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RESEARCH ARTICLE OPEN ACCESS

A Prospective, Single Center, Investigator Initiated Observational Study for Identification of Bacterial Coinfections in COVID-19 Positive Patients with Respiratory Dysfunction and Severe Pneumonia Symptoms

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Abbreviations: MIC - Minimum inhibitory concentration; ESBL - Extended spectrum β-lactamase; NDM - New Delhi metallo β-lactamase; *K.pneumoniae - Klebsiella pneumoniae; E. coli - Escherichia coli; P. aeruginosa - Pseudomonas aeruginosa; A. baumannii - Acinetobacter baumannii; S. aureus - Staphylococcus aureus; S. parasanguinis - Streptococcus parasanguinis*; ICU - Intensive care unit; MDR - Multidrug-resistance; RT-PCR - Reverse transcriptase polymerase chain reaction; PCR - Polymerase chain reaction; WHO - World Health Organisation; COVID-19 - Coronavirus disease 2019; SARS-CoV-2 - Severe acute respiratory syndrome coronavirus 2

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Abstract

This study aimed to assess bacterial co-infections in patients diagnosed with positive COVID-19 with respiratory dysfunction and severe pneumonia symptoms admitted in intensive care unit (ICU) of tertiary care hospital. This research was an observational study performed on 166 clinical bacterial isolates obtained from sputum, blood, urine of 20 critically ill COVID-19 positive patients diagnosed by RT PCR technique. Pathogens included were 82 Gram-negative and 84 Gram-positive clinical isolates. Antibiotic susceptibility was determined by broth MIC method. Among Gram-negative organisms, carbapenem resistance was found to be 54.55%, 33.33%, 93.33% in *Enterobacterales*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, respectively. Cefepime/zidebactam was found to be most active antibacterial agent tested. In Gram-positive isolates *S. aureus* and *Enterococcus* sp. were the most encountered isolates. Against *Enterococcus* sp. linezolid, daptomycin, vancomycin, tigecycline showed 100% susceptibility. For *S. aureus,* levonadifloxacin (WCK 771) was found to be most active antibiotic with 100% susceptibility followed by linezolid, teicoplanin. Presence of β-lactamases was confirmed by polymerase chain reaction (PCR) for bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{NDM}, bla_{CMY}, $bla_{OXA-48-like}$. In *E. coli*, NDM was most encountered β-lactamase whereas in *K. pneumoniae*, ESBL were predominantly detected. Dual carbapenemase i.e. NDM and OXA-48 like observed in *K. pneumoniae*. Most of the *P. aeruginosa* showed presence of OXA-4 and VEB type β-lactamase presence. Study clearly demonstrated early determination of co-infections and need of developing targeted antibacterial therapy as the highest priority. Findings showed presence of β-lactamases in bacterial pathogens that render the antibiotic resistant characteristics which significantly affect the clinical outcome and recovery of COVID-19 positive patients. Hence, it has become an urgent need to discover new antibiotics.

Keywords: COVID-19, ICU, Antibiotic, Resistance, β-lactamases, Bacterial Coinfections, Susceptibility, Comorbidity, Mortality

INTRODUCTION

In December 2019, formerly unidentified microbial agents leading to respiratory tract infections were observed in patients in Wuhan, China. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel β-coronavirus, was reported as the causative pathogen and WHO termed the disease as COVID-19.[1,2] Patients with COVID-19 positive results need admission to the intensive care unit (ICU) due to the wide spectrum of clinical complications of the disease from mild infection to respiratory failure. Even though global vaccination lead to the decrease in mortality rate, milder disease with newly emerged variants, and improvement in therapeutic options, high risk patients may still require admission in ICU admission.[3] During initial months of COVID-19 pandemic, mortality rate in ICU was reported to be found between 30%-50% depending on the mechanical ventilation, level of the ICU, and study populations. Older age, diabetes mellitus, obesity, hypertension, chronic kidney disease, oncologic, hematologic malignancy, interstitial lung disease, and secondary bacterial infections are the main risk factors of mortality among the patients with COVID-19 being treated in ICU.[4,5]

