Original Article

Study on Bacteriological Profile and Antibiotic Susceptibility Pattern of Clinical Isolates Obtained from COVID-19 Patients with Secondary Infection

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Background : Viral respiratory infections increase the risk of secondary bacterial infections owing to their need for hospitalisation, prolonged stay, the practice of Empiric Antimicrobial Prescription. This leads to worrisome Antimicrobial Resistance and such infections have a worse outcome.

Materials and Methods : This is a cross-sectional study conducted on patients admitted with COVID-19 at GEMS and Hospital from April, 2021 to June, 2021. The bacteriological profile and susceptibility pattern of the isolates obtained while investigating secondary infections in COVID-19 patients were studied.

Results : 132 positive growth samples were collected from ICU and various Wards. Maximum positive growth was found in the Intensive Care Unit (ICU) 36 (27.2%), followed by Surgery ward 27 (20.5%) and Medicine Ward 27 (20.5%). *Escherichia coli* was most commonly isolated 59 (44.7%) followed by *Klebsiella pneumonia* 28 (21.2%) and *Pseudomonas aeruginosa* 17 (12.8%). Out of 90 Gram-negative isolates, 25 (27.8%) were found to be Multi-drug Resistants and out of 11 *Staphylococcus aureus* isolates, 5 (45.5%) were MRSA.

Conclusion : This study concludes that poor infection control and irrational antibiotic prescription practices play a major role in the development of secondary infections in these patients. Standard practices need to be followed and there should be an implementation of infection prevention control measures and Antimicrobial Stewardship Programs (ASP) must reappraise the current situation.

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Key words **: Secondary infections, COVID-19, Antimicrobial pattern.**

COVID -19 patients are at a higher risk of developing Hospital-Acquired Infections (HAI), both bacterial and fungal. This is due to a prolonged hospital stay, higher chances of requiring ICU and ventilatory support and empirical antibiotics administration to ward off any HAI and also many, patients need higher antibiotics as they harbor superbugs. These patients also have impaired ability to clear infections owing to Cytokine Storm which along with the virus facilitate Secondary Bacterial Infection[3,4], resulting in negative health outcomes.

While Secondary Bacterial Infections are largely a consequence of Immune Susceptibility Viral Infections additionally inflict damage to the mucosal layer, leading to adherence of *Streptococcus pneumonia, Pseudomonas aeruginosa*, and *Haemophilus influenzae*, with biofilm formation on the linings of the airways[5]. Secondary bacterial infections are common in hospitalised, seriously ill COVID-19 patients. They contribute to 10%-30% of cases with higher frequency in the ICUs[6]. Common bacteria causing Secondary infections to include *Staphylococcus aureus, Klebsiella pneumonia, S pneumonia, Neisseria meningitides, H influenzae, Proteus, Enterobacter, Citrobacter* spp., and *Pseudomonas* spp[7]. Recent studies indicate bacterial co-infection upon admission is 3.1–3.5% of COVID-19 patients, while Secondary bacterial infections, are seen in 15% of patients[8-11], So empirical use of antibiotics is essential in severely ill patients[12,13]. Data obtained from clinical observations in China, indicate COVID-19 patients are most commonly treated with antibiotics (azithromycin, ceftriaxone, vancomycin, moxifloxacin, cefepime,) to reduce the risk of Nosocomial infections as a prophylactic strategy. But Bacterial infections occur despite the prophylactic use of antibiotics, owing to drug resistance to one or more drugs. However, the

Editor's Comment :

- Rational use of antibiotics should be strictly followed to avoid the emergence of drug-resistant pathogens.

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prophylactic use of antibiotics is not recommended by most Healthcare Institutions and Governments around the Globe, due to the rampant increase in antibiotic resistance rates, due to overuse and misuse of antibiotics[14,15]. The risk of secondary infection with Multidrug Resistant (MDR) bacteria poses additional challenges for the treatment of severely sick COVID-19 patients in ICU.

Objectives

The study was conducted with the objectives of

(1) To study the bacteriological profile of clinical isolates obtained from COVID-19 patients with secondary infection.

(2) To evaluate the antibacterial susceptibility pattern of secondary infections in COVID-19 Patients.

