The impact of the COVID-19 pandemic on Gram-negative bacteria susceptibility patterns in respiratory samples of intensive care units in the Brussels Capital Region, 2010-2021

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Published in:
American journal of infection control

DOI:
10.1016/j.ajic.2023.08.020

Publication date:
2024

MANUSCRIPT_Version2.2_20 August 2023

Title of the study:

The impact of the COVID-19 pandemic on Gram-negative bacteria susceptibility patterns in respiratory samples of intensive care units in the Brussels Capital Region, 2010-2021

Keywords:

Gram-negative bacteria; antibiotic susceptibility; respiratory samples; intensive care unit; ventilator-associated pneumonia; Coronavirus disease 2019

Word count:

Abstract: 220

Text without disclosure, references, table, and figure legend: 2860

Number of figures: 1

Number of tables: 4

Supplementary Materials: 1 additional figure, and 5 additional tables

TEXT

Introduction

The occurrence of gram-negative bacteria (GNB) resistance complicates the management of several infections, limiting therapeutical options and lowering outcomes [1, 2]. Furthermore, therapeutical regimens targeting carbapenems-resistant GNB are limited and the alternative drugs (*e.g.* colistin and tigecycline) have a narrower therapeutic index and higher toxicity [1]. Newly available β-lactam antibiotics combined with β-lactamase inhibitors might be utilized in the case of MDR GNB infections. However, their price is a limiting factor [3, 4]. Respiratory infections, especially hospital-acquired or ventilator-associated pneumonia, seem to be more frequently provoked by multi-drug resistant (MDR) GNB than soft tissue or urinary or abdominal infections [2, 5]. Additionally, hospital-acquired pneumonia is a frequent infection in the hospital population and may affect up to 2% of inpatients with a mortality of around 30%, independently of the isolation of MDR GNB [6].

The coronavirus disease 2019 (COVID-19) pandemic was associated with increased use of antimicrobials, appropriate or not, with antibiotic regimens started in up to 80-100% of the severe COVID-19 patients [7]. The most recent WHO Global Antimicrobial Resistance and Use Surveillance System report of 2020 found an increase in resistance to GNB reported by one-third of the participating countries. Nevertheless, they concluded that most of the survey members were not able to appropriately report the resistance rate as they were facing the COVID-19 pandemic and that these results should be interpreted with caution [8]. Both the intensified antimicrobial pressure due to antibiotic overconsumption, and the effort to control the COVID-19 pandemic, impeding antimicrobial stewardship programs to focus on bacterial resistance, may have increased the emergence of resistance of GNB [9, 10]. Therefore, epidemiological surveys are required to increase awareness among clinicians, assess the modification of local ecology, and finally guide therapeutic options.

Study objectives

The current study has been designed to investigate the trends of susceptibility patterns of the Gram-negative bacteria isolated from the respiratory samples collected in the intensive care units (ICU) of the Brussels Capital Region. Particularly, we hypothesized a difference in the prevalence of the drug-resistance GNB between the years before and during the COVID-19 pandemic.

Materials and methods

Study design

The study design is epidemiological, retrospective, and multicenter. The multicenter design was favored to represent the epidemiological area of the ICU of the Brussels Capital Region.

This study was elaborated following the STROBE checklist for reports of observational studies [11].

Setting

Respiratory samples collected at the ICUs of the University Hospital Brussels (UZ Brussel) Centre Hospitalier Universitaire (CHU) Saint Pierre, and the CHU Brugmann, between 1st January 2010 to 31st December 2021, were considered. In 2020, 38614 people living in the Brussels capital region were admitted to UZ Brussel, CHU Saint Pierre, or CHU Brugmann. Considering the total number of people from this region who were hospitalized (131368), the estimated catchment population of the three university centers together was 30% of the total Brussels Capital Region.

Variables

Respiratory samples of patients hospitalized in an ICU of the above-mentioned hospitals were reviewed considering all the grown bacteria. Only specimens obtained by sampling patients already located in the ICU department were considered. Whenever the same bacterium was isolated more than once from respiratory samples of the same patient, it was taken into consideration only once. Further details on bacterial de-doubling are specified in the Supplementary Materials Table 1.

GNB were classified as multi drug-resistant (MDR) if they were non-susceptible to at least one antibiotic within three different classes; extensively drug-resistant (XDR), whenever they were non-susceptible to at least one antimicrobial within all classes of antibiotic except two; pan drug-resistant (PDR), whether they were non-susceptible to all tested drugs [12]. Bacteria with resistance or intermediate resistance to an antimicrobial were both considered non-susceptible. Breakpoints for microbial drug susceptibility definition and intrinsic drug non-susceptibility are established following the criteria European Committee on antimicrobial susceptibility testing (EUCAST) [13]. All the involved centers switched to the EUCAST version 10.0 of 2020 only after 2021. During the first years of this study, independent local breakpoints were used by the microbiological laboratories of each center. These breakpoints are reported in the supplementary method of this article (Supplementary Materials Table 2).

