https://doi.org/10.29309/TPMJ/2023.30.03.7331

The Professional Medical Journal
www.theprofesional.com
2023, Volume, 30 Issue, 03

ORIGINAL ARTICLE

Bacterial Co-infections and Susceptibility patterns among admitted COVID 19 patients during 3rd wave of pandemic, in a Tertiary Care Hospital. Lahore.

Chahat Hussain[1], Muhammad Abid[2], Asad Ahmad[3], Anam Tariq[4], Shafqat Husnain Khan[5], Ayesha Bashir[6]

Article Citation: Hussain C, Abid M, Ahmad A, Tariq A, Khan SH, Bashir A. Bacterial Co-infections and Susceptibility patterns among admitted COVID 19 patients during 3rd wave of pandemic, in a Tertiary Care Hospital. Lahore. Professional Med J 2023; 30(03):336-341. https://doi.org/10.29309/TPMJ/2023.30.03.7331

ABSTRACT… Objective: To check the Bacterial Co-infections and Susceptibility patterns among admitted COVID-19 patients during 3rd wave of pandemic. **Study Design:** Descriptive Cross Sectional study. **Setting:** Department of Microbiology, Combined Military Hospital Lahore Pakistan. **Period:** May 2021 to August 2021. **Material & Methods:** Six hundred and twelve COVID-19 positive patients having positive bacterial cultures were processed, Antibiotic susceptibility testing was done by Kirby-Bauer diffusion technique, all antibiotics were reported using breakpoints recommended in clinical and laboratory standards institute (CLSI 2021). **Results:** Out of 612 patients, 348 (56.9%) were male and 264 (43.2%) were female. Mean age of the patients was 57.2 ± 14.4 years with a range of 22 to 89 years. Bacterial coinfection was present in 70.4% of the patients. Gram negative bacteria (94.4%) were more prevalent in COVID-19 patients as compared to gram positive isolates (5.6%). Antibiotic sensitivity pattern of Staphylococcus aureus showed a high resistance against penicillin, ampicillin, tetracycline and doxycycline. **Conclusion:** Our study reported a high prevalence of bacterial coinfections in COVID-19 patients infected during the third wave of pandemic. A high percentage of gram negative species were identified in our study population, this could be due to the suppression in the immunity of individuals due to severity of COVID-19 infection and already present Antimicrobial resistance.

Key words: Acinetobacter Baumannii, Antimicrobial Resistance, Bacterial Coinfection, COVID-19, E Coli, Gram Staining.

INTRODUCTION

The most feared and dreaded disease of the 21st century came to light in 2019 and was named as Corona virus disease caused by a novel member of RNA (enveloped) viruses known as SARS- CoV-2. The first case of this condition was reported in Pakistan in December 2019, since then the cases have increased then declined in a pattern. These patterns were later on categorized as waves.[1] The first wave was most critical as there was little knowledge about its infectivity, transmission and treatment modalities. It spread rapidly and mortality rate was quite high. The second wave and third wave were however much moderate and caused less deaths due to progression in the development of vaccinations and treatment protocols.[2] A new variant (Omicron) emerged in the United Kingdom. This variant was spread to 64 countries including Pakistan.[3] Since its emergence the mortality and infectivity has far exceeded than any of other ailment such as common flu. Presence of coinfection (viral, bacterial, fungal) is a very important diagnostic step as it can lead to misdiagnosis which can cause flare up in the symptoms and no responsiveness to routine therapy.[4] Pathophysiological process of COVID-19 disease further paved way for development of bacterial and fungal coinfections by underlying disruption in the macrophage function which leads to increase bacterial attachment. Two main mechanisms have been identified which include dysfunction of TLR4/5 pathway and mucosal cell death.[5] The antibiotics are usually ineffective against COVID-19. However the rationale behind

1. MBBS, FCPS Microbiology Trainee, Postgraduate Resident Microbiology, CMH, Lahore.
2. MBBS, FCPS, CI. & Classified Microbiologist, CMH, Lahore.
3. MBBS, House Officer Microbiology, CMH, Lahore.
4. FCPS Microbiology Trainee, Postgraduate Resident Microbiology, CMH, Lahore.
5. FCPS Microbiology Trainee, Postgraduate Resident Microbiology, CMH, Lahore.
6. FCPS Microbiology Trainee, Postgraduate Resident Microbiology, CMH, Lahore.