Secondary bacterial infections leading to mortality in ICUs has been understood as one of the leading causes. Duration of intubation, status, catheter insertion and duration of ICU stay are the main attributes of secondary bacterial infections. In addition, use of corticosteroids and anti-cytokine medicines negatively impact immunity results in development of secondary infections. Consequently, respiratory viral infections including influenza with secondary bacterial infections is a well-known issue; however, their role in COVID-19 still has uncertainties and complicated.[6,7] The rate of secondary bacterial infection was recorded to be 8.1% (ranging from 0%-25%) among ICU patients in a meta-analysis. This study was performed with the aim to determine the impact of secondary bacterial infections on mortality rate in the ICU and to investigate the etiology of secondary bacterial infections in COVID-19 as a result of their attributable effects, as compared to non-COVID-19 patients.[8,9]

In last three years, the world has been hit by two major COVID-19 pandemic waves. The first wave of pandemic led to overall low fatality rates and ICU admissions. However, during the second wave, the disease was often complicated owing to significantly elevated severity of infections, thereby leading to ICU admissions.[10] In the COVID-19 pneumonia during second wave, highest fatality rates were encountered. It was observed that, the concurrence of bacterial infection in COVID-19 positive patients was also an important factor associated with mortality and severity of infection. A study reported the disease, with its clinical presentation including cough, fever, and lung infiltrates, resembles bacterial pneumonia in the subset of COVID-19 patients admitted to hospitals.[11-13]

It has been reported that, a scenario of 3.5% and 28% secondary bacterial infections were observed in ICU admitted COVID-19 patients. The pathogens involved in these bacterial infections were *Haemophilus influenzae, Staphylococcus aureus* and *Streptococcus pneumoniae* being the most common isolates observed that the incidence of ICU-acquired bacterial infection was as high as 51.2%. The respiratory infections were 38.5% predominant, followed by complicated urinary tract infections (28.0%) and blood stream infections (30.7%).[14-17]

Another study reported that, bacterial infections were present in both non-COVID-19 (1001) and COVID-19 (1398) and patients (13% vs. 8%). Mostly nosocomial infections were reported in COVID-19 patients whereas patients with negative COVID-19 had community acquired infections. The severity of infections in patients with COVID-19 was found to be 81% which was also significant based on chest X-ray with higher bilateral infiltrates as compared to 48% in non-COVID-19 patients. Mortality rate was higher in patients with COVID-19 bacterial pneumonia compared to non-COVID-19 patients (15% vs. 9% respectively).[18-20]

In a survey comprising of 166 participants from 23 countries and 82 different hospitals, clinical presentation was recognized as the most important reason for the start of antibiotics. When antibiotics were started, most respondents rated as the highest the need for coverage of *Staphylococcus aureus, Klebsiella pneumoniae* and *P. aeruginosa*. Combination of fluoroquinolones and β-lactams was often deployed for the treatment of these pathogens. It is known about the piperacillin/tazobactam as the most preferred prescribed antibiotic for the patients admitted in the ICU. The mean duration of antibiotic treatment was reported to be 7.12 (SD = 2.44) days.[21-23]

In past, several studies showed the role of bacterial co-infection in COVID-19 pneumonia patients and the impact on clinical outcome. However, limited data is available assessing the frequency, identification of pathogens, resistance mechanisms expressed and impact of antibiotic therapies in predicting the clinical outcome in COVID-19 positive patients. This will help in determining the COVID-19 associated bacterial infections and help evolve treatment guidelines and identify the unmet need in treating bacterial infections in such patients.[24-30]

Hence, aim of this study was to evaluate the secondary bacterial infections and investigate their antibiotic resistance characteristics in COVID-19 positive patients admitted to ICUs. The present study also focused to determine the correlation of resistance mechanisms expressed by bacterial pathogens, antibiotic therapy used and clinical outcome in terms of mortality.

MATERIALS AND METHODS

Patient inclusion criteria

The patients admitted in ICU of 500 bedded tertiary care MGM Medical College and Hospital in Chhatrapati. Sambhajinagar, with confirmed infection of COVID-19 (RT-PCR positive and hRCT score of >6) were enrolled for the study. Experimental work of this project includes antibiotic susceptibility testing and identification of β-lactamases using PCR method was performed in central research laboratory, MGM Medical College and Hospital in Chhatrapati. Sambhajinagar. Proposed research work was carried out strictly in accordance with the ethical guidelines prescribed by Central Ethics Committee on Human Research (CECHR). The details of the proposed departmental research work were also discussed and approved by MGM-ECRHS Institutional Ethics Committee.