As the a priori speculation of the current study is an increase of resistance during the study course due to the COVID-19 pandemic, a division in periods was applied. The time between 2010 to 2021 was divided into time intervals of two years each to give an equal number of years to every period. The first study period ran from 1st January 2010 to 31st December 2011; consecutively, the second: 1st January 2012 to 31st December 2013; the third: 1st January 2014 to 31st December 2015; the fourth: 1st January 2016 to 31st December 2017; the fifth: 1st January 2018 to 31st December 2019; the sixth: 1st January 2020 to 31st December 2021. The

last study period comprises the COVID-19 pandemic, as the first non-imported COVID-19 case was reported in Belgium in March 2020.

The main outcome of the current study was a difference, between defined time points, in the prevalence of resistant GNB, particularly MDR, XDR, and PDR, in the respiratory samples obtained from patients admitted to the ICU.

Data source

Epidemiological and microbiological data were collected from laboratory databases of each hospital

Statistical methods and study size

After exploring the characteristics of isolated bacteria, chi-square tests were performed to investigate differences in the prevalence of resistant GNB patterns (MDR, XDR, and PDR) between the three above-specified periods. Thereafter, six logistic regressions were carried out to compare the prevalence of the three patterns of resistance between the first and second, and the second and third study periods.

The study size was not computed a priori. However, efforts were made to collect all the data of the respiratory specimens obtained in the ICUs of the three different university hospitals.

Analyses were performed with IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp, released in 2011.

Results

Included samples

Overall, 10577 respiratory samples obtained by 5889 patients admitted to one of the ICUs of the three considered university centers were considered (Figure 1 – Study flowchart). The most frequent specimen was endotracheal aspirate (EDTA), 7690 samples (208 of them obtained through bronchoscopy), with its percentage slightly decreasing over study time (Supplementary Materials Table 3).

Most frequently isolates

Supplementary Materials Table 4 illustrates the prevalence of isolates for each study period. The most found species was *Pseudomonas aeruginosa* (24.7%). Bacteria from the genus *Klebsiella* were the second most frequent (19.1%) with the most isolated species being *K. pneumoniae* (1497 isolates) and *K. oxytoca* (448 isolates). The third most prevalent species was *Escherichia coli* (16.8%). Thereafter, it followed the bacteria from the genus *Enterobacter* (11.3%), with the most prevalent species being *E. cloacae* (805 isolates), and *E. aerogenes* (390 isolates, recently reclassified as *Klebsiella aerogenes*).

Outcome data – Prevalence of the pattern of non-susceptibility

Globally, 3769 (37%) samples grew a bacterium classified as MDR, 894 (8%) a XDR, and 41 (0.4%) a PDR. Table 1 pictures the number of MDR, XDR, and PDR isolates in each respective period. The proportion of specimens growing an MDR bacteria grew by 5% between the second (2012-2013) and the third periods (2014-2015). The increase in MDR bacteria between those time intervals was statistically significant, chi-square value: 8.24, $p = 0.004$, and OR 1.23 (95% CI: 1.07 - 1.42) $p = 0.004$. Moreover, the percentage of MDR isolates augmented by 4% between the last time periods, and the growth was statistically significant, chi-square value: 7.03, $p = 0.008$, and OR 1.19 (95% CI: 1.05 - 1.36) $p = 0.008$. Samples isolating an XDR bacteria raised by 5% between the fifth and the sixth time periods. This difference in XDR bacteria was statistically significant, chi-square value: 20.96, $p > 0.001$, and OR 1.63 (95% CI:

1.32 - 2.02) p > 0.001. Finally, the specimens growing PDR bacteria were three and seventeen, respectively, in the fifth and sixth periods. This difference was statistically significant, chi-square value: 7.37, p = 0.006, and OR 4.69 (95% CI: 1.37 – 16.03) p = 0.014.

Prevalence of resistance to the single drugs

Supplementary Materials Table 5 illustrates the trend of non-susceptibility to the single antimicrobial during each period. Globally, resistance to all the drugs augmented during the time spans except for trimethoprim-sulfamethoxazole non-susceptibility which decreased. Species intrinsic resistant to trimethoprim-sulfamethoxazole (*e.g. Pseudomonas aeruginosa*) were excluded from the count. Regarding the anti-pseudomonal β-lactams: cefepime (FEP), meropenem (MEM), and piperacillin-tazobactam (TZP), a mild increase in non-susceptibility percentage is observed in the first part of the study. While a rise of 4%, 6%, and 10%, is noted between the last two periods respectively for FEP, MEM, and TZP (Supplementary Materials Figure 1).

