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Aerobic Bacteriological Profile and Antimicrobial susceptibility in suspected CAM (COVID Associated Mucormycosis) patients

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Abstract

Introduction: SARS-CoV-2 virus, the causative agent for the current global pandemic of 2020 was first reported to WHO on 31st December 2019 in China. Knowledge about bacterial co-infections and secondary infections caused by this is limited since it is still ongoing. In Influenza pandemics of 1918 and 2009, such infections increased the mortality rate, hence it is crucial to research about infections associated with pandemics.

Our aims were to isolate and identify the antibiotic sensitivity pattern of CAM infections including the resistance patterns like ESBL and MRSA.

Materials and Methods: All the 120 suspected CAM samples, received in Bowring & Lady Curzon Hospital during the SARS-CoV-2's second peak of April 2021 to June 2021 were retrospectively studied for their identification and antibiotic resistance patterns as per standard guidelines.

Results: The most common bacteria isolated was Staphylococcus aureus (34.96%) followed by Pseudomonas aeruginosa (27.64%). Gram Positive Cocci amounts to 42.28% and Gram-Negative Bacilli amounts to 57.72%. Gram Positive Cocci and Gram-Negative Bacilli isolates and were most sensitive to Linezolid (84.61%) and Piperacillin/Tazobactam (76.06%) respectively. Among the Gram-Negative isolates, 23.94% were ESBL producers and 24% among Staphylococcus aureus were MRSA.

Conclusion: The prevalence of CAM associated bacterial infections amount to a significant percentage, probably due to immune dysregulation and widespread use of steroids and broad-spectrum antibiotics. Hence the use of therapeutics should be monitored to achieve a therapeutic effect at the lowest dose and durations.

Keywords: CoV-2, CAM infections, Antibiotic sensitivity pattern, ESBL, MRSA

INTRODUCTION

Pandemics are threatening mankind more frequently than before. In 1918, the pandemic of Influenza resulted in ~50 million casualties worldwide which repeated in 1957, 1968, and 2009 [1]. Severe acute respiratory syndrome coronavirus (SARS-CoV) in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) in 2012 were near-pandemics which were lethal. [2]. Severe acute respiratory syndrome coronavirus 2 is the culprit behind the current pandemic of Coronavirus disease 2019 (COVID-19) [2]. It resulted in more than 100 million cases and 2 million deaths worldwide within

12 months and is still not under control. This virus was first reported to WHO 0n 31st December 2019, from the city of Wuhan in China [2].

In the 1918 and 2009 influenza pandemics, bacterial coinfection and secondary infections contributed in nearly all influenza deaths, with most common pathogens being S. pneumoniae, β-hemolytic streptococci, H. influenzae, and S. aureus [3]. These infections increased the mortality rate mainly due to shock, respiratory failure and prolonged ICU stay [3]. Also, this knowledge could have a major role in refining antibiotic management guidelines empirically [4]. Knowledge about such infections caused by the SARS- CoV-2 is limited since it is still ongoing worldwide [5]. Hence it is crucial to research about these co infections associated with pandemics timely. It is important to know the proportion of SARS-COV-2 patients with bacterial infections to make sure the responsible use of antibiotics and to reduce the overuse [5].

MATERIALS AND METHODS

We conducted a retrospective study in the period from April 2021 to June 2021 at the department of Microbiology, Bowring and Lady Curzon Medical College and Research Institute, Karnataka, India. Ethical Committee Clearance certificate was obtained from Ethical Committee of Bowring and Lady Curzon Medical College and Research Institute, Bengaluru, Karnataka. The number of samples included were 120. Inclusion criteria was Previous or current COVID-19 positive patients with Mucor mycosis with positive bacterial culture in chocolate agar plates after incubating at 37 0 C for 48 hours. Exclusion criteria was SARS-CoV-2 positive patients without Mucor mycosis. Types of samples included nasal discharges, scrapings and aspirates from sinuses in patients with rhino cerebral lesions, bronchoalveolar lavages from pulmonary lesions, and biopsy tissue from patients with gastrointestinal and/or disseminated disease.

