



Spectrum of bacteria isolated from blood of septicaemic covid19 patient as co-infection and their antibiotic resistance pattern

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Abstract

Background: In the recent coronavirus pandemic it is anticipated that most fatalities are due to co-infection or superinfection followed by sepsis specially in ICU. Moreover irrational use of broad spectrum antibiotics skyrocketing the development of multidrug resistant organisms and further deteriorating the situation.

Objectives: The objective of this study was to identify bloodstream pathogens and their antibiotic resistance pattern as it is crucial to implement antibiotic stewardship for better clinical outcome.

Materials and methods: All blood cultures were collected from suspected septicaemic patients of COVID -19 admitted in ICU. A predesigned questionnaire was used to collect relevant data. Blood cultures were done using BD BACTEC FX (USA origin). The positive blood culture were inoculated on different media as standard protocol. Identification and antimicrobial susceptibility testing of isolates were done by Kirby-Bauer disc diffusion method and BD Phoenix M50 automated system.

Results: Out of 1520 patients admitted in COVID ICU, 716 patients developed sepsis like syndrome and were subjected to blood culture. The blood culture positivity was 82/716(11.45%). All the five bacteria isolated from culture were gram negative. Burkholderia cepacia was the predominant one (45.12%). All the isolates were multidrug resistant (MDRO) and observed in 85% of cases. The rate of carbapenem resistance was seen in K.pneumoniae, E.coli, A.baumannii and Pseudomonas spp (70%,75%,100%,44.4% respectively) Burkholderia, Pseudomonas, Acinetobacter, klebsiella and E coli were found moderate to high level resistant to second, third and fourth generation cephalosporins. Overall colistin and tigecyclin were the two antibiotics showed good sensitivity against all the bacteria isolated in this study except Burkholderia which was highly sensitive to piperacillin+ tazobactam and meropenam.

Conclusion: Gram-negative bacteria were identified in COVID-19 associated sepsis and Burkholderia cepacia was cardinal among them. The presence of comorbidities, mechanical ventilation, contaminated disinfectants or nebulizer solutions, indwelling devices possibly contribute to BSIs. The prevalence of MDRO as causative agent of co-infection or superinfection is alarming and warrants the pattern of their resistance for initiating empiric antimicrobial therapy in septicaemic patients to decrease morbidity and mortality.

Keywords: COVID 19, BSIs, antimicrobial resistance, sepsis, MDRO

Introduction

It has been considered for long that viral respiratory infections predispose patients to bacterial infections which have a worse outcome than that of viral infection itself^[1]. Release of specific cytokines like IL10,IL-6,IL-17 and IL-23, reduced dendritic cells function, macrophages, natural killer cells,CD4+ and CD8+ T cells might play pivotal role in secondary bacterial infection^[2]. Several epidemiological and microbiological studies showed that most of the deaths occurring during the 1918 influenza pandemic were due to secondary bacterial infections, rather than virus itself. Similar observations were made during the later three 20th century influenza pandemics: the 1957 H2N2, the 1968–1969 H3N2 and the 2009– 2010 H1N1 pandemics^[3].

In recent COVID-19 pandemic the definite incidence of bacterial superinfections is not known so far. Limited arsenal of data revealed that 50% of the patients who have died from COVID-19 disease had sepsis due to secondary bacterial infection^[4]. Aetiology of the sepsis sometimes could be a secondary blood stream infection especially in the severely ill and patients on mechanical ventilator.⁵ Furthermore inappropriate use of empirical antibiotics to manage these infections based on earlier experiences leading to emergence of multi-drug resistant organisms and worsening the situation. Therefore the present study was undertaken to analyze the various organisms causing septicaemia in COVID-19 patients and their antibiotic resistance patterns as it would be a useful guide for clinicians initiating empiric antibiotic therapy which ultimately reduce morbidity and mortality as a whole.

Material and Methods

This retrospective observational study was carried out at the Department of Microbiology of Armed Forces Institute of Pathology (AFIP) from January 2021 to October 2021. All patients with confirmed COVID-19 (RT-PCR positive on nasopharyngeal or oropharyngeal swab) who were admitted to COVID-19 ICU of CMH, Dhaka and developed sepsis like syndrome were included. Blood samples were collected aseptically from these patients into specific blood culture bottles (BD BACTEC Culture Vials). These culture bottles were incubated at 37°C aerobically in automated blood culture machine BD BACTEC FX (USA Origin). Upon flagging positive, the bottles were taken out of the instrument, and the time to detection (TTD) was noted. All those bottles which did not flag positive within five days were considered sterile. Positively flagged bottles were subjected to Gram's stain and culture into blood agar and MacConkey agar media, followed by incubation at 37°C aerobically for 24 hours. The growth obtained were identified by colony morphology, Gram stain of the isolated colonies and conventional biochemical identification tests as per the standard protocol followed in our laboratory. Isolated and pure colonies of all bacterial isolates were again tested in BD Phoenix M50 automated identification and susceptibility testing for identification and confirmation of bacterial species. Antibiotic susceptibility testing was performed by Kirby Bauer's disk diffusion method. The zone of inhibition for different antibiotics was compared to the criteria set by the Clinical and Laboratory Standards Institute (CLSI).