Analysis of the three most represented genera

Pseudomonas aeruginosa

Pseudomonas was the most frequently isolated gram-negative bacteria with a stable rate of samples growing this bacterium between the study periods (approximately 25%). The prevalence of the main patterns of resistance and the antimicrobial resistance to the most used antimicrobials for this bacterium is illustrated in Table 2. Overall, the occurrence of MDR and XDR isolates had minor fluctuation during the study time, except between the last two periods when an increase of more than 10% was observed. Similar trends were reported for TZP and MEM with respectively, a 18% and 22% non-susceptibility rise between the fifth (2018-2019) and the sixth period (2020-2021).

Genus Klebsiella

Table 3 represents the prevalence of the patterns of resistance and the main antibiotics used for *Klebsiella*. Its prevalence progressively augmented during the study time, from 16% to 22% of the specimens. The percentage of MDR isolates had a limited fluctuation over time, while the one of XDR grew steadily. The most prevalent antimicrobial resistances were reported for amoxicillin and clavulanic acid and TZP, around 40%. The greatest growth of non-susceptibility rate, between the last two periods, was observed for TZP and temocillin (TMO). The lowest rate of resistance was found for MEM (4%).

Genus Escherichia

The third most frequently observed genus was Escherichia. Its prevalence progressively declined during the study time, from 20% to 13% of the samples. Table 4 illustrates the prevalence of the major resistance patterns and the non-susceptibility to the most used antimicrobials for this bacterium. The percentage of MDR isolates augmented the most between the second (2012-2013) and the third (2014-2015) periods (8%). While the one of XDR isolates increased the most between the fifth (2018-2019) and the sixth (2020-2021) time intervals (4%). The highest non-susceptibility rates were for AMC and TZP, respectively, roughly 50% and 27%. In contrast, the lowest rates were observed for MEM and TMO.

Discussion

Key findings

Over twelve years, the trends of GNB resistance, isolated in the respiratory samples of the ICUs of three university hospitals within the Brussels Capital Region, were observed. While a significant augmentation of MDR samples was noticed once comparing two pre-pandemic

periods (2012-2013 and 2014-2015), all three patterns of non-susceptibility (MDR, XDR and PDR) significantly increased, comparing the years before and throughout the COVID-19 pandemic (2018-2019 and 2020-2021). *Pseudomonas* spp., *Klebsiella* spp., and *Escherichia* spp. were the most observed genera, with the former being the most involved in the appearance of resistance. Three other epidemiological studies, conducted in Iraq, Mexico, and India, investigated the evolution of antibiotic resistance comparing the months before, and during the pandemic. A decrease in antimicrobial susceptibility was reported for bacteria from the genera *Escherichia*, *Pseudomonas,* and *Klebsiella* [14, 15, 16]. However, a similar study carried out at the University Hospital of Taiwan, comparing the pre and pandemic months, found a stable prevalence of the above-mentioned bacteria and resistances. A global increase in antibiotic use, particularly of carbapenem and alternative drugs for carbapenem-resistant GBN infections (e.g. colistin, fosfomycin, and tigecycline), was reported during the COVID-19 pandemic period, likely contributing to the induction of MDR bacteria [17]. Accordingly, although the participant's countries of the WHO Global Antimicrobial Resistance and Use Surveillance System reported difficulties in collecting and analyzing their data due to the pandemic, a 36% augmentation of resistance was reported for *Klebsiella pneumoniae* and 31% for *Escherichia coli* [8].

Pseudomonas aeruginosa antimicrobial non-susceptibility

A long-term study (2015-2021) from the University Hospital of Modena (Italy) observed an increased number of *Pseudomonas aeruginosa* bloodstream infections, comparing the COVID-19 pandemic with previous years, without an increase in carbapenem-resistance [10]. Similar results were found in a Spanish study, which compared bloodstream infections in the months before and during the pandemic. The number of MDR *Pseudomonas* significantly augmented, but this was unrelated to carbapenem-producing isolates [18]. Furthermore, an epidemiological study, performed in a tertiary University Hospital in Guangzhou (China), observed a year-by-

year (2018-2021) significant rise in MDR *Pseudomonas* and its resistance to TZP and MEM [19]. However, another study exploring data from the Greek Electronic System for the Surveillance of Antimicrobial Resistance found a decrease of carbapenem non-susceptible *Pseudomonas aeruginosa* by 24% between the respiratory samples obtained pre and during the pandemic (2018-2021) at the ICUs of nine Greek hospitals [20]. In the present study, during the COVID-19 years, the increase of *Pseudomonas* antimicrobial non-susceptibility concerned especially piperacillin-tazobactam and meropenem. Knowing the alarming resistance percentage to carbapenem, new alternative empiric antibiotics might be considered, whenever a *Pseudomonas aeruginosa* is isolated in an ICU patient of the Brussels Capital Region with life-threatening pneumonia [3, 4].

Other genera

In the current study, less dramatic resistance results were reported for *Klebsiella species* and *Escherichia coli,* than *Pseudomonas aeruginosa* with the non-susceptibility rate of the carbapenem being limited. Considering the relatively low level of resistance to temocillin, this antimicrobial might be a carbapenem-sparing agent whether hospital-acquired pneumonia caused by *Escherichia coli* occurres in the ICU [21, 22].

