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"Impact of COVID-19 on Antimicrobial Resistance in North Indian Asian Population: A Institutional Based Study".

Rajani Singh¹, Roshni Agarwal², Suraiya Khanam Ansari^{1*}, Vikas Mishra¹,

Assistant Professor, Department of Microbiology; Professor and Head, Department of Microbiology;

Associate Professor and Head, Department of Microbiology; Professor, Department of Microbiology.

^{1*}Department of Microbiology, GSVM Medical College, Kanpur 208002, Uttar Pradesh, India.

²Department of Microbiology, Naraina Medical College and Research Centre, Kanpur 208020,Uttar Pradesh, India.

*Corresponding Author:Dr. Suraiya Khanam Ansari

*Associate Professorand Head, Department of Microbiology, GSVM Medical College, Kanpur 208002, Uttar Pradesh, India

KEVWORDS	ABSTRACT
Antimicrohial	Introduction: Antimicrobial resistance (AMR) possess threat to the effective treatment and
resistance:	prevention of ever-amassed infections caused by viruses, bacteria, parasites and fungi. It is an
	emerging worldwide public health problem despite several technological advancements. The
SARS-COV-2, IFD,	outbreak of COVID-19 infection has additionally embroidered this problem due to the consumption
Gram-negative;	of varied antibiotics.
Pandemic phase	Aim and Objective: The present study aimed to assess the pattern of AMR developed against
	bacterial agents in the pre-and post-COVID-19 outbreak.
	Material and Methods: This single-centre hospital-based study was conducted on 967 bacterial
	isolates obtained from blood and urine samples of patients who attended the outpatient department
	(OPD) and inpatient department (IPD).A cumulative of 576 bacterial isolates including both
	specimens (blood and urine) were detected during the pandemic phase from patients infected with
	SARS-CoV-2 infection of which 71.87% were from blood and 28.12% from urine specimens. The
	Kirby Bauer disk diffusion method was used according to the CLSI guidelines 2019.
	Results: The results showed that among total bacterial isolates, the gram-negative population were
	found to be increased compared to gram-positive bacteria during both pre-COVID and during the
	COVID-19 pandemic phase. Of the total isolates, most of the blood specimens were received from
	the in-patients department (IPD) (71.87%) hospitalized for the treatment and management of
	COVID-19. Among gram-positive pathogens, Staphylococcus aureus showed more percentage
	(60.62%) followed by Enterococcus spp. (8.93%) in blood culture specimens received during the
	pandemic phase. Moreover, ciprofloxacin and cotrimoxazole showed reduced susceptibility among
	gram-negative bacteria.
	Conclusion: The results of the present study extensively reported the antimicrobial resistance
	(AMR) pattern during the pre-pandemic and COVID-19 pandemic phase and it is recommended to
	fabricate strict guidelines for the judicious use of antibiotics along with strengthening the antibiotic
	steward policies, especially in low and middle-income countries.

1. INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic was considered to be a significant worldwide challenge and has overawed our healthcare systems (1). With the increasing burden of SARS-CoV-2 infection, the global health threat of

multi-drug resistance also raised significantly thereby contributing to mortality, morbidity and economic loss (1). Among the infected patients, the admitted patients showed significant episodes of developing AMR. Recent studies supported that rise in COVID-19 infected cases worldwide, contributed to rising in

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antimicrobial therapy prescriptions and thereby the rate of AMR also raised significantly (2-5). Studies have shown that increased prescription for treatment of COVID-19 infection leads to increased AMR cases (6-7). Presentation of symptoms similar to that of viral pneumonia and bacterial diseases, guidelines and suggested existing healthcare framework the implication of antimicrobials as treatment modalities (8). The inability to distinguish between severe COVID-19 infection with other secondary bacterial and fungal infections, generated clinical uncertainties, thereby recommending broad-spectrum antibiotics to salvage the patient (9-10).