Correspondence Address:
Dr. Chahat Hussain
Department of Microbiology
CMH Lahore.
chahat@hussain@gmail.com

Article received on: 24/10/2022
Accepted for publication: 30/12/2022

this therapy is the hidden coinfections that are usually difficult to rule out due to similarity in symptoms and presentation.

A rapid increase of disease burden on the health care system was seen during this pandemic. A significant usage of antibiotics was noted which lead to the development of antimicrobial resistance. Infection control practices vary with every locality.[6] In our setup usually broad-spectrum antibiotics are used for critically ill admitted patients. There has been a long debate in literature regarding whether to use antibiotics in COVID-19 patients or not, as it can worsen the status of already ongoing antibiotic resistance in our clinical setups.[7] This study was designed with an aim to gain better understanding of the bacterial coinfections presenting with COVID-19 among patients infected in the third wave, and to identify the responsible pathogen so that adequate and targeted treatment can be carried out while minimizing the use of antibiotics and adverse effects associated with it.

MATERIAL & METHODS

It was a descriptive, cross sectional study, carried out in the department of Microbiology, Combined Military Hospital Lahore.

The study was completed over a period of four months from May 2021 to August 2021 after taking approval from ethical review committee board via letter No 308/2021.

Sample size of 612 was calculated using alpha of 0.05, Power of test 90% and confidence interval of 99% using prevalence of bilateral bacterial coinfections among COVID-19 patients to be 36% from a study conducted by Naveed et al.[8] Sampling technique used was non-probability consecutive sampling. All of the patients suffering from COVID-19 (diagnosed and tested positive during the third wave only) and admitted in Intensive care units and wards were included in the study. Patients from OPD with previously diagnosed corona virus disease in first and second wave and those not suffering from COVID-19 were excluded.

Basic demographic characteristics (age and gender), history of smoking, comorbidities and symptoms were recorded. Only one type of sample (blood, sputum, urine, throat swabs, tracheal aspirate and bronchoalveolar lavage) was collected from each patient.

After receiving the samples were kept in a biosafety cabinet and process of culture inoculation was started. Blood culture specimen were processed in BACT/ALERT 3D System (BioMerieux, France). Positive samples were subcultured on Blood agar and MacConkey agar. Urine samples were inoculated on Cysteine electrolyte deficient agar medium using bacteuric strip. Sputum samples were processed after checking microbiological fitness and inoculated on blood and MacConkey agar. Biochemical tests (catalase, coagulase, oxidase, indole, API, Dnase etc) were carried out for identification of bacteria.

Antimicrobial testing was performed based on the clinical laboratory standards (CLSI 2021) recommended criteria using Kirby-Bauer Disc diffusion method. Muller Hinton agar plates were incubated for 24h at 35 to 37°C and the results were recorded by looking at the inhibition zones. Data was analyzed using SPSS version 26.0. Mean and SD were calculated for numerical variables. Percentage and Frequency was calculated for categorical variables. Normality of data was checked using Shapiro wilk test which showed a normal distribution of data. Chi square test was used for checking association of bacterial coinfection in hospitalized COVID-19 patients, antibiotic resistance among various groups of bacterial isolates. p value of ≤ 0.05 was considered to be significant.

RESULTS

Out of 612 patients, 348 (56.9%) were male and 264 (43.2%) were female. Mean age of the patients was 57.2 $\pm$ 14.4 years with a range of 22 to 89 years. Sample distribution was as following: 257 (42%) blood samples, 175 (28.6%) urine samples, 57 (9.3%) sputum/pus, 71 (11.6%) bronchoalveolar lavage, 31 (5.1%) were throat swabs and 21 (3.4%) tracheal aspirates. Bacterial coinfection was present in 70.4% of the patients.

Basic characteristics of study participants is shown in Table-I.

Variable		Number (Percentage)
Ward of admission	COVID ward	290 (47.4%)
	Covid ITC	322 (52.6%)
Comorbidities	Hep B/ Hep C	87 (14.2%)
	Kidney Disease	18 (2.9%)
	GI problems	40 (6.5%)
	Diabetes mellitus	109 (17.8%)
	Hypertension	72 (11.8%)
	Liver disease	9 (1.5%)
	None	277 (45.3%)
Smoker	Yes	312 (51%)
	No	300 (49%)

Table-I. Basic characteristics of study population

Gram negative bacteria (94.4%) were more prevalent in COVID-19 patients as compared to gram positive isolates (5.6%). Klebsiella pneumonia was most identified, followed by E, coli, acinetobacter baumannii and pseudomonas aeruginosa. Figure-1.

