table-2 shows the pattern of bacteria isolated from different samples of Covid-19 patients. Overall, out of 98 cases of bacterial co-infection, 61 (61/98=62.2%) had infection with Gram negative bacteria while 37 (37/98=37.8%) was infected with Gram positive bacteria. Except pus, other specimens yielded mostly (55%-100%) growth of Gram negative organisms. Out of 14 pus samples, 9 (64.3%) showed growth of Gram positive bacteria. Most common organism isolated from the covid-19 patients was *Klebsiella pneumoniae* (20.4%) followed by *Enterococcus species* (14.3%), *Staphylococcus aureus* and *Pseudomonas sp* (each

Table-1: Detail characteristics of the study population (N=200)

Variables	Number	Culture positive, n (%)
Total samples	200	98 (49)
Gender		
Male	135	62 (45.9)
Female	65	36 (55.4)
Source of sample		
Outdoor patients	43	23 (53.5)
Indoor patients	88	49 (55.7)
ICU patients	69	26 (37.7)
Age group, years		
<10	8	2 (25)
11-20	10	4 (40)
21-30	38	20 (52.6)
31-40	29	15 (51.7)
41-50	32	15 (46.9)
51-60	37	19 (51.4)
> 60	46	22 (47.8)
Specimen		
Urine	85	33 (38.8)
Blood	59	29 (49.2)
Pus/Exudate	21	14 (66.7)
Tracheal aspirate	15	12 (80)
Other body fluid ^a	20	10 (50)
Comorbidity absent	170	92 (54.1)
Comorbidity present	30	6 (20)
Diabetes	11	2 (18.2)
Hypertension	17	4 (23.5)
Cancer	2	0

Note: * Other body fluids: pleural fluid, ascitic fluid, peritoneal fluid.

12.2%) Less common isolates were *Proteus sp*, *Elizabethkingia meningoseptica* and *Aerococcus viridans*.

Table-3 shows the detail antimicrobial resistance profile of Gram-negative bacteria isolated from Covid-19 patients. As per the antibiotic susceptibility testing, more than 70% of *Klebsiella pneumoniae* isolates were resistant to aminoglycosides, cephalosporins, fluroquinolones and carbapenems. All (100%) the *Acinetobacter sp*, *Proteus sp* and *E. meningoseptica* were resistant to

all antimicrobials tested. Among the gram-positive isolates, major drug resistance was noted against the fluroquinolones, macrolides and ampicillin (Table-4). None of the isolate was resistant to vancomycin and linezolid.

Among 61 Gram negative isolates, 57 isolates were tested for ESBL and carbapenemase production. Out of 57 isolates 5 (8.8%) and 24 (42.1%) were positive for ESBL and carbapenemase respectively (Table-5). Highest (58.3%) carbapenemase production was detected in *Pseudomonas sp*.

Table- 2: Pattern of bacteria isolated from different specimens of Covid-19 patients

Organism	Total n=98 n (%)	Urine (n=33) n (%)	Blood (n=29) n (%)	Pus (n=14) n (%)	TA (n=12) n (%)	Others BF* (n=10) n (%)
Gram-negative						
<i>K. pneumonia</i>	20 (20.4)	6 (18.2)	8 (27.6)	1 (7.1)	4 (33.3)	1 (10)
<i>E. coli</i>	11 (11.2)	9 (27.3)	1 (3.4)	0	0	1 (10)
<i>Enterobacter sp.</i>	4 (4.1)	1 (3)	2 (6.9)	0	0	0
<i>Citrobacter sp.</i>	3 (3.1)	1 (3)	0	0	0	2 (20)
<i>Proteus sp.</i>	1 (1)	1 (3)	0	0	0	0
<i>Pseudomonas sp.</i>	12 (12.2)	3 (9)	1 (3.4)	3 (21.4)	4 (33.33)	2 (20)
<i>Acinetobacter sp.</i>	9 (9.2)	0	3 (10.3)	1 (7.1)	4 (33.3)	1 (10)
<i>E. meningoseptica</i>	1 (1)	0	1 (3.4)	0	0	0
Total	61 (62.2)	21 (63.6)	16 (55.2)	5 (35.7)	12 (100)	7 (70)
Gram- positive						
<i>S. aureus</i>	12 (12.2)	0	9 (31)	1 (7.1)	0	2 (20)
<i>Streptococcus sp.</i>	9 (9.2)	0	0	8 (57.1)	0	1 (10)
<i>Enterococcus sp.</i>	14 (14.3)	12 (36.4)	2 (6.9)	0	0	0
<i>A. viridans</i>	2 (2)	0	2 (6.9)	0	0	0
Total	37 (37.8)	12 (36.4)	13 (44.8)	9 (64.3)	0	3 (30)

