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## Original Research Article

## Bacteriological profile and the sensitivity patterns of culture positive organisms in COVID-19 positive patients

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## ABSTRACT

**Background and Objective:** Use of antibiotics without proper confirmation carries risk of over use of antibiotics. It is associated with certain side effects like loss of natural bacterial flora present in the human body. It can lead to resistance to the bacteria due to irrational use of antibiotics. Present study was carried out to study the bacteriological profile and the sensitivity patterns of culture positive organisms in COVID-19 positive patients.

**Materials and Methods:** Hospital based cross-sectional study was carried out from March 2021 to June 2021 in the Department of Microbiology, Malla Reddy Institute of Medical Sciences (MRIMS), Hyderabad in 38 samples. These samples were referred from General Medicine Department. Culture and sensitivity was done in all cases.

**Results:** Majority of subjects belonged to the age group of 20-29 years (31.6%). The proportion of males (57.9%) was more than the proportion of females (42.1%). Most common co-morbidity was diabetes seen in 21.1% of the cases. *Klebsiella pneumoniae* was the most common organism found in four cases. Incidence of secondary bacterial infection in COVID-19 patients in the present study was 39.5%. Among the antibiotics studied in COVID-19 patients, amikacin, ciprofloxacin, imipenem, piperacillin-tazobactam, nitrofurantoin and chloramphenicol were found to be highly sensitive to most of the organisms. Antibiotics like cefepime, ampicillin, ceftazidime and cotrimoxazole were resistant to most of the organisms.

**Conclusion:** Based on the study findings, we conclude that antibiotics like amikacin, ciprofloxacin, imipenem, piperacillin-tazobactam, nitrofurantoin and chloramphenicol can be used in most of the patients with COVID-19.

**Key message:** Antibiotics should be used with caution especially in patients with COVID-19. Antibiotics like amikacin, ciprofloxacin, imipenem, piperacillin-tazobactam, nitrofurantoin and chloramphenicol can be used in most of the patients with COVID-19 while awaiting the results of culture and sensitivity. In all cases where physicians are in dilemma culture and sensitivity is highly recommended.

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## 1. Introduction

Secondary bacterial infections are common in viral infections. Especially in cases of influenza, these are common. They are found to be associated not only with

significant morbidity but also can lead to deaths in such cases. Hence, appropriate diagnosis of these secondary bacterial infections in such cases within short time is important and can help in giving rational treatment. This can prevent significant morbidity and mortality associated with such cases.<sup>1,2</sup>

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The data on the incidence of secondary bacterial infections among patients infected with SARS-CoV-2; their frequency as well as associated clinical features and other features is limited.<sup>3,4</sup> Antibiotics are not effective for the treatment of COVID-19, but there are a number of reasons for which antibiotics are given to the patients by the physicians.<sup>3</sup> Empirical use of antibiotics is permitted in COVID-19 cases. This is based on the prior experience of influenza pandemic when many patients died due to supra bacterial infection.<sup>5</sup> It is not easy to decide when the patient presents to the hospital that the patient have or have not the secondary bacterial infection during the course of the disease. Use of antibiotics without proper confirmation carries risk of over use of antibiotics. It is associated with certain side effects like loss of natural bacterial flora present in the human body, resistance to the bacteria due to irrational use of antibiotics.<sup>6</sup>

Studies on bacteriological profile are required which guide the physician decision in the treatment of patients especially treatment of COVID-19 cases. Culture and sensitivity studies help us to know which organisms are sensitive to which antibiotics. Thus, we come to know current sensitivity patterns. When we compare this with previous studies, we also come to know the changing patterns. Hence, present study was carried out to study the bacteriological profile and the sensitivity patterns of culture positive organisms in COVID-19 positive patients

## 2. Materials and Methods

### 2.1. Study design

Hospital based cross sectional study.

### 2.2. Study period

March 2021 to June 2021.

### 2.3. Settings

Department of Microbiology, Malla Reddy Institute of Medical Sciences, Hyderabad.

### 2.4. Sample size

During the study period, 38 samples were sent to the Department of Microbiology, Malla Reddy Institute of Medical Sciences, Hyderabad from General Medicine Department and all were included in the present study.

### 2.5. Ethical aspects

Present study was hospital record based study. We did not use the patient identifying information; therefore, the Institution Ethics Committee permission was not taken.