Reasons for antimicrobial resistance increase

The main reasons for the augmentation of antimicrobial resistances were: antimicrobial exposition before the ICU admission for possible bacterial superinfection [23], the empirical broad-spectrum antibiotics administered to critically ill COVID-19 patients with augmenting oxygen need, and difficulty in differentiating between the viral respiratory disease and a bacterial super-infection [10, 15], the increased time of mechanical ventilation and length of ICU hospitalization [16, 17], and the restructuring of the hospital centers due to the pandemic with also a change of main focus for the antimicrobial stewardship programs [8, 9]. Finally,

patients receiving high intensity of care during the COVID-19 pandemic (*e.g.* change to prone position) were more frequently colonized by carbapenemase-producing *Enterobacterales* in a retrospective Italian study [24]. The microbe cross-transmission through health care-workers was reported as the cause of this pattern of colonization. We believe that all the above-mentioned reasons may have led to the augmentation of non-susceptible GNB.

Antibiotic stewardship programs

All the University Hospitals involved in the current study benefit from strong antibiotic stewardship programs. A microbiologist or an infectious disease specialist counsel appropriate antibiotic use to the ICU physician more time per week in one hospital, and daily in the two other centers. Significant differences in reported antimicrobial stewardship programs in ICU were noted in a recent multinational study [25]. Implementation of these programs could be impaired in low-middle-income countries due to the lack of specific training and/or dedicated funding. Therefore, the burden of bacterial antimicrobial resistance might be even more prominent in countries lacking such programs [26].

Study strengths and limitations

The major study strength is the longitudinal observation of the antimicrobial resistance of ICU respiratory samples over 12 years. By means of this, we could better interpret the evolution of non-susceptibility that occurred throughout the years and make a comparison with the COVID-19 pandemic period. Furthermore, the multicentric design of the current study reduces the risk of overestimation of the local epidemiology and possible MDR GNB outbreaks. To the best of our knowledge, this is the first epidemiology study aiming to assess the impact of the COVID-

19 pandemic on the antimicrobial resistance reported by analyzing the respiratory samples of the Brussels Capital Regions ICUs.

Some study limitations should be mentioned. Due to the retrospective design of the current study only associations, and no causality can be determined. For instance, the augmentation of drug resistance observed in the last study period may not be caused by the COVID-19 pandemic. Only hypotheses could be made. The first COVID-19 case was admitted in March 2020 in Belgium. Nevertheless, the last study period included the month of January and February 2020. Furthermore, the three examined hospitals implemented the antimicrobial susceptibility breakpoints of EUCAST only after some years after the beginning of this study, and it might be difficult to compare the data. However, two considered hospitals (CHU Saint Pierre and CHU Brugmann) shared the microbiology laboratory, and breakpoints before the implementations of EUCAST breakpoints were described in the supplementary methods of this article. As the main focus of the study was to assess the trends of bacterial resistance, data concerning patient characteristics (*e.g.* lengths of stay in ICU, or days of mechanical ventilation) were not collected. Therefore, possible confounding factors may have altered the study results. Finally, the data on antibiotic consumption was not reported in this study. Therefore, an association between the growth of resistance to certain antibiotics and antimicrobial use could not be established based on study results.

Conclusion

The COVID-19 pandemic was associated with increasing trends of antimicrobial resistance in respiratory samples of patients admitted to the ICU in university hospitals with well-implemented antibiotic stewardship programs.

DECLARATION

Ethical approval and consent to participate

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was conducted following the study protocol, the Declaration of Helsinki, and applicable regulatory requirements. The local Institutional Review Board and Ethics Committee of the hospitals UZ Brussel, CHU Saint Pierre, and CHU Brugmann separately approved the protocol (UZ Brussel Ethics Committee reference number: EC-2022-107; CHU Saint Pierre Ethics Committee reference number: CE/22-03-07; CHU Brugmann Ethics Committee reference number: CE 2022/73). Given the retrospective nature of the study, which did not demand a deviation from standard clinical care, and the fact that all data was anonymized, informed consent from the patient or the next of kin was not essential.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

All authors declare that they have no competing interests about the contents published in this manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

MM: concept, study design, data collection, analysis, and interpretation, writing and revision, VYMD: data collection, writing and revision, DDG: data collection, writing and revision IW: data collection, writing and revision, MC: writing and revision, JJ: writing and revision, PC: writing and revision, ND: concept, study design, writing and revision. All authors have given final approval of the version to be submitted.

Acknowledgments

We would like to express gratitude for the logistic support offered by the infectious diseases coordinating study team of UZ Brussel, CHU Saint Pierre and CHU Brugmann. Particularly, we would like to thank BYG4lab which provided the INFECTIO.GLOBAL software through which data collection was performed in CHU Saint Pierre and CHU Brugmann, Mr. Witdouck Arne (Data Manager - UZ Brussel) for its help on data cleaning, and Mr. Delforge Marc (Statistician – CHU Saint Pierre) for its check on statistical analyses.