Bacterial isolates and susceptibility testing

The microbiological assessments were done on day 3, 7 and 14. Total n = 166 non-duplicate isolates were recovered from twenty COVID-19 patients. The bacterial identification was done using biochemical method and MALDI-TOF based identification was performed for all isolates. The antibiotic susceptibility of various antibiotics was determined against all the isolates according to CLSI guidelines. In addition, recently approved antibiotics such as ceftazidime/avibactam, imipenem/relebactam, ceftolozane/tazobactam and levonadifloxacin and antibiotic under phase III clinical trial cefepime/zidebactam, were also evaluated.

The commercial formulations were used for MIC testing. Zidebactam, tazobactam, levonadifloxacin, ceftolozane, relebactam, avibactam were provided by Wockhardt Research Center, India.

The susceptibilities of bacterial isolates were interpreted using CLSI breakpoints (for levonadifloxacin package insert based and for cefepime/zidebactam PK/PD based susceptibility criteria was used.

β-lactamase identification

β-lactamase genes of bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{NDM}, bla_{CMY}, $bla_{OXA-48-like}$ were amplified with PCR method using appropriate primers described previously.

RESULTS

Demography of patients

Twenty critically ill patients admitted in ICU were enrolled in the present study. They include 17 male and 3 female patients. Median age of participants was 59 ± 12.3 years. Comorbidities were present in 50% patients. The demographic descriptive analysis was presented in Table 1.

Bacterial isolates

Total 166 bacterial isolates were recovered from the sputum, blood and urine samples on various assessment days. The organisms included

Table 1. Descriptive analysis of demographic characteristics, comorbidities in patients with secondary respiratory tract infection among COVID-19 positive patients

Patient ID	Gender	Age (Years)	Comorbidities	Length of stay in ICU (Days)	End status
P1	M	69	Diabetes Mellitus	10	Deceased
P2	M	60	None	21	Survived
P3	M	55	None	19	Survived
P4	M	57	None	17	Deceased
P5	M	47	Diabetes Mellitus and Hypertension	7	Survived
P6	M	31	None	8	Survived
P7	M	57	Diabetes Mellitus	8	Survived
P8	M	56	Interstitial Lung disease	9	Deceased
P9	M	67	Diabetes Mellitus and Hypertension	33	Deceased
P10	M	70	None	10	Deceased
P11	M	53	Hypertension	6	Survived
P12	M	67	Coronary Artery disease (CAD)	1	Survived
P13	F	71	None	7	Survived
P14	F	65	None	17	Deceased
P15	M	74	None	11	Survived
P16	M	74	Diabetes Mellitus, Hypertension	12	Survived
P17	M	58	None	22	Deceased
P18	M	35	Diabetes Mellitus	6	Survived
P19	F	70	Hypertension	9	Deceased
P20	M	40	None	10	Survived
	Median	**59 ± 12.3**		**10 ± 7.2**	

Table 2. Bacterial isolates recovered from various samples from COVID-19 positive patients

Organism	Sputum	Blood	Urine
K. pneumoniae	21		1
E. coli	14		2
Pseudomonas sp.	11		
Acinetobacter sp.	10		
A. baumannii	7		
Burkholderia cenocepacia	2		
Chryseobacterium gleum	2		
E. cloacae	2		
Enterobacter	1	1	
Pluralibacter gergoviae	2		
Chryseobacterium indologenes	1		
Elizabethkingia anophelis	1	2	
Stenotrophomonas	1		
Citrobacter amalonaticus		1	
Enterococci	22	1	4
Staphylococcus aureus	16	5	2
S. epidermidis	1		
S. parasanguinis	10		
S. mitis/oralis	6		
S. pneumoniae	3		
Streptococci mittis	3		
Streptococci sp.	2		
Granulicatella adiacens	2		
Lysinibacillus fusiformis	2	2	
Abiotrophia deftiva	1		
Enterococci faecalis	1		
Rothia mucilaginosa	1		
Total	145	12	9

82 Gram-negative and 84 Gram-positive isolates. Among Gram-negative isolates, *K. pneumoniae* was encountered the most followed by *E. coli, P. aeruginosa* and *Acinetobacter* sp. complex. While among Gram-positive isolates, *Enterococci* sp. was encountered the most followed by *S. aureus* and *Streptococcus parasanguinis*. The complete description of bacterial isolates is provided in Table 2.