## 2.6. Culture and sensitivity

We received urine, blood, pus, sputum and in some cases multiple samples were received from the same patients. The samples were subjected to culture and sensitivity. Pus samples were collected with sterile disposable cotton swabs and pus aspirates in syringe. Urine and sputum samples were collected in sterile leak proof containers. Blood samples were collected in conventional BHI broth. All samples were immediately transported to the microbiology laboratory. All samples were inoculated on to Blood agar (BA), and Mac-conkey agar (MA) and the media were incubated at 37°C for 24 to 48 hrs. Identification of the culture isolates from positive cultures was done using standard biochemical tests like catalase, oxidase, indole, methyl red, voges-proskauer, citrate, Mannitol motility test and urease test.

Antimicrobial susceptibility testing of all isolates was performed and was evaluated by the standard Kirby Bauer's disc diffusion method on Mueller-Hinton agar and interpreted as per Clinical and Laboratory Standards Institute or CLSI guidelines. Standard antibiotics like amikacin (30mcg), ceftriaxone (30mcg), cefepime (30mcg), Ampicillin (30mcg), piperacillin/tazobactam (100/10mcg), Imipenem (10mcg), cotrimoxazole (25mcg), ciprofloxacin (5mcg), Vancomycin (30mcg), Chloramphenicol (30mcg), Cefoxitin (30mcg), Erythromycin (15mcg) were tested. All the culture media, biochemical media and antibiotic discs were obtained from Hi Media.

### 2.7. Statistical analysis

The data was entered in the Microsoft Excel worksheet and analyzed using proportions.

## 3. Results

**Table 1:** Distribution of study subjects as per age and sex

Variables	Number	%	
Age (years)	20-29	12	31.6
	30-39	6	15.8
	40-49	9	23.7
	50-59	3	7.9
	60-69	8	21.1
Sex	Male	22	57.9
	Female	16	42.1

Majority of the study subjects were in the age group of 20-29 years (31.6%) followed by 40-49 years (23.7%). Males (57.9%) were more than females (42.1%) (Table 1)

Diabetes was the most common co-morbidity seen in 21.1% of the cases followed by hypertension in 13.2% of the cases. 10.5% of the cases had hypothyroidism. Mixed co-morbidity like hypertension and diabetes was seen in two cases. Two cases had diabetes, hypothyroidism,

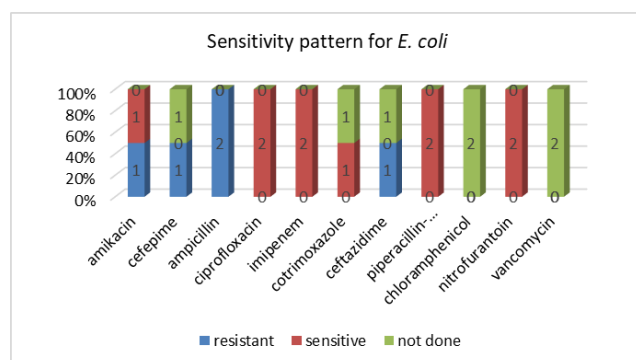
**Table 2:** Distribution of study subjects as per co-morbidities present

Co-morbidities	Number	%
Diabetes	8	21.1
Hypertension	5	13.2
Hypothyroidism	4	10.5
Hypertension and diabetes	2	5.3
Diabetes, hypothyroidism, coronary artery disease	2	5.3
Diabetes, hypertension and coronary artery disease	1	2.6
No	16	42.1

and coronary artery disease. One case had Diabetes, hypertension and coronary artery disease. (Table 2)

*Klebsiella pneumoniae* was most common organism found in this study in four cases followed by three cases each of Methicillin resistant *Staphylococcus aureus* and *Candida albicans*. The incidence of secondary bacterial infection in COVID-19 patients in the present study was 39.5%. No growth was recorded in 52.6% of the cases. (Table 3)