References

[1] H. Grundmann, C. Glasner, B. Albiger, D. M. Aanensen, C. T. Tomlinson, A. T. Andrasević *et al.* Occurrence of carbapenemase-producing Klebsiella pneumoniae and Escherichia coli in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. *Lancet Infect Dis* 2017; 17(2):153-163. doi: 10.1016/S1473-3099(16)30257-2.

[2] P. Nordmann, L. Poirel. Epidemiology and Diagnostics of Carbapenem Resistance in Gram-negative Bacteria. *Clin Infect Dis* 2019; 69(Suppl 7):S521-S528. doi: 10.1093/cid/ciz824.

[3] M. H. Kollef, M. Nováček, U. Kivistik, A. Réa-Neto, N. Shime, I. Martin-Loeches *et al.* Ceftolozane-tazobactam versus meropenem for treatment of nosocomial pneumonia (ASPECT-NP): a randomised, controlled, double-blind, phase 3, non-inferiority trial. *Lancet Infect Dis* 2019; 19(12):1299-1311. doi: 10.1016/S1473-3099(19)30403-7.

[4] R. G. Wunderink, Y. Matsunaga, M. Ariyasu, P. Clevenbergh, R. Echols, K. S. Kaye *et al*. Cefiderocol versus high-dose, extended-infusion meropenem for the treatment of Gram-negative nosocomial pneumonia (APEKS-NP): a randomised, double-blind, phase 3, non-

inferiority trial. *Lancet Infect Dis* 2021; 21(2):213-225. doi: 10.1016/S1473-3099(20)30731-3.

[5] M. Castanheira, L. M. Deshpande, R. E. Mendes, R. Canton, H. S. Sader, R. N. Jones. Variations in the Occurrence of Resistance Phenotypes and Carbapenemase Genes Among Enterobacteriaceae Isolates in 20 Years of the SENTRY Antimicrobial Surveillance Program. *Open Forum Infect Dis* 2019; 6(Suppl 1):S23-S33. doi: 10.1093/ofid/ofy347.

[6] J. P. Lynch. Hospital-acquired pneumonia: risk factors, microbiology, and treatment. *Chest* 2001; 119(2 Suppl):373S-384S. doi: 10.1378/chest.119.2_suppl.373s.

[7] R. Cantón, D. Gijón, P. Ruiz-Garbajosa. Antimicrobial resistance in ICUs: an update in the light of the COVID-19 pandemic Ventilator-Associated Pneumonia: New Definitions. *Curr Opin Crit Care* 2020; 26(5):433-441. doi: 10.1097/MCC.0000000000000755.

[8] S. Tomczyk, A. Taylor, A. Brown, M. E. A. de Kraker, A. El-Saed, M. Alshamrani *et al*. Impact of the COVID-19 pandemic on the surveillance, prevention and control of antimicrobial resistance: a global survey. *J Antimicrob Chemother* 2021; 76(11):3045-3058. doi: 10.1093/jac/dkab300.

[9] B. Tiri, E. Sensi, V. Marsiliani, M. Cantarini, G. Priante, C. Vernelli*et al*. Antimicrobial Stewardship Program, COVID-19, and Infection Control: Spread of Carbapenem-Resistant Klebsiella Pneumoniae Colonization in ICU COVID-19 Patients. What Did Not Work?. *J Clin Med* 2020; 9(9):2744. doi: 10.3390/jcm9092744.

[10] M. Meschiari, L. Onorato, E. Bacca, G. Orlando, M. Menozzi, E. Franceschini *et al*. Long-Term Impact of the COVID-19 Pandemic on In-Hospital Antibiotic Consumption and Antibiotic Resistance: A Time Series Analysis (2015-2021). *Antibiotics (Basel)* 2022; 11(6):826. doi: 10.3390/antibiotics11060826.

[11] E. von Elm, D. G. Altman, M. Egger, S. J. Pocock, P. C. Gøtzsche, J. P. Vandenbroucke. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol* 2008; 61(4):344-9. doi: 10.1016/j.jclinepi.2007.11.008.

[12] A-P. Magiorakos, A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske *et al*. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; 18(3):268-81. doi: 10.1111/j.1469-0691.2011.03570.x.

[13] S. Polsfuss, G. Bloemberg, J. Giger, V. Meyer, M. Hombach. Comparison of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI screening parameters for the detection of extended-spectrum -lactamase production in clinical Enterobacteriaceae isolates. *J Antimicrob Chemother* 2012; 67(1):159-66. https://doi.org/ 10.1093/jac/dkr400

[14] H. H. Tizkam, O. Q. Fadhil, E. Gha. Effect of COVID-19 on Bacterial Resistance. *Sys Rev Pharm* 2020; 11(11):423.

