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ORIGINAL ARTICLE

Aetiology and antibiotic susceptibility pattern of bacterial pathogens in respiratory specimens of COVID-19 patients on respiratory support: A tertiary care hospital study.

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

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ABSTRACT… Objective: To describe aetiology and antibiotic susceptibility pattern of bacterial pathogens in respiratory specimens of covid-19 patients on respiratory support in tertiary care hospital of Lahore. **Study Design:** Descriptive study. **Setting:** Microbiology Department, CMH, Lahore. **Period:** May 2021 to October 2021. **Material & Methods:** A total 107 isolates from 145 patients who were COVID-19 positive and on respiratory support were included in the study. Bacterial isolates were isolated from clinical samples according to standard protocol of culturing and incubation. Antibiotic sensitivity, carbapenamase detection was done according to CLSI 2021. Molecular identification of carbapenem resistant genes bla_{IMP} and bla_{VIM} was determined by PCR. **Results:** A total of 107 bacterial isolates were isolated from clinical specimens. A. baumannii was the most common isolated organism. Colistin and aminoglycosides were found to be the most effective antibiotics. Among carbapenem resistant isolates 89.5% were MHT positive and among these 59 (86.8%) were MBL positive. Among MBL positive isolates, 5.61% and 29.91% were positive for bla_{IMP} and bla_{VIM} respectively. **Conclusion:** Patients admitted for COVID-19 treatment are at higher risk of acquisition of secondary bacterial infections and antibiotic resistance among such pathogens is at alarming.

Key words: Antibiotic Susceptibility, Clinical Laboratory Stanadard Institute (CLSI), COVID-19, Carbapenemases, Kirby Bauer Disk Diffusion Method, Metallo Beta Lactamases (MBL).

INTRODUCTION

SARS-CoV-2 is a strain taxonomically related to severe acute respiratory syndrome related corona virus (SARSr-CoV).[1] It bears genetic resemblance to bat corona virus that is considered to be zoonotic origin.[2] It is thought that human introduction happened through an intermediate host pangolin through a spill over event in Dec 2019.[3] Confirmed report of human to human transmission via coughing and sneezing within a range of 2 meters came on 20th Jan 2020.[4] Initially labelled as 'pneumonia of unknown aetiology'' but finally on 11th Feb 2020 World Health Organization (WHO) given it a name COVID-19 which is short form derived from corona virus disease 2019.[5] On 11th March 2020 COVID-19 was declared as pandemic.[6]

With a surge in corona virus cases in the world over it was found out in a research letter published in JAMA that 21% of COVID cases had co-infection of other viral pathogens.[7] According to another research letter many hospitalized patients of COVID-19 are developing potentially lethal secondary coinfections such as pneumonia and sepsis. Studies suggest that a bacterial co-infection with a pre-existing lethal viral condition like critical COVID-19 requiring respiratory support is associated with high mortality.[8]

According to rough estimate one in every seven cases of COVID-19 suffers from bacterial infection while another study predicts that 50% of deaths are because of original COVID-19 disease while rest of 50% suffer from secondary bacterial

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

Correspondence Address:
Dr. Shafqat Husnain Khan
Department of Microbiology
CMH, Lahore.
drshafqat08@gmail.com

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infections in the course of disease.[9]

Current treatment of COVID-19 is supportive while bacterial co-infection requires definitive antibiotic treatment as per susceptibility pattern and intrinsic resistance of bacterial pathogens.[8] The aim of this study is to find out frequency and aetiology of co-infecting bacterial pathogens in respiratory pathogens in respiratory samples of patients categorized as having mild, moderate, severe or critical COVID-19 requiring oxygen support or mechanical ventilation. The objective of this study was to find out the frequency, aetiology and susceptibility pattern of bacterial pathogens in respiratory pathogens in respiratory specimens, co-infecting COVID-19 patients requiring respiratory support.

MATERIAL & METHODS

This study was conducted in CMH Lahore from May 2021 to October 2021. It was a cross-sectional descriptive study which was approved from institutional research review board via letter no 307/2021. A total of 145 patients were included in the study and written consent was obtained from the attendants of the patients. All relevant data regarding age of patient, gender, ward, disease of the patient, use of any medical devices and antibiotic consumption was collected. Any history of associated disease (diabetes mellitus, liver cirrhosis, chronic kidney disease, malignancy) was also collected. If during hospitalization two or more than two isolates were identified from the same patient, only the first isolate was included for analysis. In case of two isolates from same patient only one with higher resistance was chosen.