Statistical analysis

Data analysis was performed using SPSS software, version 26.0. Descriptive data were summarized and tabulated with continuous variables in the form of mean \pm standard deviation, Median (inter quartile range) and categorical data in the form of percentages or frequencies.

Results

Out of 1520 patients admitted in COVID ICU, CMH, Dhaka during the 12 months (Jan 2021- Dec 2021) study period. A total of 716 patients developed sepsis like syndrome and were subjected to blood culture in the department of microbiology, AFIP. The blood culture positivity rate was 82/716 (11.45%). The median age of COVID-19 patients showing a positive blood culture was 65 years (interquartile range 55-72 years) and this included 53 males and 29 females.

Table 1: Showing the culture positivity (No. of cases 716)

Total number of Sample	Growth	No Growth
716(100%)	82 (11.45%)	634(88.54%)

Table 2: Showing study of gender distribution (n=716)

Sex	No of COVID patients with Suspected septicaemia	No of COVID patients with positive bacterial culture
Male	496 (69%)	53 (65%)
Female	220 (31%)	29 (35%)
Total	716	82

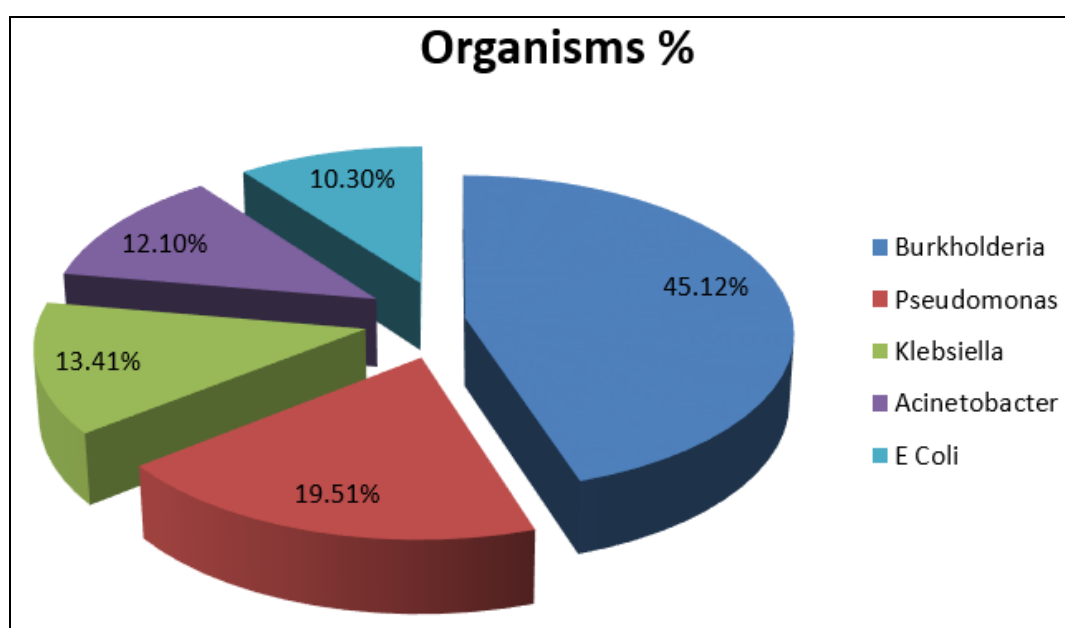


Fig 1: Frequency of bacteria isolated from culture (n=5) Antimicrobial sensitivity pattern of Burkholderia and Pseudomonas (% sensitivity).

