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Research article

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Antimicrobial Resistance Pattern of Multidrug - Resistant Bacteria Isolated from COVID-19 Patients

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1. Abstract

1.1 Aim

The primary objective of this study was to show the antimicrobial resistance pattern of bacteria isolated from COVID-19 patients.

1.2 Materials and Methods

We performed a retrospective study including 361 positive samples elevated from COVID-19 patients admitted at the Clinical Hospital of Pneumology and Infectious Diseases "By Dr. Victor Babes" Timisoara between January and December 2021.

1.3 Results

361 positive samples from a total of 1441 collected samples were analyzed.

The 361 positive samples were obtained from different cultures as follows: blood cultures (n=53), venous catheter cultures (n=7), bronchial catheter cultures (n=12), urinalysis (n=89), bronchial aspirate cultures (n=136) and wound secretion cultures (n=64).

The most frequent bacteria isolated were: *Staphylococcus aureus* (25.7 %), *Escherichia coli* (22.9 %), *Pseudomonas aeruginosa* (14.6 %), *Klebsiella pneumoniae* (12.7 %), *Staphylococcus epidermidis* (10,8 %) and *Acinetobacter baumanii* (9.6 %).

All germs were divided by their antimicrobial resistance pattern, obtaining different information. 69.2 % of *Staphylococcus epidermidis* strains were methicillin–resistant. 56.9 % of *Staphylococcus aureus* strains were methicillin–resistant. 74.2 % of *Acinetobacter baumanii* strains were carbapenemase – positive. 34.7 % of *Klebsiella pneumoniae* strains presented extended–drug resistance. 28.3 % of *Pseudomonas aeruginosa* strains were carbapenemase – positive. 26.5 % of *Escherichia coli* strains were Extended Spectrum Beta–Lactamase (ESBL) – producing.

1.4 Conclusions

The COVID-19 pandemic highly impacted the lives of all of us and, unfortunately, the profile of microbial infections and antimicrobial resistance. A high resistance level among Gram–harmful bacteria identified from COVID–19 patients were observed. The current study's findings indicate that continuous monitoring of antimicrobial resistance profile and bacterial co–infection, along with improved controlling measures, are mandatory to keep the control at a local and global level.

2. Introduction

The coronavirus disease 2019 (COVID-19) changed and challenged healthcare systems worldwide **[1]**. Bacterial co–infections, particularly with resistant bacteria, complicate the clinical presentation of COVID–19 and cause increased mortality and length of hospital stay **[2,3,4]**. Since the beginning of the COVID–19 pandemic, since no specific drugs were available to treat SARS – CoV- 2 infections, antibiotics have often been used for prophylactic and therapeutic purposes, contributing to the emergence of antimicrobial resistance **[5]**.

The symptoms of COVID–19 infection seem similar to those of atypical bacterial pneumonia **[7,8,9]**, which led to the empirical decision to administer antibiotics commonly used to treat bacterial pneumonia. Antibiotics could be helpful due to their potential antiviral **[10]**, immune–modulating, and anti–inflammatory properties. However, we have to consider that the antiviral effectiveness of some antibacterial drugs (aminoglycosides and meropenem) **[11,12]** has not been yet proven or has been proven limited **[13,14]**.

Consequently, antibiotics were over–prescribed and over – administered **[15]**.

An online survey in Australia showed that nearly 20 % of participants took antibiotics as a prophylactic measure against COVID–19 infection **[16]**. World Health Organisation (WHO) discourages antibiotic administration, both for therapeutical or preventive purposes, in patients with mild and moderate COVID–19 symptoms unless there is a clinical indication of bacterial co – infection **[17]**.

Many different definitions have been used in the medical literature to define the different patterns of bacterial resistance in healthcare–associated.

Multidrug – resistance (MDR) is acquired resistance to at least one agent in three or more antimicrobial categories. Extended–drug resistance (XDR) is characterized by non – susceptibility to at least one agent in all but two or fewer antimicrobial types, while pan-drug – resistance (PDR) is defined as non–exposure to all agents in all antimicrobial categories.

To ensure the correct applications of these terms, bacterial products must be tested against all or nearly all of the antimicrobial agents within the antimicrobial categories, and selective reporting and suppression of results should be avoided **[6]**

3. Materials and methods

We performed a retrospective study including 361 positive samples collected from COVID–19 patients admitted at the Clinical Hospital of Pneumology and Infectious Diseases "Dr. Victor Babes" Timisoara between January and December 2021. All patients were tested for SARS – CoV – 2 infections at admission (nasopharyngeal swabs were collected), and the results were reported using RT – PCR automated analysis. All patients included in the study tested positive.

361 positive samples were analyzed. The prevalence of the samples was as follows: 136 positive bronchial aspirate cultures, 89 positive urinalyses, 64 positive wound secretion cultures, 53 from blood cultures, 12 from bronchial catheter cultures, and 7 from venous catheter cultures.