Cultures were collected only if ordered by clinician in case of suspicion of bacterial infection. Samples received were nasopharyngeal swabs, broncho alveolar lavages (BAL), tracheal aspirates (TA), and sputum and nasobronchial lavages (NBL). In case of BAL sampling was performed from endotracheal tube with prior instillation of normal saline.[10] All respiratory specimens were processed according to standard protocol. These samples were incoculated on blood, chocolate and Mac Conkey's agar. After overnight incubation at 37^0C, plates were observed for bacterial growth. Isolated bacterial growth was further identified by using conventional methods (Gram stain, motility, catalase, oxidase) and standard biochemical tests and further confirmed by API 20E and API 20NE (BioMeurieux, France).[11] Antibiotic susceptibility was performed by Kirby Bauer's disc diffusion method and E-test according to CLSI 2021.

Bacterial isolates which were found resistant against carbapenems were further processed and two tests were applied additionally to find out carbapenemases enzyme and metallo beta lactamse enzyme. Carbapenemase enzyme was detected by modified Hodge test (MHT) method according to according to standard protocol of CLSI 2021 while metallo beta lactamse enzyme was detected by double disc diffusion test described previously.[12] The genomic DNAs were extracted from all phenotypically MHT and MBL positive bacterial isolates by using Thermo Genomic DNA extraction (Thermo Fisher Scientific, USA). The primer sequence for the amplification and molecular detection of IMP and VIM genes was obtained by previous studies.[13] These primer sequences were provided to local vendor and primers were prepared according to sequence. The conditions which were used for amplification of genes were; initial denaturation, denaturation, annealing, amplification and final amplification. Initial denaturation was done at 95^0C for ten minutes, one minute denaturation at 94^0C, thirty seconds annealing at 55^0C, one minute amplification at 72^0C and final amplification for ten minutes at 72^0C. A total thirty cycles were set at thermal cycler for three steps (denaturation, annealing & amplification). After the completion of PCR cycles, final products were further processed to run on 1.5% agarose gel in electrophoresis along with DNA ladder 100 bps, positive & negative controls. The gel run time was set for 30 minutes at 120 volts. After completion, gel was visualized by VU transilluminator and images were captured.

Nasopharyngeal or oropharyngeal swabs were taken from the study participants for confirmation of COVID-19. These samples were processed according to protocol. RNA was extracted from

the sample using standard extraction protocol. Detection COVID-19 virus was based on amplification of extracted COVID-19 RNA by real time PCR (RT-PCR). With every extraction and amplification of PCR internal controls both positive and negative were run. E gene and RdRP gene were selected targets as E gene demonstrates higher sensitivity so our lab has prioritize E gene as the selected target.[14]

Results were analyzed with the SPSS (version 23.0, SPSS Inc). The frequency of bacterial isolates, resistance pattern, detection of bacterial enzymes and identification of genes were presented as percentages in the figures, graphs and tables.

RESULTS

In the present study, among total 107 isolates. A. baumannii (n=27), P aeruginosae (n=16), K. Pneumoniae (n=25), E. coli (n=09), Enterobacter spp (n=12), C. ferundi (n=05) and S. marcesence (n=03) were isolated from clinical samples. It is worth mentioning here that majority of the clinical isolates (91.6%) were recovered from NBL followed by 6.5% from broncho-alveolar lavage (BAL) and 1.9% from sputum and ETT. The distribution of bacterial isolates in clinical specimens is enlisted in (Table-I).

Colistin and aminoglycosides were the most sensitive drugs against gram negative bacteria while vancomycin, linezolid and fusidic acid were the most sensitive drugs against gram positive bacteria. The antibiotic resistance pattern was given for gram negative and gram positive bacteria in separate tables, Table-II and III.

Organism	NBL	BAL	Sputum	ETT
A. baumannii (n=27)	27	-	-	-
P. aeruginosa (n=16)	15	01	-	-
K. pneumoniae (n=25)	21	02	01	01
E. coli (n=09)	09	-	-	-
C. freundii (n=05)	04	01	-	-
S. marcesence (n=03)	03	-	-	-
MRSA (n=04)	02	01	-	01
Enterobacter spp (n=12)	10	02	-	-
Bulkhulderia cepacia (n=03)	3	-	-	-
Stenotrophomonas maltophilia (n=02)	2	-	-	-
S. aureus (MSSA) (n=01)	1	-	-	-
Total (n=107)	98	7	1	1

Table-I. Distribution of bacterial isolates in clinical specimens.