Materials and Methods

This is a cross-sectional study conducted at GEMS and Hospital for 3 months from 1st April, 2021 to 30th June, 2021.

Inclusion Criteria :

Adult cases with RT-PCR positive laboratory-confirmed COVID-19 reports with Secondary Bacterial Infections that were culture positive.

Exclusion Criteria :

- Inconclusive laboratory test results (RT-PCR assay).
- Absence of clinical data in the Electronic Patient record system.
- Patient samples did not show significant growth on bacterial culture.

Data collection :

The data were collected from Electronic Patient Records of COVID-19 patients. Data including demographic details, comorbidities, date of admission, date of culture-positive results, antimicrobial susceptibility profile of isolates, antibiotics administered, etc. were collected. The collected data were analyzed using descriptive statistics like percentage, proportions, mean and SD.

Results

Out of 132 positive growth samples most patients belonged to the age group between 46-60 years followed by 31-45 years (Fig 1). Males were predominantly affected.

The samples were collected from ICU and various Wards. Most of the isolates showing positive growth were obtained from ICU patients 36 (27.2%), followed by Surgery 27 (20.5%) and Medicine 27 (20.5%) (Fig 2).

Out of 132 Positive bacterial cultures, Gram-negative organism 114 (86.4%) were the more compared to gram-positive Organisms 18 (13.6%) (Fig 3).

Among Gram-negative Isolates, *Escherichia coli* 59 (44.7%), was most common followed by *Klebsiella pneumonia* 28 (21.2%) and *Pseudomonas aeruginosa* 17 (12.8%). Among Gram-positive Organism Isolates *Staphylococcus aureus* 11 (8.3%) was most common followed by *Enterococci* 5 (3.8%)(Table 1).

In this study, a total of 90 Gram-negative

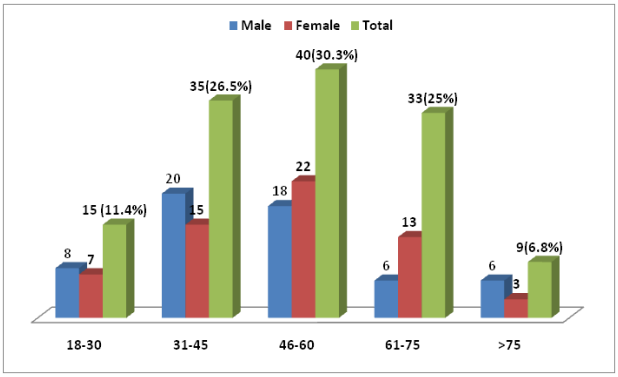

Fig 1 — Gender and Age-wise distribution of COVID-19 patients with secondary infection

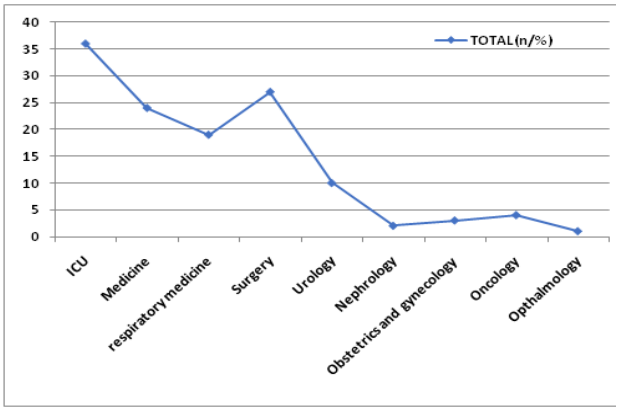

Fig 2 — Distribution of samples from various wards

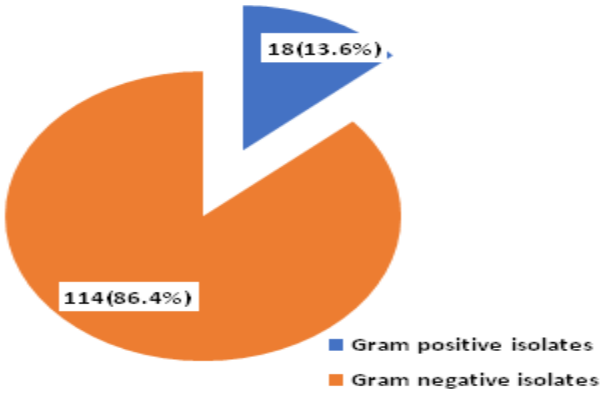

Fig 3 — Distribution of Gram positive and Gram negative isolates (n=132)