The clinical specimens (urine, blood, wound swabs, etc.) were collected from patients based on laboratory requests made by the clinicians after anamnesis, clinical examination, and presumptive diagnosis to confirm bacterial co–infection.

Blood was collected in Bactec bottles and incubated for a maximum of 7 days for aerobic and anaerobic bottles and up to 42 days for MycoF/Lytic bottles.

We used Blood agar and MacConkey agar for culturing bacteria from the other sources (urine, wound swabs, bronchial and venous catheter, bronchial aspirate), followed by incubation at 370 C for 24 hours. Additional selective and non–demanding culture mediums were also used, according to the doctors' decision in the Laboratory: Chapman agar, Chocolate agar Polyvitex, Haemophilus chocolate 2 agar, and Sabouraud agar.

Some positive samples were also tested on automatic equipment Vitek 2 Compact for identification and antibiotic sensitivity testing.

Antibiotic sensitivity testing was performed using an antibiotic diffusion test. This method is based on the principle that antibiotic–impregnated disk, placed on agar (we used Muller – Hinton agar), previously inoculated test bacterium (0.5 McFarland), pick-up moisture and the antibiotic diffuse radially through the agar medium, producing an antibiotic concentration gradient. The results of susceptibility testing were reported as "susceptible" or "resistant" according to the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

Different antibiotic were used for testing susceptibility of Gram-negative bacteria: amoxicillin and clavulanate, ampicillin, amikacin, aztreonam, cefepime, ceftazidime, ceftriaxone, cefazolin, ciprofloxacin, colistin, ertapenem, fosfomycin, gentamicin, imipenem, levofloxacin, meropenem, nitrofurantoin, piperacillin, trimethoprim/sulphamethoxazole, piperacillin–tazobactam.

For Gram-positive strains, the most frequent antibiotic tested were: erythromycin, clindamycin, moxifloxacin, cefoxitin (reported as susceptibility or resistance to oxacillin), trimethoprim/sulphamethoxazole, gentamicin, tetracycline, tobramycin, amikacin, rifampicin, linezolid, vancomycin.

4. Results

361 positive samples from a total of 1441 collected samples were analyzed. We included 217 males (60.1 %) and 144 (39.9 %) females, with a sex ratio M/F of 1.50.

The 361 positive samples were obtained from different cultures: 53 from blood cultures, 7 from venous catheter culture, 12 from bronchial catheter culture, 89 from urinalysis, 136 from bronchial aspirate culture, and 64 from wound secretion culture.

In 2021, a total number of 410 blood cultures were collected from patients. 53 of these (12.9 %) were positive. We were able to identify the following terms: Acinetobacter baumanii (n=5), Staphylococcus aureus (n=9), Klebsiella pneumonia (n=3), Pseudomonas aeruginosa (n=6), Staphylococcus epidermidis (n=7), Salmonella spp. (n=1), Bacillus spp. (n=2), Streptococcus spp. (n=2), Enterococcus faecalis (n=2), Providencia stuartii (n=1), Candida albicans (n=1) and other types of staphylococci (n=12).

Of the 5 strains of Acinetobacter baumanii identified, 100 % were carbapenemase–positive and presented extended–drug resistance. We identified 3 themes of Klebsiella pneumonia, all (100 %) presenting

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extended–drug resistance. Pseudomonas aeruginosa was determined from 6 blood cultures: 2 were carbapenemase–positive and offered prolonged drug resistance.

Other germs from the Enterobacteriaceae family were identified: extensive – drug-resistant Salmonella spp. (n=1), pan drug – resistant Providencia stuartii (n=1).

Among Gram-positive bacteria, Staphylococcus aureus was the most prevalent (n=9), followed by Staphylococcus epidermidis (n=7). 77.7 % of the Staphylococcus aureus strains presented methicillin–resistance; methicillin–resistant Staphylococcus epidermidis was reported in 71.4 % of cases. Other coagulase–negative staphylococci were isolated in 12, of which 83.3 % were also methicillin–resistant.

Streptococcus spp. was isolated from 2 blood cultures. Enterococcus faecalis was isolated in 2 cases. 2 Blood cultures were positive for Bacillus spp. 3 fungal infections, all of them with Candida albicans, were reported.

7 cultures of the venous catheter were performed, and we could isolate 9 germs. We identified one pan drug–resistant strain of Strenotrophomonas maltophilia. Pseudomonas aeruginosa was isolated from 2 cultures; 1 sample was positive for Acinetobacter baumanii (also carbapenemase–positive with extended – drug resistance); 1 strain of carbapenemase – positive Escherichia coli was reported.

