Short communication

Diagnosis of bacterial blood stream infections in coronavirus disease 2019 (COVID-19) using 16S rRNA sequencing methodJabbar S. Hassan¹, Fuad Ghazi Hassan², Ali Zaidan³¹Department of Microbiology, College of Medicine, Al-Nahrain University, Iraq² Department of Medical Microbiology/Immunity/ Al-Mustaqbal University College, Iraq³ Al-Yarmouk Teaching Hospital, Ministry of Health, Iraq

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Corresponding author: **Jabbar S. Hassan**. Email: jabbarsalman300@gmail.com**ABSTRACT**

Introduction and Aim: Patients, who are positive for Coronavirus disease 2019 in association with bacterial co-infection may be more severe, the treatment is more problematic, and the treatment cycle is prolonged overall. This study aims to investigate bacterial blood stream infection in Coronavirus disease 2019 using 16S rRNA sequencing methods.

Materials and Methods: Two ml leftover blood samples from 50 patients with laboratory confirmed RT-PCR positive for Coronavirus disease 2019, who got admitted to the intensive care unit (ICU) in Quarantine Center in Baghdad, Iraqi were enrolled in this clinical case series study.

Results: The total of 13 positive sample PCR products were sequenced. The sequencing-based identification of these isolates included: *Serratia marcescens*, *Achromobacter xylosoxidans*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Staphylococcus aureus*.

Conclusion: The significance of considering bacterial blood infection in Coronavirus disease 2019 patients even in the setting of a blood cultures are negative and clinicians ought to know about *Serratia marcescens* and *A. xylosoxidans* while treating Coronavirus disease 2019 patients due to resistant pattern to many antimicrobial agents.

Keywords: COVID-19; bacterial blood stream infection; 16S rRNA sequencing, *Serratia marcescens*, *Achromobacter xylosoxidans*.

INTRODUCTION

With COVID-19 outbreak, the escalation in patients requiring ICU admission has been vast and endangered in most critical care capacity in several hospitals worldwide (1). Clinically, most patients infected with severe acute respiratory syndrome (SARS-CoV-2) presented no severe clinical picture, but almost 5% of patients show severe (2). As a result of the virus infection and to Multiple Organ Dysfunction Syndrome (MODS) in some cases, widely patients with severe COVID-19 fulfil the Third International Consensus Definitions for Sepsis (Sepsis-3), which defines sepsis as “a life-threatening When the body's response to infection causes the host's own tissues to be damaged” (3).

Many data mentioned that the received treatment with invasive therapy is based on mechanical ventilation resulting in enhancing acquisition to secondary multidrug resistance bacterial infection (4). Patients, who are positive for COVID-19 in associated with bacterial co-infection may be more severe, the treatment is more problematic, and the treatment cycle is prolonged in overall (5).

The incidence, prevalence and kinds of bacterial infection in COVID-19-infected patients are little understood, and this has been emphasized as a major knowledge disparity throughout the outbreak (6).

Blood cultivation are yet the gold standard in the discovery of blood stream bacterial infection. However, obtaining positive cultures from COVID-19 can be difficult as these patients are receiving empirical antibiotic with the first symptoms of the disease and with the use of antibiotic treatment basic principle the fundamental numbers of cultures turn out to be negative (7, 8).

The goal of this investigation is detection of bacterial sepsis in (COVID-19) using 16S rRNA sequencing methods.

MATERIALS AND METHODS

Five months later, particularly in September 2020, two ml. leftover blood samples were taken from 50 patients who were confirmed positive with COVID-19 in the laboratory admitted to the intensive care unit (ICU) in quarantine centre in Baghdad, Iraq.

Demographics, clinical and laboratory data were accumulated from a medical record for all participants. There were 30 male patients (60%) and 20 female patients (40 %), the mean age was 47.18 +/- 17years all patients enrolled in this study were confirmed to be bacterial blood culture negative after 4-5 days incubation periods.

All samples used in the study were dealt with according to the standards set by the WHO 2020 (1).

Inclusion criteria

1. All participant tested positive for COVID-19 by RT-PCR.
2. Patients with a SpO₂ of less than 94 % on room air at sea level, a respiratory rate of more than 30 breaths per minute, a PaO₂/FiO₂ of less than 300 mmHg, or lung infiltrates greater than 50%
3. Patients whose blood cultures are negative

Exclusion criteria

Patients whose blood cultures are positive

Molecular detection and Sanger sequencing

Quick Protocol SYNTM DNA extraction Kit (Geneaid) has been used to extract high purity genomic DNA from blood samples; Approximately 1µl from both forward and reverse primers sequence to 16S rRNA (9).(5' -3') 27F-AGAGTTTGATCCTGGCTCAG and 1492R GGGTTACCTTGTTACGACTT (Bio Corp Canada) were apply to yield a DNA fragment of (1500) base pair using conventional PCR in a in total volume 20

µl of reaction mixture thus : initial denaturation at 94°C for 5min, denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, elongation at 72°C for 30 seconds, and final extension at 72°C for 7 minutes was turn 30 cycles, redundant reaction were undertaken for each specimen.