COVID-19 pandemic inadvertently hampered several necessary programs including national health programs, hospital infection control measures programs, and antimicrobial stewardshipprograms worldwide (11). Consequently, the world witnessed a surge of AMR in healthcare-associated infections (12-13). The problem of AMR was considered to be the health crisis worldwide during the pre-pandemic COVID-19 period, but due to the outbreak of COVID-19 infection worldwide, all healthcare system's efforts were focused on its management and thereby compromised antibiotic consumption and audit (14-15). Data suggested that about 50% of mortalities in the COVID-19 pandemic occurred due to secondary bacterial infections (9). It is still a matter of detailed research, on whether the COVID-19 hospitalized patients benefitted from supplementation of antibiotics. A study from the past suggested that the use of inadequate broad-spectrum empiric antibiotics in case of sepsis showed higher mortalities (16). Such differing assumptions lead to the emergence of unprecedented AMR that further compromised the global programs for combating AMR worldwide (17-18). Therefore, the present study aimed to study the pattern of AMR from clinical samples (both urine and blood) collected from pre-COVID-19 and post-COVID-19 periods.

2. Material and Methods 2.1 Ethical consideration

Ethical approval was duly obtained from theInstitutional Ethics Committee of GSVM Medical College Kanpur. Written informed consent was obtained from each participantbefore data collection.

2.2 Study design and setting

This cross-sectional observational single-centre study was performed between a period of 2019to April 2020 in the Department of Microbiology at a tertiary care hospital. Patients who attended OPDs and were admitted were included in the present study. The study was performed in Kanpur Nagardistrict, having a population size of 4581268 according to the 2011 census, which is equivalent to the population of Costa Rica and Louisiana. It is located at the coordinate of 26.4148° N, 80.2321° E on the south bank of river Ganga having a latitudeof 26.449923 and a longitude of 80.331871. Bacterial isolates were obtained from urine and blood samples for antimicrobial susceptibility patterns.

2.3 Sample size

The sample size was calculated with Epi-InfoTM version 7.1.5 StatCalc software (at 95% Confidence Interval, 80% power of test). A total of 1543bacterial isolates were identified and the antimicrobial susceptibility pattern was studied including pre-COVID-19 (n=967) and post-COVID-19 (n=576).

2.4 Sample preparation and bacterial isolate screening

Blood and urine samples obtained from patients either admitted or who attended the OPDs of our tertiary study centre were recruited into the present study. During the COVID-19 pandemic, the clinical samples from patients were transported directly to the Department of Microbiology as per the recommended guideline issued by district authorities. Blood and urine samples from clinically diagnosed/suspected/confirmed cases of COVID-19 were transported from the admitted facility to the department for analysis following strict guidelines issued by authorities.

A trained person wearing N-95 masks and personal protective equipment (PPE) handled the samples in a class-II A2 biosafety cabinet (Thermo Fisher Scientific). 10-15 mL venous blood samples were poured into the automated BACTEC aerobic blood culture bottles (Thermo Fisher Scientific) followed by incubation for 7 days. Positive flagged bottles were withdrawn for the gram staining procedure. Isolation of desired pathogens was performed from subcultures on blood and MacConkey agar plates followed by

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overnight incubation at 37^oC. Gram-stained smears of pathogens obtained from the colonies were viewed under a microscope.

Moreover, urine routine microscopy and cultures were performed from mid-stream urine samples. Briefly, semi-quantitative methods with calibrated loops containing cysteine lactose electrolyte deficient media were used to obtain CFU/mL pathogens.Bacterial growth from culture was read after 18-24 h of inoculation. An automated identification method as described previously was used for the identification of bacterial isolates (19).

2.5 Bloodstream infection definition

Bloodstream infection within recruited study subjects was defined as the presence of one positive blood culture specimen. A minimum of two positive consecutive blood cultures were required to define bloodstream infection.