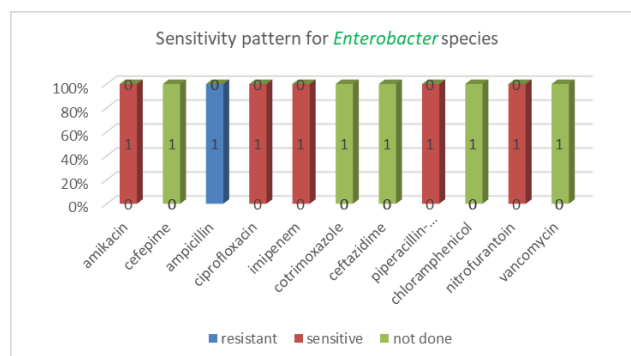
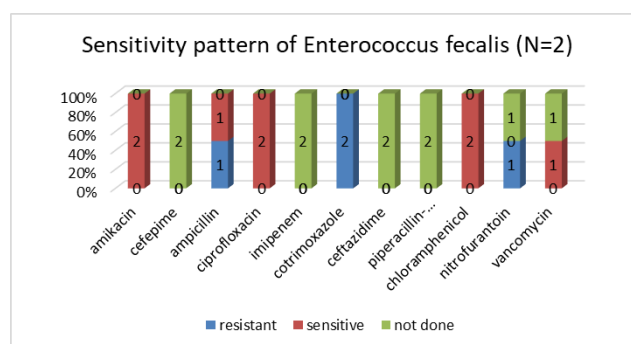
Among the antibiotics studied in COVID-19 patients, amikacin, ciprofloxacin, imipenem, piperacillin-tazobactam, nitrofurantoin and chloramphenicol were found to be highly sensitive to most of the organisms. Antibiotics like cefepime, ampicillin, ceftazidime and cotrimoxazole were resistant to most of the organisms. (Table 4)

**Fig. 1:** Sensitivity pattern for *E. coli* (N=2)

*E. coli* were found to be completely sensitive to ciprofloxacin, imipenem and nitrofurantoin and were completely resistant to ampicillin. Mixed results (one case sensitive and one case resistant) was seen in cases of amikacin. Piperacillin-tazobactam and vancomycin were not tested on *E. coli* (Figure 1)

The *Enterobacter* species was resistant to ampicillin and was found to be sensitive to amikacin, ciprofloxacin, imipenem, piperacillin-tazobactam and nitrofurantoin antibiotics. Other antibiotic sensitivity testing was not done for this species (Figure 2).

Two samples tested positive for *enterococcus fecalis*. They were resistant to cotrimoxazole and found to be

**Fig. 2:** Sensitivity pattern for *Enterobacter* species (N=1)**Fig. 3:** Sensitivity pattern of *Enterococcus fecalis* (N=2)

sensitive to amikacin, ciprofloxacin, vancomycin and chloramphenicol. In case of ampicillin, one sample was sensitive and one was resistant. (Figure 3).

Four samples were found positive for *Klebsiella pneumoniae*. In case of amikacin and imipenem it was 75% sensitive and 25% resistance. It was found to be 100% sensitive to piperacillin-tazobactam; 25% sensitive for chloramphenicol, cefepime, cotrimoxazole and nitrofurantoin; 50% sensitive for ciprofloxacin (Figure 4).

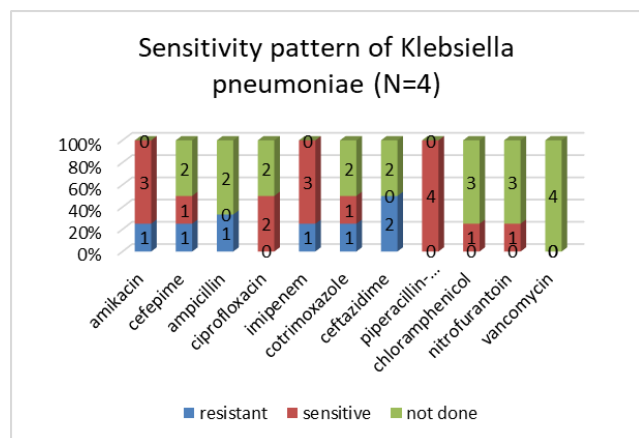
Three samples tested positive for methicillin resistant *staphylococcus aureus*. All three were sensitive to amikacin, chloramphenicol and vancomycin. All three were resistant to ampicillin. In case of ciprofloxacin, two samples were sensitive and one was resistant and in case of ceftazidime, two samples were resistant and one was sensitive. Thus, we conclude that for methicillin resistant *staphylococcus*