Bacterial Isolate	AMP	COT	TET	TGC	GEN	AMI	CIP	AMC	CRO	IMP	CT	TZP	SCF
A. baumannii (n=27)	100	92.6	74.1	74.1	70.4	88.9	100	100	96.3	85.2	0	88.9	85.2
P. aeruginosa (n=16)	NT	NT	NT	75	75	75	NT	NT	NT	62.5	0	50	37.5
K. pneumoniae (n=25)	100	96	80	76	84	84	88	96	100	80	0	84	84
Enterobacter spp (n=12)	100	58.3	NT	NT	83.3	83.3	100	100	100	75	0	75	75
E. coli (n=09)	100	100	100	100	77.8	100	100	100	100	88.9	0	100	100
C. freundii (n=05)	100	80	80	60	80	80	80	100	80	40	0	60	60
S. marcesence (n=03)	100	66.7	66.7	66.7	66.7	66.7	100	100	100	33.3	0	66.7	66.7
Bulkhulderia cepacia (n=03)	NT	66.7	66.7	100	66.7	66.7	100	100	100	100	0	100	100
Stenotrophomonas maltophilia (n=02)	NT	NT	NT	100	NT	NT	100	NT	NT	NT	0	NT	NT

Table-II. Antibiotic resistance pattern of gram negative bacteria (%)

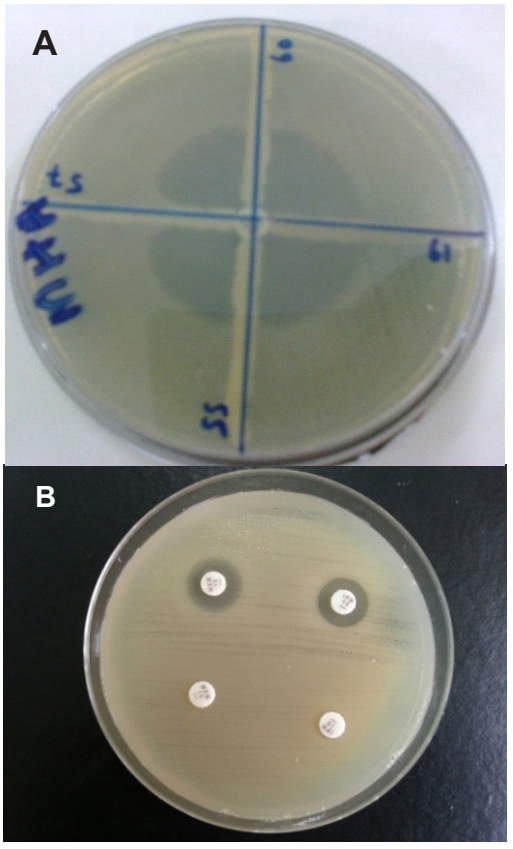
Bacterial Isolate	AMP	ERY	TOB	TET	GEN	SXT	VAN	CIP	AMC	LZD	FUS
MRSA (n=04)	100	50	50	50	50	50	0	50	50	0	0
MSSA (n=01)	100	0	0	0	0	100	0	100	0	0	0

Table-III. Antibiotic sensitivity pattern of gram positive bacteria

The Modified Hodge Test for the phenotypic detection of carbapenemase production revealed that out of 76 carbapenem resistant isolates, 68 (89.5%) isolates were positive for carbapenemase production (Figure-1A).

On the other hand, 59 (86.8%) carbapenem resistant isolates were found phenotypically positive for MBL production since they manifested increased zone of inhibition in the presence chelating agent (EDTA), indouble disc diffusion method (Figure-1B).

Figure-1: Phenotypic detection of carbapenemase and metallo-β-lactamase production by Modified Hodge test (MHT) and Combined disks diffusion (CDD) method respectively. A) The presence of clover leaf-type indentation at the intersection indicate the positive test organism while carbapenemase negative organism lack of the clover leaf-type indentation at the intersection. B) Positive results are shown by enlarged zone of inhibition in the presence of EDTA. IMI, Imipenem disc; MEM, meropenem disc; EDTA, ethylene-diamine tetra acetic acid.



producing isolates were further subjected to PCR for the detection of bla_{IMP} and bla_{VIM} genes the presence of which is considered as foremost mechanism of carbapenem resistance among gram-negative rods. A representative gel showing the PCR product of bla_{VIM} & bla_{VIM} gene is presented in Figure 2.

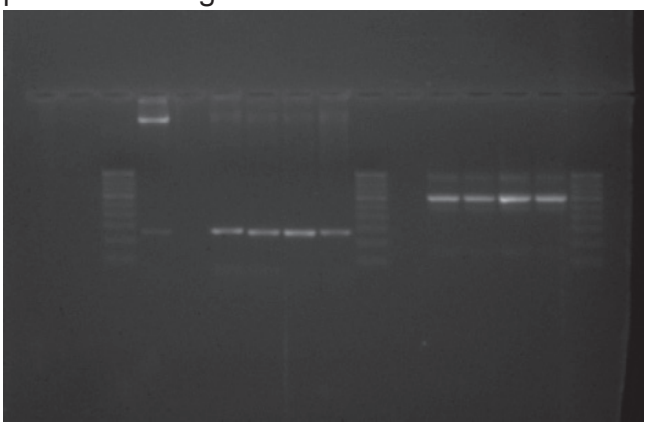


Figure-2 A representative gel displaying 261 bp and 587 bp PCR product of bla_{VIM} & bla_{IMP} gene in representative clinical isolates. M, 100 bp DNA ladder; Lane 1 (L1), positive control of bla_{VIM}; L2, negative control of bla_{VIM}; L3-6, positive test samples of bla_{VIM}. M, 100 bp DNA ladder; L7, negative control of bla_{IMP}; L8, positive control of bla_{IMP}; L9-11, positive test samples of bla_{IMP}. In the nutshell, PCR data showed that only 6 (5.61%) bacterial isolates harbor bla_{IMP} gene while 32 (29.91%) were positive for bla_{VIM} gene.