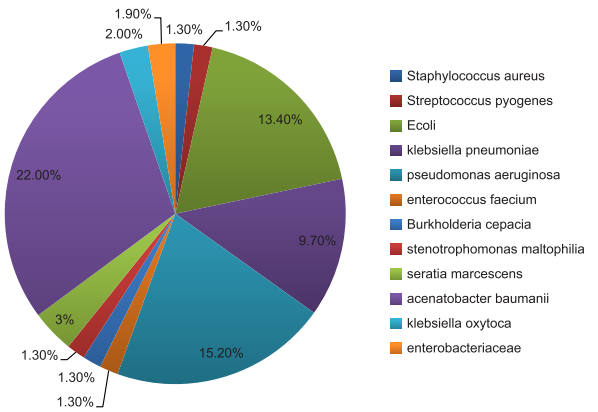

Figure-1. Clinical bacterial isolates identified among Covid 19 patients in third wave

Blood samples yielded the most positive culture results followed by urine samples. Tracheal aspirate revealed the least positive results and bronchoalveolar lavage gave the most negative results when inoculation and culture was done. Table-II.

Out of total samples tested for various antibiotic sensitivity patterns augmentin (79.5%), penicillin showed the highest resistance (74.7%) followed by ciprofloxacin (72.3%), imipenem (53.8%), meropenem (55.3%) and gentamycin (48%).

Sample	Positive (n=431)	Negative (n=181)	P-Value
Blood	199	58	0.001
Urine	139	36	
Sputum	42	15	
Throat swab	19	12	
Tracheal aspirate	12	9	
Bronchoalveolar lavage	20	51	

Table-II. Prevalence of bacterial culture posistivity among various collected samples from Covid 19 patients infected in third wave.

Antibiotic sensitivity pattern of staphylococcus aureus showed a high resistance against penicillin, ampicillin, tetracycline and doxycycline. No case of vancomycin resistance staph. aureus was noted. Streptococcus pyogenes showed greatest sensitivity against penicillin, meropenum and augmentin. Shown in Table-III and IV. Erythromycin, chloramphenicol and doxycycline is not reported in urinary samples. Nitrofurantoin is only reported in urinary samples.

Antibiotic	Resistance Percentage		
	Specie		
	Staphylococcus Aureus	Streptococcus Pyogenes	Enterococcus sp.
Cefoxitin	15.8%	NT	NT
Amikacin	100%	NT	NT
Chloramphenicol	0%	0%	19.1%
Ciprofloxacin	0%	NT	75%
Cotrimoxazole	0%	NT	IR
Clindamycin	0%	NT	IR
Erythromycin	0%	100%	NT
Linezolid	0%	NT	NT
Vancomycin	0%	0%	0%
Gentamycin	17%	NT	NT
Tetracycline	0%	41%	8%
Penicillin	100%	0%	0%
Ceftriaxone	NT	64%	IR
Levofloxacin	0%	78%	42%

Table-III. Pattern of antibiotic resistance among Gram positive bacterial specie (NT: not tested) (IR: internally resistant)

Antibiotic	Resistance Percentage			
	Specie			
	E coli (n=80)	Klebsiella pnemoniae (n=58)	Pseudomonas aeruginosa (n=91)	Acenatobacter baumanii (n=132)
Ampicillin	10%	IR	NT	NT
Augmentin	100%	92.5%	NT	NT
Ceftriaxone	100%	91.8%	35.7%	NT
Ciprofloxacin	100%	91.8%	26.3%	100%
Levofloxacin	100%	82.6%	NT	100%
Chloramphenicol	0%	17.2%	8.7%	NT
Imipenem	42.5%	67.2%	29.1%	86.3%
Meropenem	42.5%	62.7%	29.1%	89.7%
Tigecycline	47.5%	36.2%	5.4%	74.2%
Tetracycline	37.5%	46.5%	12%	61.3%
Gentamycin	37.5%	60.3%	14.2%	87.1%
Tobramycin	20%	36.2%	0%	44.6%
Colistin	0%	0%	0%	6.5%
Nitrofurantoin*	32%	100%	NT	NT
Fosfomycin*	0%	NT	NT	NT

Table-IV. Pattern of antibiotic resistance among Gram negative bacterial specie (* only in urine samples) (NT: not tested)