Note: TA – tracheal aspirate; BF – body fluids, which included pleural, ascitic and peritoneal fluids; *A. viridians* – *Aerococcus viridians*; *S. aureus* - *Staphylococcus aureus*.

Table- 3: Antimicrobial resistance patterns of isolated Gram-negative bacteria

Antimicrobial agent	<i>K. pneumoniae</i> (N=20) Resistant n (%)	<i>E. coli</i> (N=11) Resistant n (%)	<i>Enterobacter</i> <i>sp</i> (N=4) Resistant n (%)	<i>Citrobacter</i> <i>sp</i> (N=3) Resistant n (%)	<i>Pseudomonas</i> <i>sp</i> (N=12) Resistant n (%)	<i>Acinetobacter</i> <i>sp.</i> (N=9) Resistant n (%)
Amikacin	14 (70)	2(18.18)	1(25)	2(66.66)	3 (25)	9(100)
Gentamicin	14(70)	3(27.27)	1(25)	0	4 (33.33)	9(100)
Ampicillin	-*	8(72.72)	-	-	-*	-
SXT	16 (80)	4(36.36)	1(25)	2 (66.66)	-	9(100)
Ciprofloxacin	20(100)	7(63.63)	1(25)	0	5 (41.66)	9(100)
Ceftriaxone	17(85)	7(63.63)	1(25)	3 (100)	-	9(100)
CAZ	17(85)	7(63.63)	1(25)	1(33.33)	5 (41.66)	9(100)
CAZ +CA	14(70)	5(45.45)	1(25)	1(33.33)	5 (41.66)	9(100)
PIP+TAZ	14(70)	3(27.27)	1(25)	2(66.66)	3 (25)	9(100)
Amox +CA	17(85)	3(27.27)	-	-	-	-
Meropenem	14(70)	2(18.18)	1(25)	1(33.33)	6 (50)	9(100)

Note: SXT - Co-trimoxazole, CAZ – ceftazidime; CA- clavulanic acid ; PIP – Piperacillin; TAZ – tazobactam; Amox – Amoxycillin.

Table-4: Antimicrobial resistance patterns of isolated Gram-positive bacteria.

Antimicrobial agent	<i>Enterococcus sp</i> (N=14)	<i>Staphylococcus aureus</i> (N=12)	<i>Streptococcus sp</i> (N=9)	<i>A. viridans</i> (N=2)
	Resistant, n (%)	Resistant, n (%)	Resistant, n (%)	Resistant, n (%)
Amikacin	-*	-	-	2 (100)
Gentamicin	-	6 (50)	-	-
Ampicillin	11 (78.57)	-	0	2 (100)
Co-trimoxazole	-	7 (58.33)	3 (33.33)	2 (100)
Ciprofloxacin	13 (92.85)	9 (75)	1 (11.11)	0
Erythromycin	13 (92.85)	8 (66.66)	0	2 (100)
Clindamycin	-	8 (66.66)	0	2 (100)
Vancomycin	0	0	0	0
Linezolid	0	0	0	0