2.6 Antimicrobial susceptibility testing

The antimicrobial susceptibility testing (AST) of the collected specimens was performed by the previously described method of Kirby Bauer disk diffusion according to the CLSI guidelines 2019 (20). Quality control procedures were followed for microscopy and AST using the ATCC strains (Staphylococcus aureus 25923 and Escherichiacoli 25922).

2.7 Statistical Analysis

Statistical analysis was done with Prism Version 9.0 (GraphPad by Dotmatics,San Diego, CA 92108). Descriptive categorical variables were evaluated in the form of frequencies and confidence intervals (CIs) at 95%. Quantitative variables were assessed in the form of mean and standard deviation using the student's "t" test. The p-value <0.05 was considered to be significant.

3. Results

In the present study the total number of specimens received in the Department of Microbiology laboratory during the pre-pandemic was significantly more (N=5628) compared to the pandemic phase (N=3985). A sharp decline in bacterial growth was seen during the COVID-19 pandemic phase (14.44 vs. 17.18%). A cumulative of 576 bacterial isolates including both specimens (blood and urine) were detected during the pandemic phase from patients infected with SARS-CoV-2 infection of which 71.87% were from blood and 28.12% from urine specimens. The distribution of the bacterial isolates within the specimens of different age groups showed significant variation when compared to the pre-pandemic phase among infants below 12 months and >12 months of age group (p 0.000) Table 1.

Bacterial isolate distribution is not dependent on gender and is found to be non-significant in specimens from the pre-and post-pandemic phase of COVID-19 (p 0.620). There was significant variation found among the specimens collection site as 99.47% of specimens were collected from the in-patient department (IPD) during the ongoing pandemic phase compared to the precovidphase. A significant variation was also observed in the pattern of specimen collection in both phases from IPD, out-patient department (OPD) and intensive care unit (ICU) (p 0.009) Table 1.

Moreover, during the pandemic phase, a significant increase in the receiving proportion of blood culture specimens (71.87%) was observed compared to the urine specimens (27.60%). The percentage of bacterial isolates in blood samples among the specimens received in a covid phase was higher (71.87%) compared to the isolates from the pre-covid phase (60.28%). Similarly, urine samples showed fewer isolates (28.12%) in covid phase specimens compared to pre-covid phase (37.84%) (Table 1).



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Graph 1: Comparative distribution of commonly isolated bacteria from blood and urine specimens in the year 2019 and 2020.

In the present study (Graph 1), significant differences were observed in the number of specimens for culture received in the pre-COVID and post-COVID era including both blood and urine.*Staphylococcus aureus* showed a significant presence compared to other Grampositive bacteria in blood culture in specimens received

from the pre-COVID phase and IPD admitted patients suffering from the COVID-19 pandemic. Moreover, *Klebsiella pneumonia* and Pseudomonas *aeruginosa* showed more percentages 7.48% and 3.14% respectively compared to other Gram-negative bacteria.



Graph 2: Comparison of antimicrobial susceptibility pattern of Gram negative in blood samples between pre-COVID and COVID period (a) *Escherichia coli* (b)*Acinetobacter baumanii*(c) *Citrobacter freundii*(d) *Enterobacter aerogenes*.

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In the antimicrobial susceptibility pattern of blood culture isolates, ciprofloxacin and cotrimoxazole showed reduced susceptibility among the Gram negative bacteria (Graph 2). *Klebsiella pneumoniae* was less susceptible to aminoglycosides, fluoroquinolones and carbapenems. On the other hand, *Pseudomonas aeruginosa* isolates showed alarming resistance to imipenem along with third-generation cephalosporins.



Graph 3: Comparison of antimicrobial susceptibility pattern of Gram positive bacteria in blood samples between pre-COVID and COVID period (a) *Enterococcus spp.* (b)*Staphylococcus aureus* (c)*Coagulase negative staphylococcus spp.*

Staphylococcus aureus depicted reduced susceptibility to all classes of the drug during the pandemic period. Significant increases in MRSA during the COVID-19 period with decreases in susceptibility to commonly prescribed drugs such as clindamycin, erythromycin and fluoroquinolones were observed as shown in Graph 3.