Bacterial isolation and identification were done according to standard guidelines. Antimicrobial sensitivity was determined by Kirby Bauer's disc diffusion method on Mueller Hinton agar (MHA) as per the guidelines of CLSI 2021 M100Ed30E2021[6]. Antibiotic discs used for sensitivity testing were Amikacin (AK) 30µg, Amoxycillin-clavulanic acid (AMC) 20/10µg, Ampicillin/sulbactum (A/S) 10/10 µg, Aztreonam (AZM) 30 µg, Cefuroxime (CXM) 30 µg , Ceftriaxone (CTR)30 µg, Ceftazidime (CAZ) 30 µg, Ceftazidime-clavulanate (CAC) 30/10 µg, Cefoxitin (CX) 30 µg, Cotrimoxazole (COT) 1.25 µg/23.75 µg, Ciprofloxacin (CIP)5 µg, Clindamycin (CD) 2 µg, Chloramphenicol (C) 30 µg, Erythromycin (E) 15 µg, Imipenem (IPM)10 µg, Linezolid (LZ) 30 µg, Pipercillin-Tazobactum (PIT) 10/10 µg, Vancomycin (VA) 30 µg [6].Quality controls used were Staphylococcus aureus (ATCC 25923), E. coli (ATCC 25922) and P. aeruginosa (ATCC 27853) throughout the study for culture and antimicrobial susceptibility testing.

ESBL producing strains were screened using Ceftazidime (30 µg), those with zone diameter ≤22 mm was taken as screening positives [6]. It was followed by double disc synergy test with Ceftazidime (30 µg) and Ceftazidime-clavulanic acid (30 µg/10 µg) drug discs supplied by Himedia labs. Those with >5mm increase in the diameter of the zone of clavulanic acid compared to disc without clavulanate in the lawned MHA plate was taken as positive for ESBL production. MRSA detection using Himedia labs Cefoxitin disc (30 µg) diffusion method. Zone diameter ≤ 24 mm were taken as MRSA and ≥25 mm was taken as MSSA according to CLSI 2021 M100Ed30E2021[6].

Statistical Analysis: The results were expressed as percentages for analysis of various epidemiological details and also for analyzing the distribution of different bacterial isolates and their antibiotic sensitivity pattern. Results were interpreted using Microsoft excel.

RESULTS

Bacterial growth was detected in 120 covid Associated Mucormycosis (CAM) suspected patients, of which the most common bacteria isolated was Staphylococcus aureus (49 isolates, 41 %) followed by Pseudomonas aeruginosa (26 isolates, 22 %). Gram Positive Cocci amounts to 42.28% and Gram-Negative Bacilli amounts to 57.72%. Gram Positive Cocci isolates were most sensitive to Linezolid (92.22%) and Vancomycin (89.80%) (*Table 2*). Gram-Negative Bacilli isolates were most sensitive to Amikacin (87.40 %) and Piperacillin/Tazobactam (87.32%) (*Table 3*). Among the Gram-Negative isolates, 23.94% were ESBL producers (*Fig.1*) and 24% among Staphylococcus aureus were MRSA (*Fig. 2*).