[15] L. E. López-Jácome, D. Fernández-Rodríguez, R. Franco-Cendejas, A. Camacho-Ortiz, M. Del Rayo Morfin-Otero, E. Rodríguez-Noriega *et al*. Increment Antimicrobial Resistance During the COVID-19 Pandemic: Results from the Invifar Network. *Microb Drug Resist* 2022; 28(3):338-345. doi: 10.1089/mdr.2021.0231.

[16] V. Saini, C. Jain, N. P. Singh, A. Alsulimani, C. Gupta, S. A. Dar *et al*. Paradigm Shift in Antimicrobial Resistance Pattern of Bacterial Isolates during the COVID-19 Pandemic. *Antibiotics (Basel)* 2021; 10(8):954. doi: 10.3390/antibiotics10080954.

[17] C.-C. Lai, S.-Y. Chen, W.-C. Ko, P.-R. Hsueh. Increased antimicrobial resistance during the COVID-19 pandemic. *Int J Antimicrob Agents* 2021; 57(4):106324. doi: 10.1016/j.ijantimicag.2021.106324.

[18] M. Torrecillas, V. D. Gumucio, A. Padullés, F. Tubau, D. Marco, E. Shaw *et al*. Antimicrobial use and aetiology of bloodstream infections in critically ill patients during early stages of SARS-CoV-2 pandemic. *Infect Prev Pract* 2022; 4(4):100241. doi: 10.1016/j.infpip.2022.100241.

[19] J. Lyu, H. Chen, J. Bao, S. Liu, Y. Chen, X. Cui *et al*. Clinical Distribution and Drug Resistance of Pseudomonas aeruginosa in Guangzhou, China from 2017 to 2021. *J Clin Med* 2023; 12(3):1189. doi: 10.3390/jcm12031189.

[20] M. Polemis, G. Mandilara, O. Pappa, A. Argyropoulou, E. Perivolioti, N. Koudoumnakis et al. COVID-19 and Antimicrobial Resistance: Data from the Greek Electronic System for the Surveillance of Antimicrobial Resistance-WHONET-Greece (January 2018-March 2021). Life (Basel) 2021; 11(10):996. doi: 10.3390/life11100996.

[21] R. De Jongh, R. Hens, V. Basma, J. W. Mouton, P. M. Tulkens, S. Carryn. Continuous versus intermittent infusion of temocillin, a directed spectrum penicillin for intensive care patients with nosocomial pneumonia: stability, compatibility, population pharmacokinetic studies and breakpoint selection. *J Antimicrob Chemother* 2008; 61(2):382-8. doi: 10.1093/jac/dkm467.

[22] H Habayeb 1, B Sajin, K Patel, C Grundy, A Al-Dujaili, S Van de Velde. Amoxicillin plus temocillin as an alternative empiric therapy for the treatment of severe hospital-acquired pneumonia: results from a retrospective audit. *Eur J Clin Microbiol Infect Dis* 2015; 34(8):1693-9. doi: 10.1007/s10096-015-2406-x.

[23] J. Van Laethem, S. Wuyts, S. Van Laere, J. Koulalis, M. Colman, M. Moretti *et al*. Antibiotic prescriptions in the context of suspected bacterial respiratory tract superinfections in the COVID-19 era: a retrospective quantitative analysis of antibiotic consumption and identification of antibiotic prescription drivers. *Intern Emerg Med* 2022; 17(1):141-151. doi: 10.1007/s11739-021-02790-0.

[24] B. Tiri, E. Sensi, V. Marsiliani, M. Cantarini, G. Priante, C. Vernelli *et al*. Antimicrobial Stewardship Program, COVID-19, and Infection Control: Spread of

Carbapenem-Resistant Klebsiella Pneumoniae Colonization in ICU COVID-19 Patients. What Did Not Work? *J Clin Med* 2020; 9(9):2744. doi: 10.3390/jcm9092744.

[25] R. El-Sokkary, H. Erdem, R. Kullar, A. U. Pekok, F. Amer, S. Grgić *et al*. Self-reported antibiotic stewardship and infection control measures from 57 intensive care units: An international ID-IRI survey. *J Infect Public Health* 2022; 15(9):950-954. doi: 10.1016/j.jiph.2022.07.009.