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Graph 4: Comparison of antimicrobial susceptibility pattern of Gram negative in urine samples between pre-COVID and COVID period (a) *Escherichia coli* (b)*Acinetobacter baumanii*(c) *Citrobacter freundii*(d) *Enterobacter aerogenes*.

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Graph 5: Comparison of antimicrobial susceptibility pattern of Gram positive bacteria in blood samples between pre-COVID and COVID period (a) *Enterococcus spp.* (b)*Staphylococcus aureus* (c)*Coagulase negative staphylococcus spp.*

In the present study it was observed that *Escherichia coli* and *Acinetobacter baumannii* showed increased resistance to most of the drugs, while the susceptibility pattern for *Klebsiella pneumoniae* did not vary in both periods. Although *Pseudomonas aeruginosa* isolates were less in number, the resistance patterns for various antibiotics were not significantly different except for piperacillin-tazobactum and cefepime (Graph 4-5).

4. Discussion

Frequent use of antibiotics in patients with COVID-19 threatens to exacerbate antimicrobial

resistance. Antibiotics were commonly used for the management and treatment of COVID-19 infection worldwide across healthcare settings even though aware of the fact that such antibiotics were not affected against viruses, especially against SARS-CoV-2. It was seen that almost 80% of patients hospitalized with COVID-19 infection received antibiotics. This fact was also applicable to the non-symptomatic patients under home isolation, they also even received antibiotics. Antibiotics can be the life saviour but simultaneously posed threat to society in the form of developing AMR against broad-spectrum antibiotics in healthcare

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settings. Widespread use of antibiotics hampered the antimicrobial stewardship policy. AMR development also contributed to uncontrolled use of over the counter availability of antibiotics and steroids that worsen the

Over-the-counter availability of drugs, indiscriminate use of steroids and multiple shopping further worsened the situation to the extent, that the patients with moderate symptoms received multiple courses of antibiotics.Moreover, the inability to differentiate between bacterial and viral infection along with the severity of manifestation among the patients suffering from COVID-19 infection prompted the physicians to initiate broad-spectrum antibiotics causing hurdles in the implementation of antimicrobial stewardship policies. A recent media report published in the Indian Express expressed by the former director general of the Indian Council of Medical Research (ICMR) reported increased drug resistance due to indiscriminate use of antibiotics during the COVID-19 pandemic (21). In one of the recently published studies, it was shown that among the COVID-19 patients presented bacterial coinfection (3.1-3.5%) and secondary bacterial infections (15%) post-hospitalization reported AMR in India, which further contributed to the increased mortality (22). The results are in full agreement with the finding of our study, in which a higher number of samples received from patients hospitalized with IPD presented AMR. This is due to the secondary infections acquired by the admitted patients that especially contributed to their negative health outcomes and prompted the clinicians to combat such events by the use of antibiotics that further resulted in an increased burden of AMR in the Indian population (23). In another Indian study, 84% of patients presented AMR in admitted patients with COVID-19 infection, among them 13% were reported with secondary bacterial infections (24).

The results of our study showed that there was a significant increase in the proportion of blood culture samples received (71.87%) to the department of microbiology for culture compared to the urine culture samples (21.82%)during the COVID-19 pandemic from patients admitted to IPD. Similar observations were made by the authors of the recently published study (25). Moreover, our study was conducted in tertiary care hospital setting on COVID-19 patients with moderate to severe symptoms. During the pandemic phase, there was an increased surge of bacterial

aetiology using blood culture, so our results also showed more blood samples for culture compared to urine during this pandemic phase. However, the positivity of urine cultures is found more compared to the blood cultures as demonstrated by another recently published study (26). The study showed that there was a 38% increase in the surge of the blood culture during the COVID-19 phase compared to urine cultures (26).