The amplification products of 13 positive samples were purified and sequenced for bacterial identification using Sanger sequencing method for 16Sr RNA gene, (PCR/Sanger-Seq) which was performed by using BigDye™ in the MacroGen; Inc. is a South Korea public biotechnology company and the data obtained were matched with the online database using BLAST.

RESULTS

Conventional PCR assay based on 16Sr RNA gene was used for the detections of bacterial sepsis with amplicon produce 1500 bp, results proved that 13(26%) out of 50 samples were PCR positive (Fig. 1).

Fig.1: Agarose electrophoresis of PCR amplification for 16S RNA gene; lanes 1-5,6,7,10-18 positive samples(1500bp); lanes (2,3,4,8,9) negative samples

The total of 13 positive samples PCR products were sequenced; The sequencing-based identification of these isolates included: *Serratia marcescens*, *Achromobacter xylosoxidans*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Staphylococcus aureus*.

It was found that 5(38.4%) of sequencing-based identification identify as *Serratia marcescens*,3 (23%) as *Achromobacter xylosoxidans*,2 (15.3%) belongs to *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively, while only 1(7.6%) belong to *Acinetobacter baumannii*. (Fig. 2).

Fig. 2: Sequencing-based identification of bacterial isolates using Sanger sequencing

DISCUSSION

The rapid spread of severe acute respiratory syndrome, (SARS-CoV-2), through the world leads to an unusual medical situation and all medical personnel are faced with a public health concern (2) SARS-CoV-2 coinfection with other microbes, such as viruses, bacteria, and fungi, is a major factor in COVID-19, and it can make diagnosis, treatment, and prognosis more challenging, and even increase the disease symptom and mortality (10).

Blood cultures are important method for the identification and management of bloodstream infections among patients with suspected bacteremia, reports are missing on their usefulness for patients with suspected or confirmed COVID- 19, because many patients with severe COVID-19 are given empiric antibiotics to treat potential bacterial coinfections, the rate of bacterial septicemia in these patients remains unknown (11).

In the present investigation, 13 (26%) out of 50 samples were positive by PCR based 16S rRNA gene, which improved that 16S rRNA PCR assays can possibly make an essential dedication to understanding patient management by detecting the presence of bacterial pathogen in medical specimens, this method is reasonably simple and reliable in that it produces accurate results that can be applied to samples obtained during antimicrobial treatment.

In the present study, 16S rRNA sequencing result may give a clear picture about the bacterial blood infection in COVID-19 patients; in which the most widely recognized causes of bacterial sepsis in our study were Gram negative bacteria (11/13) (88.6%) then Gram positive (2/13) (15.3%), such result was similar to other investigation during the COVID-19 pandemic as study by Sepulveda J et al who reported that in 28,011

COVID-19 patients, Gram negative bacteria is the common causes of bacteremia (12).

This investigation shows that the most widely recognized bacteria in COVID-19 sepsis based on 16SrRNA sequencing methods were *Serratia marcescens* (38.4%), *Achromobacter xylosoxidans* (23%), *Pseudomonas aeruginosa* and *Staphylococcus aureus* (15.3%) *Acinetobacter baumannii* (7.6%).

This contradicts Sepulveda et al., (12), who found that *Escherichia coli* (16.7 %), *Staphylococcus aureus* (13.3 %), *Klebsiella pneumoniae* (10.0 %), and *Enterobacter cloacae* complex (8.3%) were the most common causes of bacteremia in COVID-19 patients.

It is remarkable in this study that *Serratia marcescens* more frequent as causative agents in COVID-19 sepsis, *S. marcescens* is one of the most opportunistic pathogens that emerged as nosocomial infection in ICU and non-intensive care unit patients (13). The ability of *S. marcescens* to production a beta-lactamase, which imparts resistance to broad-spectrum beta-lactam antibiotics, is a key characteristic. *S. marcescens* lead to bacteremia in 2.0 % of patients admitted in hospitals (14).

The three bacterial strains belong to *Achromobacter xylosoxidans*, an opportunistic pathogen that can cause serious infections, especially in immunocompromised people, according to the current study which used a sequencing method. It's been discovered in new-borns.

and in patients with cancer, neutropenia, these bacteria can be inaccurate in diagnosis with other non-lactose fermenting Gram negative bacteria mainly *pseudomonas spp.* Previously, antibiotics such as first- and second-generation cephalosporins, ampicillin, Aztreonam, aminoglycosides, tetracyclines, and Rifampin had little effect on *Achromobacter xylosoxidans* (15, 16). Many studies have found an increased prevalence of septicemia caused by *A.*

xylosoxidans, and these findings are consistent with that (17,18).