Organisms were isolated, 25 (27.8%) were MDR isolates. Out of the 11 *Staphylococcus aureus* Isolates, 5 (45.5%) were MRSA

The antimicrobial susceptibility pattern of Isolated Organisms is presented in tabular form. (Table 2) *Escherichia coli* was predominantly isolated which showed the highest resistance third-generation Cephalosporins, Ceftriaxone 51 (86.4) and Ceftazidime 48 (81.3) and β-lactam-β-lactamase inhibitor combinations, piperacillin/tazobactam 47 (79.6).

Table 1 — *Site-Specific Etiological Distribution of Pathogens*

Organism Isolated	Blood	Sputum	Urine	Pus	Others	Total(n/%)
Escherichia coli	6 (31.6)	11 (36.7)	28 (71.8)	10 (31.2)	4 (33.3)	59 (44.7%)
Klebsiella pneumoniae	3 (15.8)	12 (40)	5 (12.8)	5 (15.6)	3 (25)	28 (21.2%)
Serratia	1 (5.3)	-	-	-	-	1 (0.8%)
Salmonella spp.	-	-	-	-	1(8.3)	1 (0.8%)
Proteus mirabilis	-	-	-	1 (3.1)	-	1 (0.8%)
Pseudomonas aeruginosa	2 (10.5)	4 (13.3)	2 (5.1)	6 (18.8)	3 (25)	17 (12.8%)
Acinetobacter baumannii	2 (10.5)	3 (10)	-	2 (6.2)	-	7 (5.3%)
Staphylococcus aureus	3 (15.8)	-	-	8 (25)	-	11 (8.3%)
Enterococci	1 (5.3)	-	4 (10.2)	-	-	5 (3.8%)
Streptococcus spp.	1 (5.3)	-	-	-	1 (8.3)	2 (1.5%)
TOTAL	19	30	39	32	12	132

Discussion

This cross-sectional study was conducted on laboratory-confirmed COVID-19 patients who developed a Secondary Bacterial Infection. Patients in whom samples were found to have Colonizing Bacteria without clinically significant infection or contaminants were excluded.

In our study there was a high male to the female ratio similar to several studies which have reported similar results[16,17], the reason might be due to gender-based lifestyles and behavioral differences like smoking, etc which affect the level of pre-existing diseases such as Heart disease, Chronic lung disease, and cancer.

The most common bacteria detected in the current study were Gram-negative Bacteria 114 (86.4%) followed by Gram-positive Bacteria 18 (13.6%). Gram-negative infections have dominated as far as the type of organisms is concerned and this is similarly seen in studies reported from other parts of the World describing Superinfections or Secondary Bacterial Infections[14,16]. And also study by Mustafa Karatas, *et al* where *Acinetobacter baumannii* was the main pathogen in the respiratory infections of COVID-19 patients (9.76%)[18].

Another study also showed Gram-negative Bacteria were commonest (78.03%), eg, *Klebsiella pneumoniae* (29.3%,) *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *E coli*[19].

In a study conducted by Surbhi Khurana *et al*, *K pneumoniae* (33.3%) was the predominant pathogen, followed by *A. baumannii* (27.1%)[20].

It has been found that the COVID pandemic had strong implications for antimicrobial resistance. Indiscriminate use of antibiotics has strongly increased the incidence of Multidrug-resistant Bacteria. In our study, out of the total hospitalised patients with Secondary infections, there were 25 (27.8%) MDR.