**Table 3:** Distribution as per organisms isolated

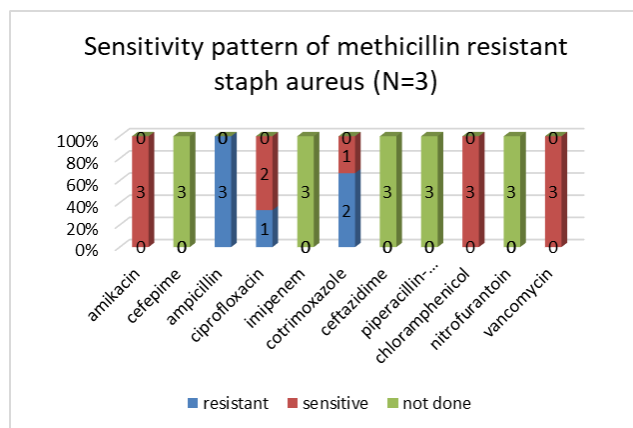
Organism isolated	Specimen					Total	%
	Urine	Blood	Pus	Urine & blood	Sputum		
<i>Candida albicans</i>	2	0	0	1	0	3	7.9
<i>E. coli</i>	1	0	0	1	0	2	5.3
<i>Enterobacter species</i>	0	0	0	0	1	1	2.6
<i>Enterococcus fecalis</i>	1	1	0	0	0	2	5.3
<i>Klebsiella pneumoniae</i>	1	1	0	2	0	4	10.5
<i>Methicillin resistant staph aureus</i>	0	1	2	0	0	3	7.9
<i>Non albicans candida</i>	1	0	0	1	0	2	5.3
<i>Pseudomonas aeruginosa</i>	0	0	1	0	0	1	2.6
	No growth					20	52.6

**Table 4:** Summary of sensitivity pattern of antibiotics on total number of samples tested

Antibiotics	Sensitive		Resistant		Total	
	Number	%	Number	%	Number	%
Amikacin	11	84.6	2	15.4	13	100
Cefepime	1	25	3	75	4	100
Ampicillin	1	11.1	8	88.9	9	100
Ciprofloxacin	10	90.9	1	9.1	11	100
Imipenem	7	77.8	2	22.2	9	100
Ceftazidime	0	0	3	100	3	100
Piperacillin-tazobactam	8	100	0	0	8	100
Nitrofurantoin	4	80	1	20	5	100
Cotrimoxazole	3	37.5	5	62.5	8	100
Chloramphenicol	6	100	0	0	6	100
Vancomycin	3	75	1	25	4	100



**Fig. 4:** Sensitivity pattern of *Klebsiella pneumoniae* (N=4)



**Fig. 5:** Sensitivity pattern of methicillin resistant *staphylococcus aureus* (N=3)

*aureus* ampicillin should not be used. (Figure 5)

One sample tested positive for *pseudomonas aeruginosa* and it was found to be resistant to cefepime while sensitive

for amikacin, ciprofloxacin, imipenem, piperacillin; while the sensitivity was not done in other cases (Figure 6)

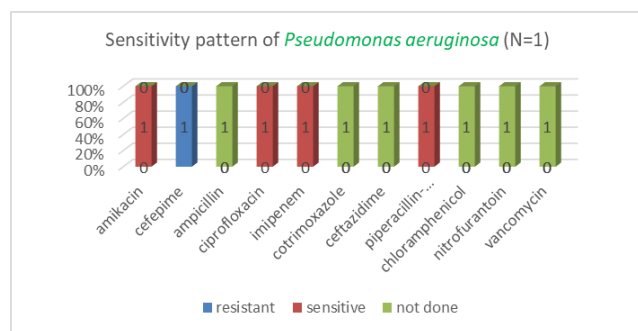


Fig. 6: Sensitivity pattern of *Pseudomonas aeruginosa* (N=1)

#### 4. Discussion

Majority of subjects belonged to the age group of 20-29 years (31.6%). The proportion of males (57.9%) was more than the proportion of females (42.1%). Most common co-morbidity was diabetes seen in 21.1% of the cases. *Klebsiella pneumoniae* was the most common organism found in four cases. Incidence of secondary bacterial infection in COVID-19 patients in the present study was 39.5%. Among the antibiotics studied in COVID-19 patients, amikacin, ciprofloxacin, imipenem, piperacillin-tazobactam, nitrofurantoin and chloramphenicol were found to be highly sensitive to most of the organisms. Antibiotics like cefepime, ampicillin, ceftazidime and cotrimoxazole were resistant to most of the organisms.