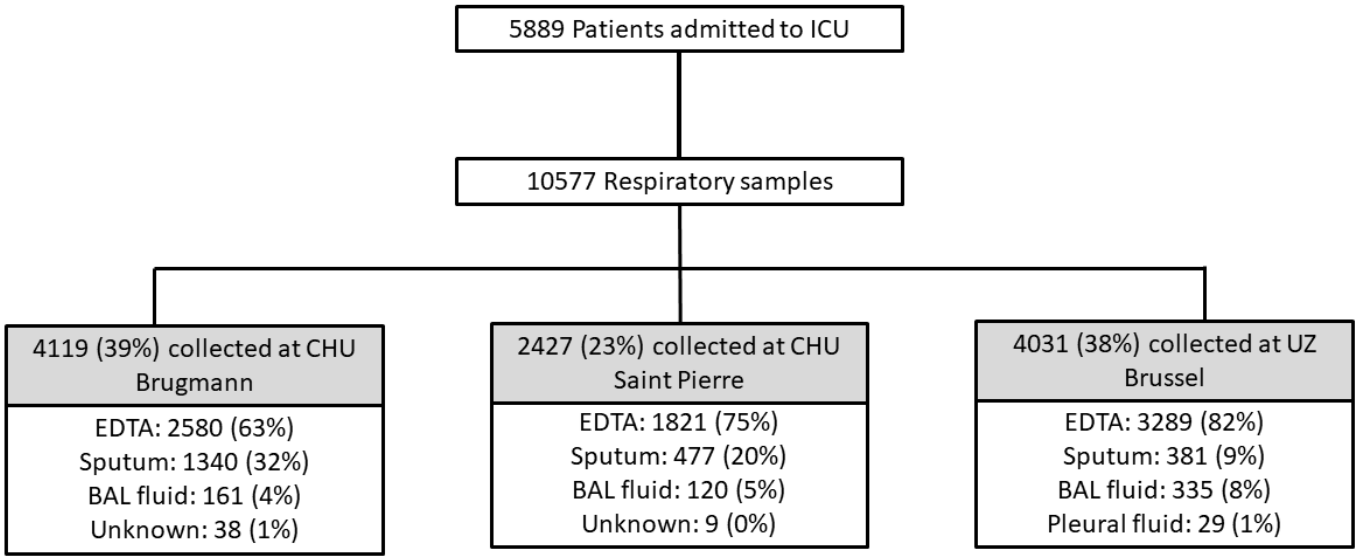
[26] C. J. Murray, K. S. Ikuta, F. Sharara, L. Swetschinski, G. R. Aguilar, A. Gray *et al*. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet* 2022; 399(10325):629-655. doi: 10.1016/S0140-6736(21)02724-0.

FIGURES

Figure Legend

Fig. 1 – Study Flowchart. BAL: broncho-alveolar lavage; EDTA: endotracheal aspiration; ICU: intensive care unit.

Figure 1



TABLES

Table Legend

Table 1 – Trends of the patterns of non-susceptibility during the study periods. Chi-square tests were applied to estimate significant differences in the pattern of non-susceptibility between the first and the second, and the second and the third study periods. The red color is used to underline a significant augmentation compared with a previous period ($p < 0.05$). MDR: multi drug-resistant; XDR: extensively drug-resistant; PDR: pan drug-resistant.

Table 2 – Prevalence of resistance patterns and antimicrobial non-susceptibility in the six study periods for the bacteria of the genus *Pseudomonas*. MDR: multi drug-resistant; XDR: extensively drug-resistant; PDR: pan drug-resistant.

Table 3 – Prevalence of resistance patterns and antimicrobial non-susceptibility in the six study periods for the bacteria of the genus *Klebsiella*. *Klebsiella aerogens* isolates were not included due to previous nomenclature. MDR: multi drug-resistant; XDR: extensively drug-resistant; PDR: pan drug-resistant.

Table 4 – Prevalence of resistance patterns and antimicrobial non-susceptibility in the six study periods for the bacteria of the genus *Escherichia*. MDR: multi drug-resistant; XDR: extensively drug-resistant; PDR: pan drug-resistant.

Table 1

Parameters	Total samples n: 10577	1st period (2010-2011) n: 1632	2nd period (2012-2013) n: 1511	3rd period (2014-2015) n: 1909	4th period (2016-2017) n: 1572	5th period (2018-2019) n: 1784	6th period (2020-2021) n: 2169
MDR	3769 (37%)	525 (32%)	483 (32%)	700 (37%)	585 (37%)	626 (35%)	850 (39%)
XDR	894 (8%)	115 (7%)	109 (7%)	128 (7%)	123 (8%)	145 (8%)	274 (13%)
PDR	41 (0.4%)	2 (0.1%)	8 (0.5%)	9 (0.5%)	2 (0.1%)	3 (0.2%)	17 (1%)

Table 2

Parameters	Total samples growing Pseudomonas spp. n: 2612	1st period (2010-2011) n: 427	2nd period (2012-2013) n: 355	3rd period (2014-2015) n: 453	4th period (2016-2017) n: 409	5th period (2018-2019) n: 432	6th period (2020-2021) n: 536
MDR	935 (36%)	144 (34%)	93 (26%)	137 (30%)	153 (37%)	155 (36%)	253 (47%)
XDR	479 (18%)	72 (17%)	41 (11%)	63 (14%)	84 (20%)	70 (16%)	149 (28%)
PDR	15 (1%)	2 (0.5%)	5 (1%)	4 (1%)	0 (0%)	0 (0%)	4 (1%)
Ceftazidime non-susceptibility	823 (32%)	132 (31%)	80 (22%)	132 (29%)	137 (34%)	118 (27%)	224 (42%)
Ciprofloxacin non-susceptibility	683 (31%)	118 (33%)	55 (20%)	96 (27%)	79 (31%)	105 (24%)	230 (43%)
Cefepime non-susceptibility	753 (29%)	122 (29%)	66 (19%)	118 (26%)	110 (27%)	117 (27%)	220 (41%)
Piperacillin-tazobactam non-susceptibility	829 (32%)	103 (25%)	80 (23%)	129 (28%)	131 (32%)	128 (30%)	258 (48%)
Meropenem non-susceptibility	745 (28%)	104 (24%)	78 (22%)	89 (20%)	114 (28%)	110 (25%)	250 (47%)

