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Original Article

Superadded Bacterial Infections in COVID–19 Patients; Antimicrobial Susceptibility and Association with Serological Markers

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ABSTRACT

Objective: To determine the frequency and antimicrobial susceptibility pattern of pathogens responsible for superadded bacterial infection in COVID-19 patients and correlate the association of these infections with serological markers.
Study Design: Cross-sectional study.
Place and Duration of study: Department of Pathology, Combined Military Hospital, Multan Pakistan, from Jan to Dec 2021.
Methodology: A total of 290 patients having positive RT-PCR for SARS CoV-2 were included. All samples were processed per Clinical Laboratory Standard Institute (CLSI) protocols. API 20E and API 20NE were used for the identification of Gram-negative rods. Antimicrobial susceptibility testing was performed by the modified Kirby Bauer disc diffusion method. Serological markers, including C-reactive protein (CRP), total leucocyte count (TLC) and serum Ferritin, were determined and compared for significance in positive and negative culture cases.
Results: A total of 75 patients had positive bacterial cultures. Among these, 42(56%) were blood culture, 26(35 %) were respiratory culture and 7(9%) were urine culture. Commonly isolated organisms were *Acinetobacter baumannii, Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa*, i.e., 23(31%), 20(27%), 13(17%), and 12(16%) respectively. CRP, TLC and S. ferritin were markedly raised in superadded bacterial infection compared to patients with COVID-19 infection only.
Conclusion: The frequency of superadded bacterial infections in COVID-19 patients is high. The pathogens isolated in these cases were multidrug-resistant, reflecting mostly hospital-acquired flora. The association of serological markers in depicting superadded infection is statistically significant and may be used to screen for superadded bacterial infection in COVID-19 patients.

Keywords: COVID-19, RT-PCR, Serological markers, superadded bacterial infection.

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INTRODUCTION

In the hindsight of COVID-19, the superadded infections are a big concern as using steroids puts the patients at increased risk. COVID-19 infection is more prevalent in extreme age, which also is a factor in weakened immunity.[1,2] Altered immunity with prolonged hospital stay results in the acquisition of hospital acquired infections.[3,4]

Both bacterial and fungal superadded infections are reported in COVID-19 patients. However, the frequency of bacterial infections is higher.[5,6] The hospital-acquired pathogens are known to have multidrug resistance against common antimicrobials. They need timely detection for early resolution by culture-directed antimicrobial therapy.[7] Hospital-acquired infections are most commonly caused by Gram-negative bacteria, especially Enterobacterales, *Candida* spp and *S. aureus*.[8] Serological markers play an important role in diagnosing bacterial infections as the microbiological culture takes a longer turnaround time while relying upon an aseptic sampling technique and processing.[9,10]

In the prevalent COVID-19 scenario, attention has been drawn to serological markers to monitor COVID-19 progression and provide heads-up for an element of superadded bacterial infection. Therefore, this study was conducted to assess cases of superadded bacterial infection and antimicrobial susceptibility patterns of causative pathogens while correlating with levels of serological markers.

METHODOLOGY

The cross-sectional study was conducted at Combined Military hospital (CMH) Multan Pakistan from January to December 2021 after approval from Hospital IERB Committee (File no 13/Trg/2020 dated 28 December 2020). The sample size was calculated using the WHO sample size calculator, taking a

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confidence level of 95%, and reported prevalence of COVID-19 infection at 7.2%.[11] The estimated sample size came out to be 290 patients.

Inclusion criteria: RT-PCR confirmed COVID-19 admitted patients of either gender with suspected bacterial infection were included in the study.

Exclusion Criteria: Suspected COVID-19 patients or HRCT-positive patients were not included in the study.