SARS-CoV-2 infected patients showed a lower occurrence of bacteremia unlike in patients who suffered from severe influenza as documented by previously published studies (27-29). Taking account of viral sepsis the precise reason behind this decreased episode of bacteremia in SARS-CoV-2 infected patients remains uncertain as observed from blood culture reports (25). Our results demonstrated the higher prevalence of Gram-positive bacteria in the samples received during the COVID-19 pandemic although the pattern was found to be the same as in the pre-pandemic phase.

Our study results are in concordance with the findings obtained by the authors of a previously published study (30). Among the screened Gram-positive bacteria, the Staphylococcus aureusis among the most common bacteria detected in samples received during the COVID-19 pandemic from patients admitted in IPD. This study is in accordance to the previously published studies as they have also reported a higher prevalence of Staphylococcus aureus during the COVID-19 phase (31-32). A previously published study reported that antibiotic resistance developed in Staphylococcus aureus might be contributed by two major pathways either through the mechanism by horizontal transfer of some resistant determinants including plasmids, transposons and the staphylococcal cassette through mutations their chromosome or in chromosomal genetic material (33). It has been found that prolongedhospital stay during the COVID-19 pandemic along with extensive use of steroids and broad-spectrum antibiotics witnessed the higher occurrence of Staphylococcus aureus as a common source of bloodstream infections.

Our results also showed that *Staphylococcus aureus* showed less sensitivity to antibiotics including ciprofloxacin, imipenem, cotrimoxazole, tetracycline, linezolid, clindamycin, cefoxitin and erythromycin. These results are in concordance with the consensus

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data provided by the Indian Network for Surveillance of Antimicrobial Resistance (INSAR). On the contrary, *Pseudomonas aeruginosa* showed higher occurrence among the group of Gram-negative bacteria in samples received from admitted patients from IPD during the COVID-19 pandemic phase similar to the observations made by the authors of a previously published study (34). Among the group of Gram-negative bacteria, Acinetobacter baumanii presented reduced susceptibility against antibiotics such as cefotaxime.

non-judicious The prescriptions of antibiotics contributed to this alarming condition of antimicrobial resistance during the COVID-19 pandemic phase compared to the preceding time (35). World Health Organization (WHO) stated that the overuse of such antibiotics offers opportunities to the bacteria to acquire drug resistance through various mechanisms that will lead the world to an emergency (36-37). Our study also focussed on the trends of bacterial population and species compared between the pre-pandemic and during the COVID-19 pandemic phase. Our study also demonstrated the potential role of AMR developed during the COVId-19 pandemic phase in samples received from patients admitted to IPDs. However, the burden of infection is still not alarming in the study because of several factors region including discontinuation of antibiotics after COVID-19 vaccine shots, limited supply of antibiotics and a holistic approach for home isolated non-symptomatic patients using traditional medicines.

To ensure the appropriate use of antibiotics and to control AMR, more efforts need to be forged to monitor and improve antibiotic stewardship activities at the community level [38]. More education and awareness campaigns and activities may be worthwhile.

5. Conclusion

As the entire world faced the threat of the COVID-19 pandemic, there is still a major threat of antimicrobial resistance lurking behind the community. AMR posed threat to all patients admitted to the hospital facilities by putting their lives at risk, irrespective of comorbidities and other medical conditions during the COVID-19 pandemic. Strict antimicrobial stewardship policies may reduce the occurrence of AMR during COVID—19 pandemic management. Countries worldwide should make a repository of available AMR data on a singleuser platform for physicians and policymakers for framing strict guidelines. Further qualitative and quantitative research is needed to explore the occurrence of secondary bacterial infections in patients infected with SARS-CoV-2 along with its management using broad-spectrum antibiotics. Low and middleincome countries should strictly monitor the misuse of antibiotics in primary healthcare settings especially having large population sizes to avoid any future medical emergency.

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Conflicts of interest: There is no any conflict of interest associated with this study

Consent for publication: All authors have given consent for publication

Authors' contributions: All the authors equally contributed the work.

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