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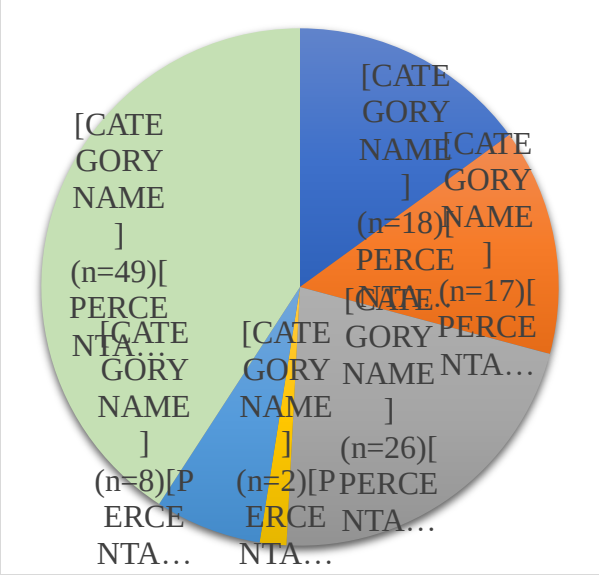
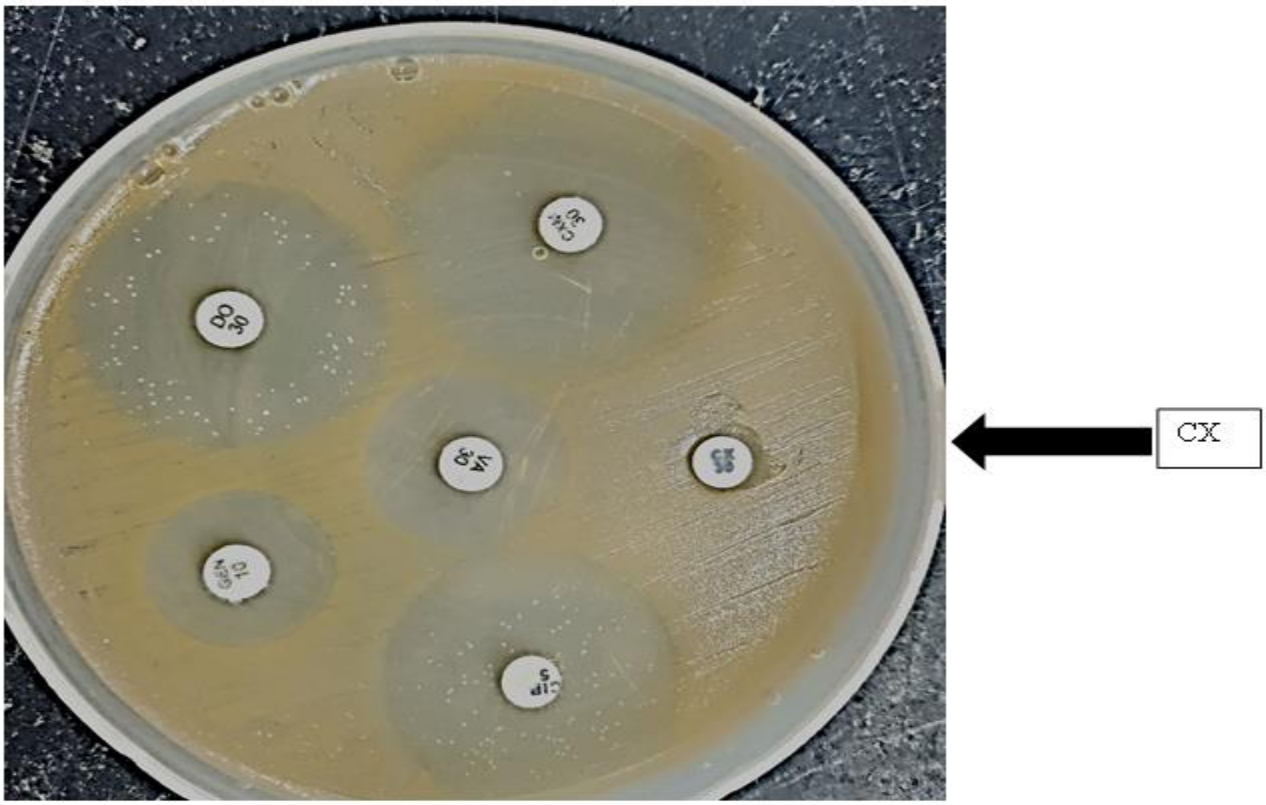


"Graph 1: Distribution of positive bacterial cultures in CAM suspected patients."



"Figure 1: Result of a positive MRSA detection test by Kirby-Bauer disc diffusion method using cefoxitin 30 µg on MHA. Description: CX is Cefoxitin (resistant) (black arrow)."