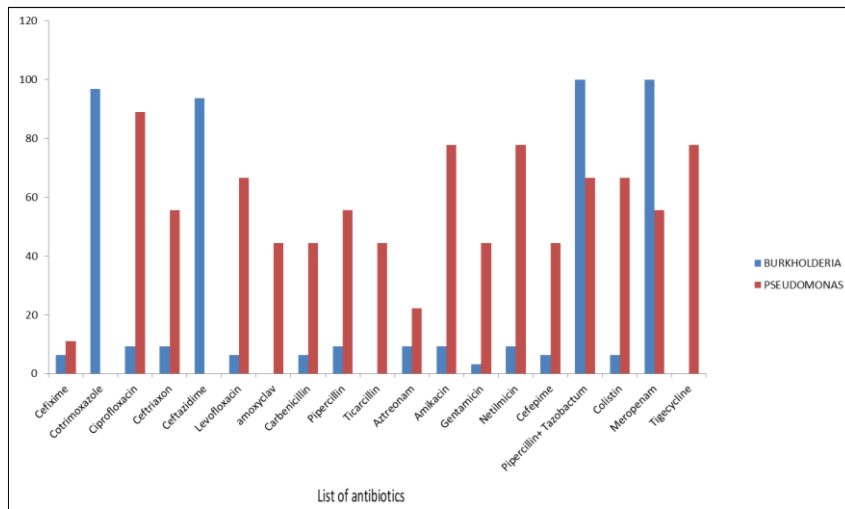


Fig 2: Antimicrobial sensitivity pattern of Klebsiella, Acinetobacter and E.Coli (% sensitivity).

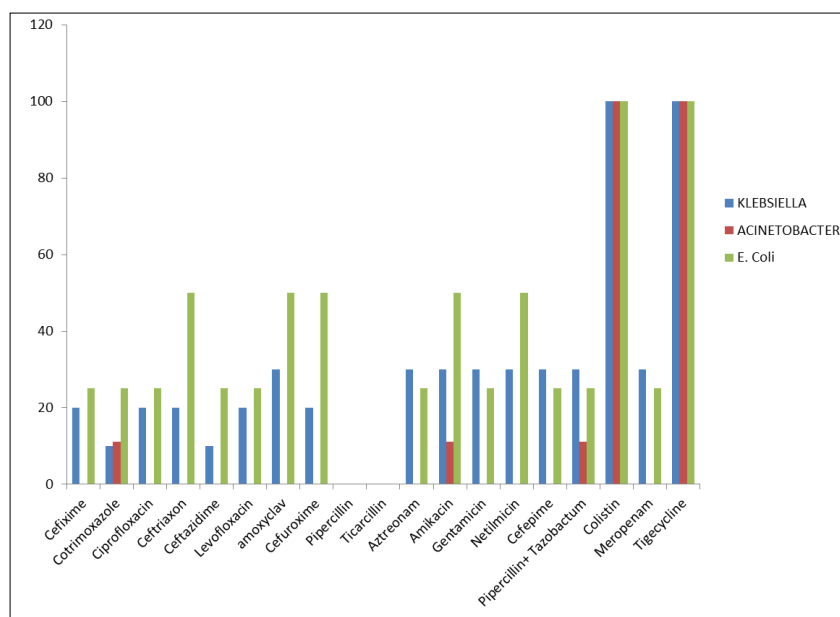


Fig 3

All the five bacteria isolated from culture were gram negative. Burkholderia cepacia was the predominant one (45.12%). All the isolates were multidrug resistant (MDRO) and observed in 85% of cases. The rate of carbapenem resistance was seen in K.pneumoniae, E.coli, A.baumannii and Pseudomonas spp (70%, 75%, 100%, 44. 4% respectively). Burkholderia, Pseudomonas, Acinetobacter, klebsiella and E coli were found moderate to high level resistant to second, third and fourth generation cephalosporins. Overall colistin and tigecyclin were the two antibiotics showed good sensitivity against all the bacteria isolated in this study except Burkholderia which was highly sensitive to piperacillin+ tazobactam and meropenam.

Discussion

Patients with severe covid-19 are prone to develop sepsis like syndrome owing to cytokine storm. The risk factors for secondary blood stream infection in a patient might depend either on comorbid conditions or ongoing drug treatment [6]. Additionally, several invasive procedures making a longer hospital stay rendering them vulnerable to HAIs [7]. Both these conditions have a number of overlapping clinical and biochemical features. Blood culture thus constitutes an important investigation for bringing more clarity into clinical judgment. While most patients with COVID-19 are treated with empirical antibiotics for potential bacterial co-infections, very little data exist regarding rate of bacteremia among this patients. In this study, we found the overall prevalence of BSIs in ICU patients to be 11.45%. A recent study from India found the prevalence of BSIs in ICU patients to be 8.5% [5]. In another study by Daniel Roberti *et al.* also found the incidence of secondary BSIs was 0.9% to 7.7%.⁶ both the studies showed similar result as ours. However, a study from gujrat, India was found higher incidence of BSIs compared to ours and it was 22.28%.⁶ we found a 73% mortality rate in severe COVID-19 pneumonia patients with BSIs. Previous studies have reported 21–68% mortality rate in this group of patients [8-11] which is almost same as our study. Comorbidity also played an important role in mortality, with nearly half of

the patients in this study having diabetes and hypertension. Few studies also showed high COVID-19 associated mortality in the male gender ^[12, 13]. Our report did not find a similar result. Seventy Seven out of Eighty Two patients (94%) had an indwelling device. we found significantly elevated CRP and procalcitonin levels in patients who developed BSIs.