Antibacterial agents used for treatment

COVID-positive patients with suspected bacterial infections were prescribed antibacterial agents based on symptoms. Ceftriaxone was the most prescribed antibiotic followed by meropenem, piperacillin/tazobactam and colistin. Among newly approved agents ceftazidime/avibactam and levonadifloxacin were prescribed to five and one patient respectively. Detailed description of antibiotic prescription is provided in Supplementary Table S1.

Clinical outcomes

The overall mortality was 40.0% (08/20). The comorbidities among survived and deceased and the mortality was similar (50.0%) in patients with and without comorbidities. There was no associated higher mortality with diabetes, hypertension of coronary artery disease presented in Table S2.

Table 3. Antibiotic susceptibility (%) for Gram-negative bacterial isolates recovered from COVID-19 positive patients

Antibiotic	Susceptibility (%)		
	Enterobacterales (N = 33)	*Pseudomonas* (N = 9)	*Acinetobacter* sp. (N = 15)
Cefepime	27.27	88	26.67
Cefepime/zidebactam*	100	100	66.67
Ceftazidime	15.15	66.67	6.67
Ceftazidime/avibactam	48.48	88.88	NT
Colistin	NA	NA	NA
Imipenem	45.45	66.67	13.33
Meropenem	45.45	77.78	6.67
Piperacillin/tazobactam	33.33	88.88	25
Aztreonam/avibactam#	87.88	100	NT
Amikacin	45.45	100	33.33

*Cefepime/zidebactam tested in 1:1 ratio as per CLSI, PK/PD based breakpoints were used
#aztreonam/avibactam current breakpoint of aztreonam was applied
NA - breakpoint are not available, NT - not tested

Antibiotic susceptibility

Antibiotic susceptibility was determined by broth MIC method using CLSI guidelines. Among Gram-negative organisms carbapenem resistance was 54.55%, 33.33% and 93.33% in *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* respectively. Cefepime/zidebactam was the most active antibacterial agent tested as shown in Table 3.

Table 4. Antibiotic percentage susceptibility for Gram-positive *Enterococci* sp. and *Staphylococci* sp. isolates recovered from COVID-19 positive patients

Antibiotic	Susceptibility (%)	
	Staphylococci (n = 21)	*Enterococci* (n = 21)
Linezolid	95.24	100
Daptomycin	NT	100
Vancomycin	100	100
Levofloxacin	42.86	38.81
Ampicillin	NT	66.67
Tigecycline	NT	100
Synercid	NT	38.81
WCK 771 (Levonadifloxacin)	100	38.81
Teicoplanin	95.24	NT
Cefoxitin	23.81	NT
Minocycline	80.95	NT
Clindamycin	66.67	NT

Only one *S. epidermidis* was isolated and it was found to be susceptible to vancomycin, minocycline, linezolid, clindamycin, and was resistant to cefoxitin and WCK 771

In Gram-positive isolates *S. aureus* and *Enterococcus* sp. were the most encountered isolates. Against *Enterococcus* sp. linezolid, daptomycin, vancomycin and tigecycline showed 100% susceptibility. While for *S. aureus* levonadifloxacin (WCK 771) was the most active antibiotic with 100% susceptibility followed by linezolid and teicoplanin as shown in Table 4.

Against *Streptococcus* sp. clindamycin was the most active agent followed by nafithromycin, whereas solithromycin and levofloxacin showed limited activity. Among *Streptococcus* sp. Amoxicillin/clavulanic acid susceptibility was observed among 50% isolates whereas penicillin was 100% non-susceptible against *Streptococci* sp. as shown in Table 5.

β-lactamase in Gram-negative isolates

The presence of β-lactamases was confirmed by performing polymerase chain reaction for bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{NDM}, bla_{CMY}, $bla_{OXA-48-like}$. In *E. coli* NDM was the most encountered β-lactamase whereas in *K. pneumoniae* ESBL were predominant. Dual carbapenemases i.e. NDM and OXA-48 like were observed in *K. pneumoniae*. All *A.baumannii* isolates showed the presence of OXA-23 and OXA-58, including three showed the presence of NDM as well in Table 6 and Figure. Most of the *P. aeruginosa* showed presence of OXA-4 and VEB type β-lactamase presence as shown in Table 7 and Figure.