Organism	bla_{VIM} (n=)	bla_{IMP} (n=)
A. baumannii (n=27)	9 (33.33%)	2 (7.41%)
P. aeruginosa (n=16)	6 (37.50%)	1 (6.25%)
K. pneumoniae (n=25)	10 (33%)	3 (12%)
E. coli (n=09)	3 (33.33%)	-
C. freundii (n=05)	1 (20%)	-
Enterobacter spp (n=12)	3 (25%)	-
Total (n=107)	32 (29.91%)	6 (5.61%)

Table-IV. Distribution of bla_{VIM} and bla_{IMP} positive gram-negative rods.

The Table-IV briefly describes the distribution of bla_{IMP} and bla_{VIM} genes in gram negative rods under this study.

DISCUSSION

The secondary infections in patients infected with COVID-19 are very common and become

more lethal when COVID-19 patients seek ventilator support. But still, the role of antibiotics and its resistance is yet to be elucidated in such patients for progression of disease.[15] Therefore, the present study was aimed to find out etiology and antibiotic susceptibility pattern of bacterial pathogens in respiratory specimens of covid-19 patients on respiratory support in tertiary care hospital of Lahore during January-December 2021. We also checked the burden of bla_{IMP} and bla_{VIM} genes as these genes are responsible for carbapenem resistant in bacterial isolates.

Acinetobacter baumannii was isolated the most common (20.1%), (30.4%) and (90%) isolated organism among all the clinical isolates according to the study conducted in Cape Town, South Africa[16], Italy[17] and Iran[18] respectively, which is according to our study. Unlikely, a study conducted in Switzerland[15] isolated Pseudomonas aeruginosa (46%) as most common isolate.[15] Similarly, a study from Germany and Iran revealed the Klebsiella spp (47.82% & 25.59%) were the most common isolated gram negative bacteria from the samples of COVID-19 patient which is also in accordance to our study.[19,20] This discrepancy may be due to the fact that difference in geographical location might be a factor of different bacterial pathogen isolation.[21]

Majority of the gram negative isolates were resistant to ampicillin, amoxicillin + clavulanic acid, ceftriaxone, piperacillin + tazobactam and the same has been documented by the studies done Gysin et al.,.[15] They reported that piperacillin/tazobactam (65.6%), cefepime (56.3%), ceftazidime (46.9%) and meropenem (50.0%) were resistant drugs against gram negative isolated from COVID-19 patients. In our study, aminoglycoside and colistin was the most sensitive drugs against the isolated gram negative isolates which is in accordance with the study conducted by Gysin et al.[15], Sharifipour et al.,[18] and Mahmoudi et al.,[20] Moreover, studies conducted by Mahmoudi et al.,[20] and Sharifipour et al.,[18] reported that vancomycin is most effective antibiotic against MSSA and MRSA which is accordance to our study.

We reported in our study that 89.5% carbapenem isolates were positive for MHT test and this is according to the researches published in Pakistan[13], Nepal[22] and Sudan[23] where 93%, 69.1% and 72% isolates were showed positivity for MHT test respectively. Likewise, 86.6% MHT positive isolates were found to be positive for MBL detection and this is according to the study conducted by Akhtar et al.,[13] (89%) in Pakistan recently.

According to current study, only 5.6% MBL positive isolates were shown to harbor bla_{IMP} gene which is according to different researches conducted in Pakistan (3.3%)[13], Iran (3.48%)[24] and India (2.08%).[10] It is worth mentioning that 29.91% MBL positive isolates harbored bla_{VIM}gene and results of the current study is according to the researches conducted in Pakistan (32.5%)[13], Iran (33%)[24] and Greece (37.6%).[25]

CONCLUSION

It is concluded that Patients admitted for COVID-19 treatment are at higher risk of acquisition of secondary bacterial infections and antibiotic resistance among such pathogens is at alarming. It is also worth mentioning that MBL-positive gram-negative rods harbor bla_{IMP} and bla_{VIM} genes are distributed among such clinical settings of hospitals of Lahore region, Pakistan.

Conflict of Interest

None declared.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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