However, the utility of inflammatory markers for predicting BSIs and empirical initiation of antibiotics in these patients remains debatable. All the isolates we found in this report were Gram-negative microorganisms. In this study, we observed the relatively high proportion of Burkholderia (45.12%) followed by Pseudomonas, Klebsiella, Acinetobacter and E.coli (19.51%, 13.41%, 12.10%, and 10.30% respectively). Similar observations were described by a multicentric study from India with a predominance of Gram-negative pathogens (78%).¹⁰ A study done by Rachana Patel *et al.* gujrat, India showed higher incidence of Gram negative secondary blood stream infection (43.24%) where they found E. coli as predominant isolates followed by Klebsiella pneumonia, Acinetobacter spp and Pseudomonas spp. Predominance of Gram negative pathogens could be due to the invasive device associated infections during hospitalization due to mechanical ventilation and central venous catheter implantation in these patients. Conversely, Elabaddi *et al.* and a few other studies reported the increased prevalence of Gram-positive microorganisms, particularly Staphylococcus aureus (prevalence varies from 44 to 79.6%) in COVID-19 ICU patients ^[9, 11]. This heterogeneity in prevalence and distribution of micro-organisms may attribute to different patient settings, the number of patients on mechanical ventilation, duration of hospital stays and follow-up and isolation of the pathogen from other specimens (like respiratory, urine and pus samples) in addition to BSIs. The prevalence of Burkholderia is currently increasing as a causative agent of hospital-acquired infections owing to commonly distributed in soil, water, fruits vegetables as well as contaminants of pharmaceutical preparation, medical equipments, nebulizer solutions especially Ipratropium Bromide. In a recent study Hoque MM and *et al* reported outbreak of Burkholderia cepacia due to contaminated nebulizer solution at a tertiary care hospital in Dhaka ^[14]. Similar results were observed by S. vathshalan *et al.* of National Hospital, Srilanka ^[15].

Regarding the pattern of antibiotic resistance all the isolates were were multidrug resistant (MDRO).The rate of carbapenem resistance was seen in K. pneumoniae, E. Coli, A.baumannii and Pseudomonas species and the rate was 70%,75%, 100%and 44.4%,respectively. Mortality among patients with K.pneumoniae and A.baumannii infections was 76% and 63% respectively.These results were in line with the previously documented high mortality associated with carbapenem-resistant A.baumannii and K.pneumoniae. Burkholderia cepacia was 100% resistant to amoxyclav and ticarcillin and was 100% sensitive to piperacillin+ tazobactam and meropenam. Klebsiella pneumonia was found highly resistant to ceftazidime and co-trimoxazole and showed 100% sensitivity to colistin and tigacycline. Acinetobacter was highly resistant to most of the antibiotics except colistin and tigacycline. A study from India by Naveenraj *et al.* showed that Acinetobacter was highly resistant to Ceftriaxone and PIT while Klebsiella showed highest resistance against Aztreonam ^[16] In our study Pseudomonas spp. was resistant to cefixime and ceftazidime and highly sensitive to ciprofloxacin and amikacin. Another study from Iran by Koorosh Ahmadi *et al.* showed Pseudomonas spp was highly sensitive to imipenam.¹⁷Our study showed E.coli was highly resistant to azithromycin and colistin. Few reports showed a decreased prevalence of MDRO due to effective implementation of infection control practices; in contrast, there are studies that showed a high transmission of MDRO due to prolonged ICU stay and use of multiple antibiotics in COVID-19. ¹⁸⁻²⁰

Conclusion

BSIs in COVID-19 are associated with poor outcome. This study revealed the prevalence of gram-negative bacteria as causative agents of sepsis in the ICU settings and their antibiotic resistance pattern. We found Burkholderia as an predominant cause of septicaemia in ICU admitted COVID-19 patients which is resistant to almost all common antibiotics. Other gram negative organisms shown in this study are also resistant to most of the conventional antibiotics used in ICU. Therefore our study aims to identify the bloodstream pathogens in critically ill COVID 19 patients and their antibiotic resistance pattern to encourage implementing antibiotic stewardship guide and to improve clinical outcome.

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