Bacterial infection was suspected in cases where a spike of fever was reported after achieving thermal regulation or the patient's general condition deteriorated based on systemic symptoms. Nasopharyngeal swabs of all patients were collected and transported in viral transport media (VTM). According to manufacturer instructions, the extraction and amplification of viral nucleic acid for SARS-CoV-2 PCR was done on automated systems. According to the manufacturer's instructions, the total leucocyte count was done using a fully automated Sysmex KX21 haematology analyzer. A venous blood sample was used for C-reactive protein, and it was analyzed by Roche Cobas c501 analyzer based on the spectrophotometer technique. S. ferritin values were ascertained on a fully automated Roche Cobas e411 immunoassay analyzer based on the electrochemi-luminescence technique.

Antimicrobial culture and susceptibility testing were performed using Clinical Laboratory Standard Institute (CLSI) 2020 guidelines.13 Blood cultures were processed in an automated BACT alert system. Once flagged positive, they were dealt with on recommended agar. Respiratory and urine specimens were also dealt with per guidelines on nutrient, enriched and differential agar. Blood, MacConkey and chocolate agar were primarily used along with supplementary media as per guidelines. After sample processing for culture, staining was done for presumptive identification. Inoculated culture plates were incubated for 18-24 hrs at 35 ± 2o C.

The samples that yielded growth were subjected to biochemical tests after Gram staining. Catalase, Coagulase, DNase, bile esculin, arabinose and growth in 6.5% NaCl were performed for Gram-positive organisms. Analytical profile index (API) 20 E and API 20 NE (bioMerieux, Inc.), were used to identify Gram-negative rods. Antimicrobial susceptibility testing was performed by modified Kirby Bauer disc diffusion method on Muller Hinton agar (MHA). No more than six antimicrobial discs were placed on each agar plate. MHA plates were incubated for 18-24 hours at 35±2oC. Zone sizes were measured, and antimicrobial susceptibility was determined per CLSI 2020.[12]

Statistical Package for Social Sciences (SPSS) version 25.0 was used for the data analysis. Descriptive statistics were used to enumerate the frequency of bacterial infection in COVID-19 patients, the type of organism and their antimicrobial susceptibility profile. The association of serological markers with a superadded bacterial infection in COVID-19 vs culture-negative COVID-19 was done by using the Pearson correlation coefficient and chi-square test. The *p*-value ≤0.05 was considered significant.

RESULTS

The positive superadded bacterial infection in COVID-19 patients was among 75 cases (26%). Among culture-positive samples, 42(56%) were central line/ blood culture specimens, 26(35%) were respiratory specimens, and 7(9%) were urine specimens. The distribution of cases according to the causative organism was shown in Table-I.

Table-I: Specimen and Isolates Distribution (n=75)

Isolate	Total
Blood(n=39)	
A. Baumannii	17(43%)
K. pneumoniae	8(20%)
P. aeruginosa	4(10%)
S. aureus	10(26%)
Respiratory (n=24)	
K. pneumoniae	10(42%)
P. aeruginosa	8(33%)
A. baumannii	6(25%)
Urine (n=12)	
Enterococcus	3(25%)
E. coli	4(33%)
K. pneumoniae	2(17%)
S. aureus	3(25%)

Among Gram-negative organisms, *A. baummanni* was the most common, while Gram-positive *S. aureus* (Methicillin-resistant) was the commonest. Isolates yielded in our study were mostly multi-drug resistant, and their susceptibility profile was summarized in Table-II.

Among Gram-negative isolates, resistance was very high against Penicillins, Cephalosporins and Carbapenems, while for Gram-positive isolates, Penicillins, Macrolides, and Quinolones were mostly resistant. Out of 13 *S. aureus* isolates, 10(77%) were found to be Methicillin-Resistant *S. aureus* (MRSA). The multi-drug resistant pattern was indicative of infection being hospital-acquired.