DISCUSSION

Pakistan has a unique location when it comes to sharing borders with two epicenters of this corona pandemic, China and Iran. Knowledge about epidemiology, diagnosis, transmission, management and clinical manifestations of COVID-19 is quite ambiguous. Many studies and trials are being carried out for better understanding of this new emerging disease.[9] Mustafa et al in his study identified that only 1.4% of the patients had secondary bacterial infection or bacterial coinfection along with covid 19.[10] This ratio is very less as compared to our study. It may be due to widespread antibiotic use and concurrently developing antimicrobial resistance among individuals during 1st and second wave. This poses a severe threat to patients admitted in intensive care units as their chances of getting hospital acquired infections raises significantly. Veroken et al reported a relatively high incidence of bacterial coinfection (40.6%) in COVID-19 patients.[11] Baccolini et al reported a prevalence of 56.7% of bacterial coinfections and hospiatla acquired infections in COVID-19 patients.[12] Thus it can be seen that prevalence of bacterial coinfections in COVID-19 patients may vary from country to country.

Clancy et al and Lehmann et al in their studies showed the incidence of bacterial coinfections ranging from 3-30%.[13,14] Nosheen et al in her study revealed that 72% of the patients had hospital acquired bacterial infections. Majority of the causative organisms were of gram negative type (82%) with Acinetobacter, E coli, Enterrococcus and Klebsiella pneumoniae among the most frequently found organisms.[15] Fattorini et al in his study conducted in Italy concluded that 1.3% patients reported with evidence of antibiotic resistant super infections, with mycoplasma pneumonia, staphylococcus aureus, streptococcus pneumonia among the isolated specie.[16] Westblade et al in his study showed that coinfections occurred in less than 4% of the patients admitted with COVID-19 infection. Staphylococcus aureus, Streptococcus pneumonia and Hemophilus influenza were the commonest organisms found on admission, however after prolong hospitalization the ratio of pseudomonas aeruginosa and Klebsiella pneumonia increases.[17]

Naveed et al in his study showed that staph aureus was highly resistant against tetracycline, streptococcus pyogenes against penicillin and pseudomonas aeruginosa against ciprofloxacin.[8]

In our study multiple drugs were 100% ineffective against certain bacterial species. This is an alarming situation for doctors in our health care sector. Chen et al reported that only 1% of the COVID-19 patients showed positive results for presence of bacterial coinfection but 71% of the patients had been put onto empirical antibiotic therapy.[18] Similarly Zhou et al reported that 95% of COVID-19 patients were given different antibiotic regimens, where as only 15% were later on confirmed for superinfections.[19]

Symptomology of various respiratory and infectious disease generally overlap with patients presenting with COVID-19 disease. Some specific signs such as presence of anosmia, fever, cough and extreme fatigue could support the above diagnosis. Research has been going on to establish the clinical significance of various biomarkers in order to identify COVID-19 patients with and without added bacterial co infections. WHO clinical guidelines for management of COVID-19 patients clearly state to not prescribe antibiotics in mild infection with low or no susceptibility of superinfection. Waseem et al reported that 100% of the patients were prescribed antibiotics before admission to the isolation facility.[20] If the same practice continues we might land up in a pandemic bigger and worse than COVID-19 itself and it will be termed as post Covid Antimicrobial resistance.

Strict compliance to the rulings for antibiotic prescription should be followed and judicial and evidence based utilization of drugs should be carried out. Antibiotic steward ship programs should be done at public and private sector clinic and hospitals in order to raise awareness among healthcare professionals regarding this upcoming challenge.

CONCLUSION

Our study reported a high prevalence of bacterial coinfections in COVID-19 patients infected during the third wave of pandemic. A high percentage of gram negative species were identified in our study population, this could be due to the suppression in the immunity of individuals due to severity of COVID-19 infection and already present antimicrobial resistance.

REFERENCES

1. Ciotti M, Ciccozzi M, Terrinoni A, Jiang WC, Wang CB, Bernardini S. **The COVID-19 pandemic.** Crit Rev Clin Lab Sci. 2020 Aug 17; 57(6):365-88.

2. Imran M, Khan S, Khan S, Uddin A, Khan MS, Ambade P. **COVID‐19 situation in Pakistan: A broad overview.** Respirology (Carlton, Vic.). 2021 May 31.