Amikacin non-susceptibility	486 (19%)	73 (17%)	33 (9%)	72 (16%)	86 (21%)	69 (16%)	153 (28%)

Table 3

Parameters	**Total samples growing *Klebsiella spp.*** **n: 2021**	**1st period (2010-2011)** **n: 263**	**2nd period (2012-2013)** **n: 257**	**3rd period (2014-2015)** **n: 350**	**4th period (2016-2017)** **n: 331**	**5th period (2018-2019)** **n: 336**	**6th period (2020-2021)** **n: 484**
MDR	740 (37%)	70 (27%)	96 (37%)	149 (43%)	115 (35%)	118 (35%)	192 (40%)
XDR	214 (11%)	16 (6%)	25 (10%)	42 (12%)	12 (4%)	43 (13%)	76 (16%)
PDR	14 (1%)	0 (0%)	0 (0%)	4 (1%)	0 (0%)	1 (0.3%)	9 (2%)
Amoxicillin clavulanic acid non-susceptibility	778 (39%)	89 (34%)	98 (38%)	150 (43%)	113 (34%)	132 (39%)	196 (40%)
Cefotaxime non-susceptibility	510 (25%)	42 (16%)	69 (27%)	117 (33%)	65 (20%)	93 (28%)	124 (26%)
Cefepime non-susceptibility	454 (23%)	41 (16%)	58 (23%)	96 (27%)	46 (14%)	88 (26%)	125 (26%)
Ciprofloxacin non-susceptibility	508 (25%)	49 (19%)	64 (25%)	97 (28%)	82 (25%)	89 (27%)	127 (26%)
Temocillin non-susceptibility	356 (18%)	9 (4%)	20 (8%)	76 (22%)	61 (18%)	66 (20%)	124 (26%)

Piperacillin-tazobactam non-susceptibility	740 (37%)	78 (31%)	97 (38%)	148 (42%)	99 (30%)	121 (36%)	197 (41%)
Meropenem non-susceptibility	90 (4%)	1 (0.4%)	8 (3%)	34 (10%)	12 (4%)	13 (4%)	22 (4%)
Amikacin non-susceptibility	125 (6%)	9 (3%)	22 (9%)	25 (7%)	13 (4%)	20 (6%)	36 (8%)

Table 4

Parameters	**Total samples growing Escherichia spp.** **n: 1778**	**1st period (2010-2011)** **n: 326**	**2nd period (2012-2013)** **n: 261**	**3rd period (2014-2015)** **n: 340**	**4th period (2016-2017)** **n: 294**	**5th period (2018-2019)** **n: 273**	**6th period (2020-2021)** **n: 284**
MDR	849 (48%)	128 (39%)	114 (44%)	178 (52%)	148 (50%)	136 (50%)	145 (51%)
XDR	38 (2%)	2 (0.6%)	4 (1%)	1 (0.3%)	9 (3%)	6 (2%)	16 (6%)
PDR	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Amoxicillin clavulanic acid non-susceptibility	865 (49%)	135 (41%)	121 (46%)	167 (49%)	138 (47%)	152 (56%)	152 (53%)
Cefotaxime non-susceptibility	273 (15%)	39 (12%)	41 (16%)	52 (15%)	40 (14%)	46 (17%)	55 (19%)
Cefepime non-susceptibility	248 (14%)	37 (11%)	33 (13%)	44 (13%)	37 (13%)	48 (18%)	49 (18%)

Ciprofloxacin non-susceptibility	478 (27%)	87 (27%)	71 (27%)	110 (32%)	74 (25%)	67 (24%)	69 (24%)
Temocillin non-susceptibility	171 (10%)	3 (1%)	14 (6%)	31 (9%)	46 (16%)	42 (15%)	35 (12%)
Piperacillin-tazobactam non-susceptibility	455 (27%)	75 (27%)	67 (27%)	104 (31%)	73 (25%)	58 (21%)	78 (28%)
Meropenem non-susceptibility	9 (0.5%)	0 (0%)	0 (0%)	0 (0%)	4 (1%)	2 (1%)	3 (1%)
Amikacin non-susceptibility	38 (2%)	4 (1%)	4 (1%)	4 (1%)	10 (3%)	5 (2%)	11 (4%)