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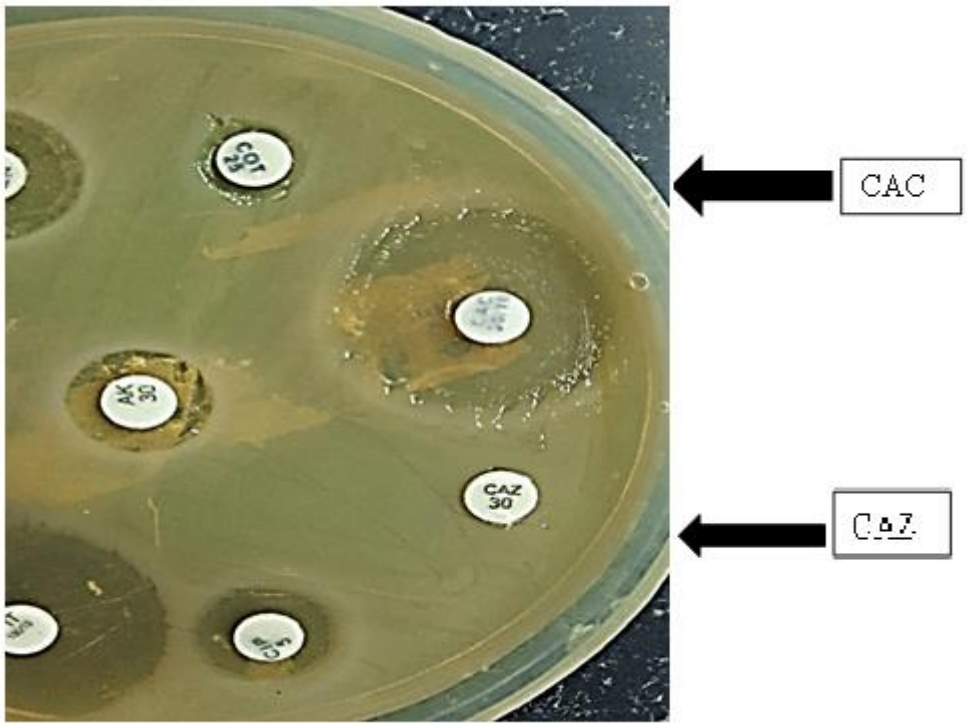

"Figure 2: ESBL detection by Double Disc Diffusion test. (CAZ: Ceftazidime 30 µg; CAC: Ceftazidime +Clavulanate 30 µg/ 10 µg) (black arrows)"

DISCUSSION

Huang and Zhou et al. published a study involving 191 COVID-19 patients from Wuhan, China. Co-infections and secondary infections were identified in 10% and 15%, respectively [5]. Mechanical ventilation in ICU was required in 31 % of them. 50% of non-survivors and only 1% of survivors were diagnosed with a bacterial infection. They diagnosed bacterial infections in patients who had positive culture or clinical features of pneumonia or bacteremia [5]. In London, a retrospective case series of hospitalized SARS-CoV-2 patients was analyzed from two hospitals (20 February–20 April 2020). 27 (3.2%) out of 836 patients had positive bacterial cultures 0–5 days post admission. It rose to 51 (6.1%) out of 836 throughout admission [7]. In another study published by Westblade and Simon et al, the bacterial infections were detected in <4% of SARS-COV-2 patients during admission. Staphylococcus aureus, Streptococcus pneumoniae, and Hemophilus influenzae were the most common pathogens isolated among the culture from these patients [3]. In the epidemiological analysis done by Jennifer M. Farrell and Y. ZhaoTo et al, mortality rate of COVID or post COVID patients with bacterial infection is higher than those without. They used different casual structures of biostatistics in which 3 out of 4 gave similar results and fourth one showing no significant relation between mortality and co infections [8]. All these studies have a common word in conclusion about optimized use of antimicrobial therapy to prevent drug resistance and monitoring the use of corticosteroids [5,7].