Table-II: Result of antimicrobial susceptibility testing for main culture positive isolates (n=75)

Percentage of isolates resistant to each antimicrobial				
Gram Negative Isolates				
Antimicrobial	*A. baumannii* n=23(31%)	*K. pneumonia* n=20(27%)	*P. aeruginosa* n=12(16%)	*E. coli* n=4(5%)
Ampicillin	IR	IR	IR	100%
Amoxicillin-Clavulanic acid	IR	88%	IR	75%
Cefipime	85%	95%	45%	100%
Ceftriaxone	83%	90%	IR	100%
Ceftazidime	91%	90%	41%	75%
Tazobactam-Piperacillin	67%	77%	31%	50%
Imipenem	68%	68%	42%	25%
Meropenem	69%	70%	41%	25%
Ciprofloxacin	88%	89%	64%	75%
Gentamicin	80%	83%	47%	50%
Doxycycline	24%	62%	IR	50%
Polymyxin B	8%	10%	8%	25%
Gram Positive Isolates				
Antimicrobial	*S. aureus* n=13(17%)		*E. spp* n=3(4%)	
Penicillin	98%		67%	
Ampicillin	98%		67%	
Amoxicillin-clavulanic acid	77%		67%	
Cloxacillin	77%		NT	
Erythromycin	73%		34%	
Clindamycin	28%		IR	
Trimethoprim sulfomethoxazole	40%		IR	
Doxycycline	26%		34%	
Ciprofloxacin	66%		IR	
Gentamicin	20%		IR	
Linezolid	8%		34%	
Rifampicin	7%		NT	
Vancomycin	0%		34%	

IR= Intrinsic resistance, NT= Not tested

Mean serological markers in COVID-19 bacterial culture-negative patients were TLC: 8 x 109 /L, CRP: 83 mg/dl, S. ferritin: 857 ng/ml, while for COVID-19 bacterial culture-positive cases were TLC: 18 x 109/L, CRP: 141 mg/dl, S. ferritin: 1380 ng/ml. Statistical significance of the association of serological markers with a superadded bacterial infection in COVID-19 patients was calculated using a bivariate Pearson equation, and the *p*-value was < 0.01.

DISCUSSION

The SARS-CoV-2 virus is evolving rapidly, leading to new variants, which are more contagious and resilient to innate and acquired immunity.[13,14] This has led to the ongoing fourth wave of this pandemic. The increasing number of cases is already stretching the healthcare system beyond its capacity, and these superadded bacterial infections will increase the workload multifold.[15,16]

This study found Superadded bacterial infections in 26% of COVID-19 cases with a suspected bacterial infection. This is quite a high number considering that a study by Basetti *et al*. reported a prevalence of 14% in critically ill patients with COVID-19. In contrast, another study by Lansbury *et al*. on co-infections in people with COVID-19 reported prevalence of 7%.[17,18]

In our study, most superadded infections were of central line-associated bloodstream infection(CLABSI) followed by respiratory involvement. A study by Musuuza *et al*. reflected the same, with CLABSI being the most common healthcare-associated infection. Ventilator-associated pneumonia (VAP) is the second most prevalent since COVID-19 patients require ventilation and frequent suctioning predisposing them to VAP.[9]

Etiological pathogen depends upon the site of infection, which was the case in this study. In blood culture specimens, *A. baumannii* and *S. aureus* were the most common. In respiratory specimens, *A. baumannii* and *K. pneumoniae* were most prevalent, while *Enterococcus* spp, *S. aureus* and *E. coli* led to catheter-associated urinary tract infection (CAUTI).

Serological markers play an important role in diagnosing infection, but their use in identifying superadded bacterial infection in an ongoing viral infection has been evaluated infrequently. This study showed a marked difference in average levels of serological markers, including TLC, CRP and S. ferritin in COVID-19 versus COVID-19 with a superadded bacterial infection.

CONCLUSION

With the evolution of new strains of SARS CoV-2, hospital stays are getting prolonged, predisposing patients to nosocomial superadded bacterial infections. Clinical correlation with a rising trend of serological markers should be included in the diagnostic algorithm for superadded infection in COVID-19 patients. Strict infection control practices should be implemented at all times to prevent nosocomial infections. Furthermore, serological markers' static or decreasing trend can rule out superadded infection for effective antimicrobial stewardship.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

RKA & SA: Study design, drafting the manuscript, data interpretation, approval of the final version to be published.

FA & SS: Data acquisition, data analysis, critical review, approval of the final version to be published.

WH & MQS: Concept, critical review, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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