Table 5. Antibiotic percentage susceptibility for Gram-positive *Streptococci* sp. isolates recovered from COVID-19 positive patients

Antibiotic	Susceptibility (%)		
	S. pneumoniae (n = 4)	*S. mitis/oralis* (n = 7)	*S. parasanguinis* (10)
Azithromycin	25	14.28	10
Clindamycin	100	85.72	80
WCK 4873 (Nafithromycin)	100	85.72	40
Solithromycin	100	28.56	40
Levofloxacin	100	28.56	50
Amoxicillin/clavulanic acid	50	0	50
Penicillin-G	0	0	0
WCK 771	100	42.84	50

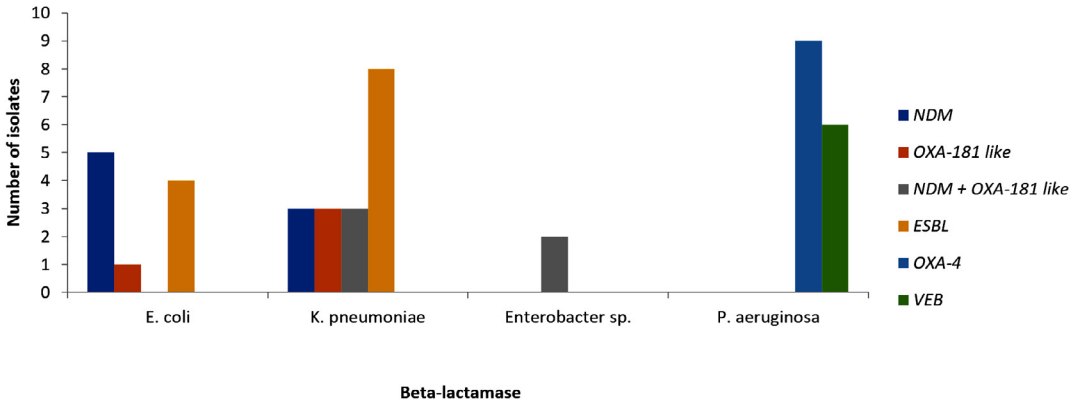

Figure. Figure depicts the presence of β-lactamse enzymes in the Gram-negative isolates obtained from patients for the determination of their antibiotic resistance

Table 6. Activity of recently approved/pipeline and older antibiotics against *Acinetobacter* spp. (n = 11) recovered from COVID-19 positive patients

Acinetobacter IDs	β-lactamases	MIC (mg/L)				
		FEP	FEP-ZID 1:1	IPM	IPM+ 4234-4	SUL
MGM-16	OXA-23, OXA-58	8	4	4	0.25	4
MGM-19	OXA-23, OXA-58	4	4	4	0.5	8
MGM-21	OXA-23, OXA-58	16	4	16	32	>128
MGM-32	OXA-58	0.06	0.06	1	0.5	128
MGM-21	OXA-23, OXA-58	128	32	128	32	64
MGM-87	OXA-23, OXA-58	>128	32	>128	128	128
MGM-115	OXA-23, OXA-58	>128	32	>128	128	128
MGM-113	NDM, OXA-23, OXA-58	>128	>128	>128	>128	128
MGM-160	NDM, OXA-23, OXA-58	>128	>128	>128	>128	128
MGM-161	OXA-23, OXA-58	128	64	>128	>128	>128
MGM-162	NDM, OXA-23, OXA-58	>128	>128	>128	>128	128

FEP : Cefepime; ZID : Zidebactam ; IPM: imipenem; WCK4234: a potent class D (OXA carbapenemase) β-lactamase inhibitor; SUL: sulbactam

DISCUSSION

A viral pneumonia with an unusual outbreak, COVID-19 was considered as major health concern and accountable for 7 million deaths worldwide. In addition to COVID-19, bacteria and fungi are reported to cause Coinfections in critically ill patients, which increase its morbidity and mortality. In an observational study of COVID-positive patients, 58.8% bacterial culture positive rates were reported by Shiralizadeh et al.[5]

In present study median stay in ICU was 10 ± 7 days; undoubtedly this duration for bacteria was an excellent opportunity to infect the patients. Furthermore, sampling was done multiple times to evaluate the bacterial coinfection in the patients, which resulted in increased culture positivity rates. With emphasis on secondary bacterial