Note: *: Antibiotic was not tested or intrinsic resistant;

Table-5: Rate of ESBL and carbapenemase producing bacteria

Organism	Total number	Positive for	
		Only ESBL, n (%)	Carbapenemase
<i>K. pneumoniae</i>	20	2 (10)	9 (45)
<i>E. coli</i>	11	2 (18.18)	2 (18.18)
<i>E. meningoseptica</i>	1	1 (100)	0
<i>Pseudomonas sp.</i>	12	0	7 (58.33)
<i>Acinetobacter sp.</i>	9	0	5 (55.55)
<i>Enterobacter sp.</i>	4	0	1 (25)
Total	57	5 (8.8)	24 (42.1)

Discussion

In the present study, bacterial co-infection was found in 49% patient. The rate was higher than many reported studies [13-18]. Those studies reported bacterial co-infection from 4% to 20% in Covid-19 patients. However on the contrary, Alshrefy *et al* [19] and Sreenath *et al* [20] reported almost similar rate of bacterial co-infection (42.4% and 47.1%) like ours. Covid-19 affects all age group patients which include from pediatric to geriatric age groups but older age group patient is infected more compared to other age group. In the present study, there was no significant increase of bacterial isolation rate with the increase of age. Mean age of the Covid-19 patients in current study was 47.13 years which was lower compared to various studies done on bacterial co-infection in Covid-19 admitted patients. The reported mean

age of patients in other studies ranged from 56 to 74 years [14,16,17,21-24].

Majority of the Covid-19 patients need ventilator support as well as urinary catheterization during their ICU stay. Due to use of various immunosuppressive drugs, ICU patients have more chance to develop the bacterial infections. Several studies reported 28% to 83% bacterial infection in ICU admitted Covid-19 patients [14,15,21]. In the present study, 37.7% specimens from ICU patients had positive bacterial growth.

Several studies reported Gram negative bacteria as predominant infecting agents in Covid-19 patients. Bacterial co-infection in Covid-19 patients due to Gram negative organism varied from 75% to about over 90% [22,16,15] while it was around 40% by Gram positive bacteria [16,22]. Similarly, in the

current study we also found Gram negative bacteria as the predominant (62.2%) offending agents for causing co-infection in our Covid-19 cases. Only, 37.4% cases were infected by Gram positive bacteria. Among the Gram negative bacteria, *K. pneumoniae* was the most commonly isolated bacteria followed by *Pseudomonas* and *Acinetobacter* species. Same types of bacteria were most commonly isolated from Covid-19 cases by others [15,16,19,24]. As found by other studies [16,20], we also observed enterococci and *S. aureus* as the most commonly isolated Gram positive bacteria.

Resistance against antimicrobial agents is a global health burden in current time. With the extensive use of antimicrobials, multi-drug resistant isolates have arisen globally. During the covid-19 pandemic, antibiotics are extensively used by the clinicians. Over 70% of *Klebsiella pneumoniae*, the most commonly isolated bacteria in our series, was resistant to all the antimicrobial agents tested. Similar high rate of resistance was exhibited by our isolated *Acinetobacter* sp and Gram positive bacteria to several antibiotics tested. This could be due to prevalence of such drug resistant bacteria in the local community. The increasing exposure to healthcare environments and invasive procedures, as well as increased antibiotic usage, raises the potential for emergence of multidrug resistant bacteria [15,22,24]. The present study had some limitations. The study was conducted at a single center over a short period and the sample size was small.

The present study has demonstrated that about half of the Covid-19 cases suffer from bacterial co-infections and many of those are caused by multidrug resistant bacteria. However, it is still unclear what exact roles co-infections and/or super infections play in patients with COVID-19 cases. Accurate and quick detection of bacterial co-infection with antibiotic susceptibility testing, particularly for severe infections, can assist clinicians to effectively treat Covid-19 patients with better clinical outcomes.

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Conflict of Interest

None of the author has any conflict of interest.

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