3. Khan A, Bibi S, Kanwal H. **Omicron: A new face of COVID‐19 pandemic.** Health Sci Rep. 2022 Mar; 5(2).

4. Mubashar S, Mukhtar T, Khan NA. **Coronavirus disease (COVID-19) with special reference to Pakistan: A Review on its Different Aspects.** Pak J Zool. 2021 Oct 1; 53(5).

5. Atif M, Malik I. **Why is Pakistan vulnerable to COVID‐19 associated morbidity and mortality?** A scoping review. Int J health Plann Manage. 2020 Sep; 35(5):1041-54.

6. Bilal H, Khan MN, Rehman T, Hameed MF, Yang X. **Antibiotic resistance in Pakistan: A systematic review of past decade.** BMC Infect Dis. 2021 Dec; 21(1):1-9.

7. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P. **Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study.** The Lancet Infect Dis. 2010 Sep 1; 10(9):597-602.

8. Ahmed N, Khan M, Saleem W, Karobari MI, Mohamed RN, Heboyan A, Rabaan AA, Mutair AA, Alhumaid S, Alsadiq SA, Bueid AS. **Evaluation of bi-lateral co-infections and antibiotic resistance rates among COVID-19 patients.** Antibiotics. 2022 Feb 19; 11(2):276.

9. Wu YC, Chen CS, Chan YJ. **The outbreak of COVID-19: An overview.** J Chin Med Assoc. 2020 Mar; 83(3):217.

10. Mustafa ZU, Saleem MS, Ikram MN, Salman M, Butt SA, Khan S, Godman B, Seaton RA. **Co-infections and antimicrobial use among hospitalized COVID-19 patients in Punjab, Pakistan: Findings from a multicenter, point prevalence survey.** Pathog Glob health. 2022 Jan 2:1-7.

11. Verroken, A.; Scohy, A.; Gérard, L.; Wittebole, X.; Collienne, C.; Laterre, P.-F. **Co-infections in COVID-19 critically ill and antibiotic management: A prospective cohort analysis. Crit. Care 2020, 24, 410.**

12. Baccolini, V.; Migliara, G.; Isonne, C.; Dorelli, B.; Barone, L.; Giannini, D.; Marotta, D.; Marte, M.; Mazzalai, E.; Alessandri, F.; et al. **The impact of the COVID-19 pandemic on healthcare-associated infections in intensive care unit patients: A retrospective cohort study.** Antimicrob. Resist. Infect. Control 2021, 10, 87.

13. Clancy CJ, Nguyen MH. **COVID-19, superinfections and antimicrobial development: What can we expect?** Clin Infect Dis. 2020; 71(10): 2736- 2743.

14. Lehmann CJ, Pho MT, Pitrak D, Ridgway JP, Pettit NN. **Community acquired co-infection in COVID-19: A retrospective observational experience.** [published online ahead of print July 1, 2020]. Clin Infect Dis. 2020:ciaa902

15. Nasir N, Rehman F, Omair SF. **Risk factors for bacterial infections in patients with moderate to severe COVID□19: A case□control study.** J Med Virol. 2021 Jul; 93(7):4564-9.

16. Fattorini L, Creti R, Palma C, Pantosti A. **Bacterial coinfections in COVID-19: An underestimated adversary.** Ann Ist Super sanita. 2020 Sep 11; 56(3):359-64.

17. Westblade LF, Simon MS, Satlin MJ. **Bacterial coinfections in coronavirus disease 2019.** Trends Microbiol. 2021 Oct 1; 29(10):930-41.

18. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, Qiu Y, Wang J, Liu Y, Wei Y, Yu T. **Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study.** The lancet. 2020 Feb 15; 395(10223):507-13.

19. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L. **Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: A retrospective cohort study.** The lancet. 2020 Mar 28; 395(10229):1054-62.

20. Waseem M, Rafiq M, Munir A, Kamal Z, Aziz N, Iqbal MJ. **Antibiotics prescription pattern in COVID-19 patients presenting in DHQ Teaching Hospital Sahiwal; Is Pakistan heading towards Post-COVID Antibiotic Resistance Era?.** J Rawalpindi Med Coll. 2021 Aug 31; 25(1):105-9.

AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Chahat Hussain	Corresponding author.	
2	Muhammad Abid	Principal contribution.	
3	Asad Ahmad	Practical contribution.	
4	Anam Tariq	Practical contribution.	
5	Shafqat Husnain Khan	Practical contribution.	
6	Ayesha Bashir	Practical contribution.	