Mahmouidi H<sup>7</sup> found that the incidence of secondary bacterial infections was 12.5% which is quite low compared to the present study and may be due to differences in the settings of the study or host immunity and other factors that affect this aspect. Like present study, they also found that *Klebsiella* species was the most common organism isolated. They observed that *Enterobacteriaceae* isolates were highly resistant to cotrimoxazole, piperacillin, ceftazidime and cefepime. Amikacin was found to be antibiotic of choice as it has shown 100% sensitivity which is similar to the results of the present study.

Lansbury L et al<sup>8</sup> included 30 studies with a sample of 3834 patients and found that the incidence of secondary bacterial infection in COVID-19 patients was 7%. This is very low compared to the present study findings and may be due to the fact that the sample size in the present study was low. They also noted that ICU patients had higher incidence of 14%. *Mycoplasma pneumoniae*, *Pseudomonas aeruginosa* and *Hemophilus influenzae* were the most common isolates. Thus, they concluded that antibiotics should not be used routinely in COVID-19 patients.

Saini V et al<sup>9</sup> studied 494 bacterial isolates of COVID-19 cases from Delhi, India. Among them, gram negative was 55.5% and remaining were gram positive. Coagulase Negative *Staphylococcus* and *Staphylococcus aureus* were most common from blood isolates. *Escherichia coli* and

*Staphylococcus aureus* were the most common from urine samples. They noted that there was an increase of 40% in the anti-microbial resistance compared to the pre pandemic era. This may be due to over use of antibiotics.

Gohel K et al<sup>10</sup> carried out an organism profile study in 1440 blood samples. Incidence of positive blood culture was 9.2% among. Gram positive organisms were seen in 58.3% of the cases. *Staphylococcus aureus* was the most common among the gram positive organisms. Among gram negative organisms, *enterobacteriaceae* was most common. Fungal isolates accounted for 1.5% which is very low compared to present study of 13.5%. They noted that antibiotics like vancomycin was useful for gram positive organisms similar to the findings of the present study.

Halder A et al<sup>11</sup> found that the *Klebsiella* was the most common organism in patients with COVID-19 and this finding is similar to the finding of the present study where we found that *Klebsiella* was the common isolate. They reported isolates of *Acinetobacter* in few cases. They also noted that the antibiotic resistance was more in COVID-19 cases especially with *Acinetobacter*. *E. coli* was also common but it was not found out to be drug resistant.

Mengistu A et al<sup>12</sup> studied 5823 samples from 18 studies in their systematic review. They found that 31.9% were positive for blood culture. Majority were gram positive organisms in 57.8% of the cases. Among the gram positive organisms, *staphylococcus aureus* was most common and among the gram negative organisms, *Klebsiella* species was most common. In the present study, we also found that *Klebsiella* species was the most common isolate in COVID-19 patients. Ampicillin, amoxicillin, ceftriaxone, cotrimoxazole were not found to be working i.e. high resistance and we also noted that ampicillin had highest resistance.

Habyarimana T et al<sup>13</sup> observed that the incidence of secondary bacterial infection was 12% which is lower than that reported by us in the present study. Gram negative organisms were predominant in their study and most common species was *Klebsiella*. This finding is similar to the finding of the present study. They also reported that ampicillin had the highest resistant like in the present study.

Abeba A et al.<sup>14</sup> studied 856 samples of blood. On analysis they found that the incidence of bacteremia was 19.7%. Males were more than females. We also observed that males were more than females in the present study.

#### 5. Conclusion

Incidence of secondary bacterial infection in COVID-19 patients in the present study was high i.e. 39.5%. Among the antibiotics studied in COVID-19 patients, amikacin, ciprofloxacin, imipenem, piperacillin-tazobactam, nitrofurantoin and chloramphenicol were found to be highly sensitive to most of the organisms. Antibiotics like cefepime, ampicillin, ceftazidime and cotrimoxazole

were resistant to most of the organisms. Hence we recommend to use antibiotics like amikacin, ciprofloxacin, imipenem, piperacillin-tazobactam, nitrofurantoin and chloramphenicol and avoid cefepime, ampicillin, ceftazidime and cotrimoxazole.

## 6. Source of Funding

None.

## 7. Conflict of Interest

The authors declare no conflict of interest.

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