Table 7. Activity of recently approved/pipeline and older antibiotics against *P. aeruginosa* (n = 9) recovered from COVID-19 positive patients

		MIC (mg/L)									
P. aeruginosa IDs	β-lactamases	FEP	FEP/ ZID 1:1	CAZ	CAZ/ AVI	CST	IPM	IPM/ REL	MEM	PIP/ TAZ	AMK
MGM-4	OXA-4, VEB	64	1	>128	>128	1	1	0.5	2	>128	8
MGM-15	OXA-4	64	4	>128	16	0.5	>128	>128	>128	>128	>128
MGM-27	OXA-4, VEB	8	2	8	2	1	32	2	8	8	4
MGM-95	OXA-4, VEB	2	0.25	64	64	64	8	>64	16	2	1
MGM-98	OXA4, OXA-2, VEB	2	2	2	2	1	2	1	0.5	4	4
MGM-125	OXA4, OXA-2, VEB	4	2	16	2	1	1	0.25	0.25	8	4
MGM-137	OXA-4, VEB	2	1	4	2	0.5	0.5	0.12	0.12	16	2
MGM-145	OXA-4	1	1	2	2	1	0.5	0.12	0.25	8	2
MGM-71	OXA-4, VEB	16	0.25	>64	4	0.5	16	32	1	>64	2

FEP: cefepime; ZID: zidebactam; CAZ: ceftazidime; AVI: avibactam; CST: colistin; IPM: imipenem; REL: relebactam; MEM: meropenem; PIP/TAZ: piperacillin/tazobactam; AMK: amikacin

infection of the COVID-positive patients, among Gram-negative isolates, *K. pneumoniae* (13.25%) was the most common organism followed by *A. baumannii* complex (10.24%). Similar observation was reported from Turkiye and Wuhan in two independent retrospective studies conducted during 2020 and 2022 by Stoian et al[1] and Chen et al.[2] In Gram-positive isolates *Enterococcus* (16.26%) and *S. aureus* (13.85%) were most frequently encountered.

Infection with *E. coli, S. aureus* and *A. baumannii* was observed significantly higher percentage in deceased patients. It was confirmed in a study performed by Liu et al. that the co-infection of extensively antibiotic-resistant *A. baumannii* and avian influenza A virus in the patients with invasive mechanical ventilation is a key factor for the high mortality and severity of the disease.[3] It was note withstanding that only one antibiotic was prescribed to all the discharged patients whereas more than two antibiotics were prescribed for patients who died. This clearly indicates currently available antibacterial therapy against MDR pathogens is inadequate and more specifically infection caused by β-lactamase producing pathogen the treatment decisions have become scarce and thus multiple amalgamations were used in the anticipation of cure. Nevertheless β-lactamases such as bla_{NDM}, $bla_{OXA\_48\ like}$ either alone or in combinations shows increasing trends in India among *Enterobacterales*. In case of *Acinetobacter baumannii* bla_{NDM}, bla_{OXA_23} presents the toughest treatment challenge.

CONCLUSION

The study clearly demonstrated early determination of co-infection and the need to develop targeted antibacterial therapy as the highest priority. Findings showed significance of presence of potential β-lactamases in bacterial pathogens that render the antibiotic-resistant characteristics which significantly affect the clinical outcome and recovery of COVID-19 positive patients.

Moreover, there is an urgent need to discover and develop new antibiotics as an unmet medical need to reduce the burden of infectious diseases, which can tackle the bacterial infections caused by β-lactamase producing organisms.

Clinical outcomes and significance

The overall mortality was 40.0% (08/20). The comorbidities among survived and deceased and the mortality was similar (50.0%) in patients with and without comorbidities. There was no associated higher mortality with diabetes, hypertension of coronary artery disease presented in Table S2.

SUPPLEMENTARY INFORMATION

Supplementary information accompanies this article at https://doi.org/10.22207/JPAM.19.4.29

Additional file: Additional Table S1-S2.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or in the supplementary files.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, MGM Medical College and Hospital, (Chhatrapati Sambhajinagar) Aurangabad, Maharashtra, India, vide identification number as MGM-ECRHS/2020/01.

INFORMED CONSENT

Written informed consents were obtained from participating patients before enrolling them into the study.

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