Gram-positive bacteria were also isolated: 2 strains of coagulase–harmful staphylococci (both presenting methicillin-resistance) and 1 bacterial isolate of Enterococcus spp. Fungal infection with Candida albicans was positive in 1 sample isolate.

12 bronchial catheter samples were brought and tested in the Microbiology Laboratory, from which we could isolate 19 germs.

We identified Proteus vulgaris in 1 sample, a strain with extended – drug resistance. Klebsiella pneumonia was isolated from 2 pieces, of whom 1 model (50 %) presented extended–drug resistance.

Pseudomonas aeruginosa was identified in 3 samples, 1 (33.3 %) being carbapenemase–positive and presenting extended–drug resistance. Acinetobacter baumanii was the most prevalent bacteria isolated (n=7). 85.7 % (6/7) were carbapenemase–positive and gave prolonged–drug resistance, while 1 isolate (14.3 %) was pan drug–resistant.

Escherichia coli was determined from 1 sample.

Gram-positive bacteria were also isolated: Staphylococcus aureus in 3 cases (66.6 % with methicillin - resistance) and other coagulase–negative staphylococci in 1 case, also being methicillin–resistant.

Candida albicans was identified in 1 case.

We examined a total number of 635 urinalyses, of which 89 (14.01 %) were positive.

Escherichia coli was the most prevalent bacteria isolated (n=39 – 43.8 %); 13 Escherichia coli strains were ESBL–producing strains. Pseudomonas aeruginosa was isolated from 11 samples (12.3 %), and 9 strains (81.8 %) were carbapenemase–positive. 8 of the 9 carbapenemase – positive strains presented extended – drug resistance.

11 samples tested positive for Klebsiella pneumoniae: 6 strains of ESBL–producing Klebsiella pneumoniae (54.5 %) and 3 carbapenemase–positive samples (27.2 %). 7 of the pieces (63.6 %) presented extended – drug resistance.

Proteus spp. was identified in 8 samples, 3 (37.5 %) presenting extended–drug resistance. Acinetobacter baumanii was determined from 2 bacterial isolates, both of the strains being carbapenemase–positive and extended–drug resistant.

Other Gram-negative bacteria were identified: Serratia spp. (n=2, with 1 case of extended – drug-resistant bacteria); Citrobacter spp. (n=2); Providencia stuartii (1 pan drug–resistant strain), Myroides spp. (1 extended – drug-resistant strain).

Gram-positive bacteria were also identified: 8 strains of Enterococcus spp. and 1 song of methicillin–sensitive Staphylococcus aureus. The samples also tested positive for fungal infections: Candida tropicalis (n=1), Candida krusei (n=1), and Candida glabrata (n=1).

We examined 69 wound secretion cultures, 64 of which (92.7 %) were positive. From the 64 positive samples, we were able to isolate 103 germs.

Acinetobacter baumanii and Escherichia coli were the most prevalent Gram-negative bacteria isolated (11 cases of each germ). 7 of the 11 samples of Acinetobacter baumanii (63.6 %) were carbapenemase–positive and extended–drug resistant. 5 of the 11 Escherichia coli strains (45.4 %) were ESBL–producing strains. 2 of them (40 %) were extended–drug resistant.

Pseudomonas aeruginosa was isolated in 8 cases (12.5 %), 1 being carbapenemase–positive and extended–drug resistant. Klebsiella pneumonia and Proteus spp. were identified in an equal number of 6 samples. 4 strains (66.6 %) of Klebsiella pneumoniae were ESBL–producing strains, and 1 strain (16.6 %) was carbapenemase–positive; also, 4 of them presented extended–drug resistance.

Extended – drug resistant Proteus spp. was determined in 2 cases (33.3 %). Other Gram-negative bacteria were isolated: Morganella morganii (n=1), Citrobacter koseri (n=1), Serratia marcescens (n=2, with 1 strain with extended–drug resistance), Stenotrophomonas maltophilia (n=1), Providencia stuartii (n=1).

Among Gram-positive bacteria, Staphylococcus aureus was the most prevalent (n=39). Methicillin – resistance was determined in 48.7 % of cases. Coagulase–negative staphylococci were positive in 10 samples, 50 % being methicillin–resistant. Enterococcus spp. was identified in 4 wound secretion samples. Fungal infections were isolated from 2 pieces.

We examined 308 bronchial aspirate cultures, 136 of which were positive (44.1 %). From the 136 positive samples, we were able to isolate 164 germs.

Escherichia coli was the most prevalent bacteria isolated (n=31 – 22.7 %). Only 3 strains (9.6 %) were ESBL–producing strains. 2 (66.6 %) were extended–drug resistant. Klebsiella pneumoniae was identified

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in 24 samples (17.6 %), with 1 strain (4.1 %) carbapenemase–positive and extended–drug resistant.