CONCLUSION

The prevalence of CAM associated bacterial infections amount to a significant percentage, probably due to immune dysregulation and widespread use of steroids and broad-spectrum antibiotics. Hence the use of these therapeutic agents should be monitored to achieve a therapeutic effect at the lowest dose and shortest durations. Stringent measures to avoid hospital acquired infections should be taken.

REFERENCES

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1. Morens, D. M., Daszak, P., Markel, H., & Taubenberger, J. K. (2020). Pandemic COVID-19 Joins History's Pandemic Legion. mBio, 11(3), e00812-20. https://doi.org/10.1128/mBio.00812-20
2. World Health Organization [homepage on internet] Europe. Available from https://www.euro.who.int/en/health-topics/health- emergencies/coronavirus-covid-19/novel-coronavirus-2019-ncov
3. Westblade, L. F., Simon, M. S., & Satlin, M. J. (2021). Bacterial Coinfections in Coronavirus Disease 2019. Trends in microbiology, 29(10), 930–941. https://doi.org/10.1016/j.tim.2021.03.018
4. Langford BJ, So M, Raybardhan S, Leung V, Westwood D, MacFadden DR et al. Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. Clin Microbiol Infect. 2020 Dec;26(12):1622-1629. doi: 10.1016/j.cmi.2020.07.016.
5. Fattorini L, Creti R, Palma C, Pantosti A; Unit of Antibiotic Resistance and Special Pathogens; Unit of Antibiotic Resistance and Special Pathogens of the Department of Infectious Diseases, Istituto Superiore di Sanità, Rome. Bacterial coinfections in COVID-19: an underestimated adversary. Ann Ist Super Sanita. 2020 Jul-Sep;56(3):359-364. doi: 10.4415/ANN_20_03_14.
6. clsi.org/ [homepage on the internet] CLSI 2021 M100Ed30E2021. Available from https://clsi.org/standards
7. Hughes S, Troise O, Donaldson H, Mughal N, Moore LSP. Bacterial and fungal coinfection among hospitalized patients with COVID-19: a retrospective cohort study in a UK secondary-care setting. Clin Microbiol Infect. 2020 Oct;26(10):1395-1399. doi: 10.1016/j.cmi.2020.06.025. Epub 2020 Jun 27.
8. Farrell Jennifer M., Zhao Conan Y., Tarquinio Keiko M., Brown Sam P.Causes and Consequences of COVID-19-Associated Bacterial Infections.Frontiers in Microbiology volume=12.year=2021.doi=10.3389/fmicb.2021.682571

No. of males	79
No. of females	41
No. of Tissue samples	38
No. of Nasal swabs	75
No. of Sputum samples	6
No. of urine samples	1
No. of blood samples	0
No. of patients in the age group	
<20 years	1
20-30 years	6
30-70 years	109
> 70 years	4

"Table 1: General Statistics of patients included in the study"

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Antibiotic tested	No. of samples showing sensitivity	percentage of sensitivity
Vancomycin	44	89.79
Linezolid	49	100
Gentamicin	37	75.51
Erythromycin	6	12.24
Clindamycin	24	48.98
Ciprofloxacin	30	61.22
Cefuroxime	20	40.82
Amoxclav	28	57.14
Chloramphenicol	48	97.96
Penicillin	2	4.08
Azithromycin	8	16.33
Doxycycline	32	65.31
Cefoxitine	12	24.48

Table 2: Antibiotic Sensitivity pattern of Gram-positive bacterial isolates.

Antibiotic tested	No. of samples showing sensitivity	percentage of sensitivity
Chloramphenicol	34	47.88732
Ceftazidime	22	30.98592
Ceftazidime/Clavulanic acid	34	47.88732
Aztreonam	38	53.52113
Amikacin	62	87.32394
Ampicillin/Sulbactam	28	39.43662
Amoxicillin-Clavulanic Acid	20	28.16901
piperacillin-Tazobactam	62	87.32394
Cefepime	50	70.42254

"Table 2: Antibiotic sensitivity pattern of Gram-negative Bacterial isolates.

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