Table 2 — *The antimicrobial resistance pattern of isolated organisms is presented in tabular form*

Antibiotics	*S aureus* (n=11)	Strepto-coccus spp.(n=2)	Entero-cocci (n=5)	*E coli* (n=59)	*Klebsiella* (n=28)	*Serratia* (n=1)	Salmonella (n=1)	*Pseudo-monas* (n=17)	*Acinetobacter baumannii* (n=7)	Proteus miabilis (n=1)
Penicillin	8 (72.7)	2 (100)	4 (80)	-	-	-	-	-	-	-
Amoxiclav	-	-	1 (20)	-	-	-	-	-	-	-
Piperacillin-Tazobactam	-	-	-	47 (79.6)	23 (82.1)	0	-	7(41.1)	2 (28.6)	0
Cefoxitin	5 (45.5%)	-	-	-	-	-	-	-	-	-
Cefuroxime	-	-	-		17 (60.7)	1 (100)	0	8 (47.0)	5 (71.4)	0
Ceftazidime	-	-	-	48 (81.3)	15 (53.5)	1 (100)	0	3 (17.6)	6 (85.7)	1 (100)
Ceftriaxone	4 (36.3)	1 (50)	2 (40)	51 (86.4)	23 (82.1)	1 (100)	0	9 (52.9)	6 (85.7)	1 (100)
Gentamicin	5 (45.4)	0	1 (20)	51 (86.4)	24 (85.7)	0	1 (100)	8 (47.0)	5 (71.4)	1 (100)
Amikacin	3(27.2)	0	1 (20)	49 (83.0)	23 (82.1)	0	1 (100)	7 (41.2)	5 (71.4)	1 (100)
Ciprofloxacin	4 (36.3)	1 (50)	1 (20)	38 (64.4)	18 (64.2)	1 (100)	1 (100)	11 (64.7)	7 (100)	1 (100)
Clindamycin	0	0	2 (40)	-	-	-	-	-	-	-
Chloramphenicol	9 (81.8)	1 (50)	1 (20)	-	-	-	-	-	-	-
Erythromycin	8 (72.7)	1 (50)	1 (20)	-	-	-	-	-	-	-
Linezolid	0	0	0	-	-	-	-	-	-	-
Vancomycin	0	0	0	-	-	-	-	-	-	-
Imipenem	-	-	-	4 (6.8)	3 (10.7)	0	0	3 (17.6)	2 (28.6)	0
Meropenem	-	-	-	3 (5)	2 (7.1)	0	0	4 (23.5)	1 (14.3)	0
Polymyxin B	-	-	-	2 (3.3)	1 (3.6)	0	0	2 (11.8)	0	-
Colistin	-	-	-	2(3.3)	1 (3.6)	0	0	1 (5.9)	0	-

Escherichia coli was predominantly isolated which showed the highest resistance to third-generation cephalosporins, ceftriaxone 51 (86.4 %) and ceftazidime 48(81.3%) and β-lactam-β-lactamase inhibitor combinations, piperacillin/tazobactam 47 (79.6%).

Not only overuse of antibiotics but intrinsic characteristics of patients themselves also have a strong impact on antimicrobial resistance. As hospitalized COVID-19 patients often are comorbid subjects who went through several hospitalizations during previous months, leading to colonization by Multiple Drug Resistant (MDR) and also Impaired Immune System. All these factors together lead to a full-blown infection with resistant bacteria. Several studies have indicated that not only use of excessive and inappropriate use of antibiotics but also hygiene education, social distancing, wearing of face masks, regular hand washing, isolation of infected cases, Personal Protective Equipment (PPE) employments, contact precautions are strongly recommended, however evidence for this is weak.

Limitations :

As this study was conducted in a single center, therefore findings can't be generalized to other parts of the Country, as the prevalence of MDR pathogens is often specific to each Hospital.

Conclusion :

In our study maximum, positive growth was isolated from ICU 36 (27.2%), *Escherichia coli* was most commonly isolated 59(44.7%). Out of 90 Gram-negative isolates, 25(27.8%) were found to be MDR producers and among 11 *Staphylococcus aureus*, 5(45.5%) were Methicillin-Resistant Staphylococcus Aureus (MRSA).

From this study it has been found that poor infection control and irrational antibiotic use lead to Secondary infections, standard practices in ICU need to be followed, (use of PPEs, double gloves, frequent hand washing, etc), there should be an implementation of Infection Prevention Control Measures in hospitals and Antimicrobial Stewardship Programs (ASP) should take effort to measure and improve antibiotic prescribing and use to effectively treat infections, protect patients from harms caused by unnecessary antibiotic use and combat antibiotic resistance and clinicians could also de-escalate treatment as soon as patients condition improves. All these together may decrease the overall mortality rate.

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