From 23 samples (16.9 %), we were able to isolate Pseudomonas aeruginosa. Only 2 strains (8.6 %) were carbapenemase–positive and extended–drug resistant. Acinetobacter baumanii was isolated in 10 samples (7.3 %), 5 (50 %) being carbapenemase–positive and comprehensive – drug resistant.

Other Gram-negative bacteria were isolated: Achromobacter spp. (n=1), Serratia marcescens (n=2), Proteus spp. (n=3), Enterobacter spp. (n=1), Citrobacter spp. (n=1).

Among Gram–positive bacteria, Staphylococcus aureus was the most prevalent (n=42 – 30.8 %). Methicillin – resistance was determined in 61.9 % of cases. Staphylococcus epidermidis was isolated from 23 samples (16.9 %), with 17 patients (73.9 %) presenting methicillin – resistance. Other Gram–positive bacteria identified were Streptococcus spp.(n=1) and Enterococcus spp. (n=1). 1 fungal infection with Aspergillus spp. was reported.

5. Discussion

Bacterial co–infections among patients hospitalized with COVID–19 are a significant health problem.

Among the Gram–harmful bacteria, *Escherichia coli* (n=83) and *Pseudomonas aeruginosa* (n=53) are the most prevalent bacterial isolates with high rates of antimicrobial resistance determined. They were followed by *Klebsiella pneumoniae* (n=46) and *Acinetobacter baumanii* (n=35), presenting very high antibiotic resistance rates.

In a study conducted by Russell et al., it has been proven that from the family of *Enterobacteriaceae, Escherichia coli* is the most common bacteria isolated from blood cultures **[18]**. In our study, *Pseudomonas aeruginosa* and *Acinetobacter baumanii* are the most frequent isolates causing bloodstream infections among COVID–19 patients. Higher resistance rates were found in *Acinetobacter baumanii* (74.2 %, n=26): 25 strains presented extended–drug resistance, and 1 pressure-given pan-drug – resistance. This finding was also reported in the study by Jie Li et al. **[19,20]** and also in the survey by Ehsan Sharifipour et al. **[21]**.

Other bacteria, such as *Escherichia coli*, showed resistance in 31.8 % of ESBL–positive bacterial strains (7 of 22 ESBL–cheerful songs presented extended–drug resistance). A study by Mahmoudi et al. showed that *Escherichia coli* gave more excellent resistance to cephalosporins and piperacillin–tazobactam **[22]**. In the survey conducted by Ravichandran et al. **[20]**, many isolates were resistant to cephalosporins, and few were resistant to imipenem and meropenem. In our study, the highly resistant bacterial strains were also resistant to cephalosporins, imipenem, and meropenem.

Pseudomonas aeruginosa showed 28.3 % resistance (15/53). Similar data were reported in the study conducted by Qu et al. **[23]**

In our study, we isolated 3 strains of *Providencia* spp, out of which 66 % (n=2) were pan drug–resistant. Similar findings were highlighted in the study conducted by McGann et al. **[24]**

Among the Gram–positive bacteria, *Staphylococcus aureus* was the most prevalent bacterial isolate determined (n=93). 56.9 % of the strains were methicillin–resistant. The data are confirmed by the study conducted by Rezasoltani et al. **[25]**.*Staphylococcus epidermidis* was also isolated in 39 samples, of which 69.2 % were methicillin–resistant (n=27).

In this study, we analyzed a significant number of samples collected from COVID–19 patients, being able to present an appropriate microbiological and antibiotic resistance pattern. However, the study was conducted in a single center. We mention that microbiological and antibiotic patterns can vary from one location to another.

6. Conclusion

The COVID–19 pandemic has widely impacted not only our everyday habits of us but also the profile of microbial infections and antimicrobial resistance **[15]**.

Bacterial and fungal co–infections are common and place a significant threat to the patient with COVID – 19 disease. At the same time, COVID – 19 disease increases the risk of bacterial and fungal co–infections.

An effective antimicrobial regimen remains critical for the successfully treatment of COVID–19 [20]. Investigation of bacterial co–infections and antibiotic patterns can further help in improved health of COVID–19 patients and help us understand the viral and bacterial pathogen interaction within the host **[26]**.

The battle with COVID–19 may accelerate the worsening of antibiotic–resistant pathogens. The rising number of multidrug–resistant bacteria and our decreasing capacity to eradicate them render us more vulnerable to bacterial infections and weaken us during viral pandemics **[27]**.

Overall, it is essential to limit the risk of infections and the spread of these resistant strains by controlling nosocomial diseases accurately and bringing secondary infections caused by resistant bacteria that can increase the mortality rate in COVID–19 critical patients into attention **[21]**.

The findings of the current study suggest that continuous monitoring of bacterial co–infection and resistance patterns and improving infection control measures are essential to control COVID–19 at a local and global level.

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