CONCLUSION

In conclusion, the importance of considering bacterial blood infection in COVID-19 patients even in the setting of a blood cultures is negative and clinicians ought to know about *Serratia marcescens* an *A. xylosoxidans* while treating COVID-19 patients due to resistant pattern to many antimicrobial agents.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

1. World Health Organization. Laboratory biosafety guidance related to coronavirus disease (COVID-19): interim guidance, 13 May 2020. World Health Organization; 2020.
2. Mullis, K., Erlich, H. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*. 1988; 239(4839): 487-491.
3. Quah, P., Li, A., Phua, J. Mortality rates of patients with COVID-19 in the intensive care unit: A systematic review of the emerging literature. *Crit Care* 2020; 24: 285.
4. Singer, M., Deutschman, C., Seymour, C., Shankar, M., Annane, D., Bauer, M. *et al.*, The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA* 2016; 315: 801-810.
5. Shah, N., Greenberg, J., McNulty, M., Gregg, K., Riddell, J., Mangino, J., Bacterial and viral co-infections complicating severe influenza: incidence and impact among 507 US patients, 2013e14. *J Clin Virol* 2016; 80:12e9
6. Martín, I., Sanchez, A., Diaz, E., Granada, R., Zaragoza, R., Villavicencio, C. *et al.*, Community-acquired respiratory coinfection in critically ill patients with pandemic 2009 influenza A(H1N1) virus. *Chest* 2011; 139: 555e62
7. Yang, X., Yu, Y., Xu, J., Shu, H., Liu, H., Wu, Y., *et al.*, Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centred, retrospective, observational study. *The Lancet Respiratory Medicine*. 2020 May 1; 8(5): 475-481.
8. Huttner, B., Catho, G., Pano-Pardo, J.R. COVID-19: don't neglect antimicrobial stewardship principles! *Clin Microbiol Infect* 2020; 26: 808e10.
9. Cates, J., Lucero, C., Dahl, R., Schirmer, P., Garg, S., Oda, G., *et al.*, Risk for In-Hospital Complications Associated with COVID-19 and Influenza—Veterans Health Administration, United States, October 1, 2018–May 31, 2020. *Morbidity and Mortality Weekly Report*. 2020 Oct 23; 69(42): 1528.
10. Alhazzani, W., Möller, M., Arabi, Y., Loeb, M., Gong, M., Fan, E., *et al.*, Surviving Sepsis Campaign: guidelines on the management of critically ill adults with Coronavirus Disease 2019 (COVID-19). *Intensive care medicine*. 2020 May; 46(5): 854-887.
11. Ruuskanen, O., Lahti, E., Jennings, L.C. Viral pneumonia. *The Lancet*. 2011 Apr 9; 377(9773): 1264-1275.
12. Sepulveda, J., Westblade, F., Whittier, S., Satlin, J., Greendyke, W., Aaron, J., *et al.*, Bacteremia and blood culture utilization during COVID-19 surge in New York City. *Journal of clinical microbiology*. 2020 Jul 23; 58(8).
13. Bizzarro, M. J., Dembry, L. M., Baltimore, R. S. Case-control analysis of endemic *Serratia marcescens* bacteremia in a

- neonatal intensive care unit *Arch Dis Child Fetal Neonatal Ed*. 2007; 92: F120–F126. doi: 10.1136/adc.2006.102855
14. Lefort, A., Righi, S., Jauréguy, F., Bégué, T., Robineau, M., Bouchaud, O., *Serratia marcescens* prosthesis infection successfully treated with meropenem after imipenem failure. *Journal of Infection*. 2005 Aug 1;51(2): E45-E47.
15. Turgutalp, K., Kiykim, A., Ersoz, G. Fatal catheter-related bacteremia due to *Alcaligenes* (*Achromobacter*) *xylosoxidans* in a hemodialysis patient. *International urology and nephrology*. 2012 Aug 1; 44(4): 1281-1283.
16. Turel, O., Kavuncuoglu, S., Hosaf, E., Ozbek, S., Aldemir, E., Uygur, T. Bacteremia due to *Achromobacter xylosoxidans* in neonates: clinical features and outcome. *Brazilian Journal of Infectious Diseases*. 2013 Aug; 17(4): 450-454.
17. Aisenberg, G., Rolston, K.V., Safdar, A. Bacteremia caused by *Achromobacter* and *Alcaligenes* species in 46 patients with cancer (1989-2003). *Cancer*. 2004; 101: 2134-2140.
18. Gomez, J., Suárez, I., Ríos, J., Peña, P., Miguel, G., Jose, M., *et al.*, *Achromobacter xylosoxidans* bacteremia: a 10-year analysis of 54 cases. *European Journal of Clinical Microbiology and Infectious Diseases*. 2003 Jun; 22(6): 360-363.