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RESEARCH ARTICLE

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Protective effect of SARS-CoV-2 preventive measures against ESKAPE and *Escherichia coli* infections

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Abstract

Background/Objectives: We investigated whether behavioral precautions adopted during Coronavirus disease (COVID-19) pandemic also influenced the spreading and multidrug resistance (MDR) of ESKAPEEc (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* [AB], *Pseudomonas aeruginosa*, *Enterobacter* spp and *Escherichia Coli*, [EC]) among Intensive Care Unit (ICU) patients.

Subjects/Methods: We performed a single-center retrospective study in adult patients admitted to our COVID-19-free surgical ICU. Only patients staying in ICU for more than 48 hours were included. The ESKAPEEc infections recorded during the COVID-19 period (June 1, 2020 - February 28, 2021) and in the corresponding pre-pandemic period (June 1, 2019 - February 28, 2020) were compared. An interrupted time series analysis was performed to rule out possible confounders.

Results: Overall, 173 patients in the COVID-19 period and 132 in the pre-COVID-19 period were investigated. The ESKAPEEc infections were documented in 23 (13.3%) and 35 (26.5%) patients in the pandemic and the pre-pandemic periods, respectively ($p = 0.005$). Demographics, diagnosis, comorbidities, type of surgery, Simplified Acute Physiology Score II, length of mechanical ventilation, hospital and ICU length of stay, ICU death rate, and 28-day hospital mortality were similar in the two groups. In comparison with the pre-pandemic period, no AB was recorded during COVID-19 period, ($p = 0.017$), while extended-spectrum beta-lactamase-producing EC infections significantly decreased ($p = 0.017$). Overall, the ESKAPEEc isolates during pandemic less frequently exhibited multidrug-resistant ($p = 0.014$).

Conclusions: These findings suggest that a robust adherence to hygiene measures together with human contact restrictions in a COVID-19 free ICU might also restrain the transmission of ESKAPEEc pathogens.

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KEYWORDS
ESKAPEEc, hand hygiene, health care-associated infections, multidrug resistance, personal protective equipment, SARS-CoV-2

1 | INTRODUCTION

The ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) are endowed with resistance mechanisms allowing them to 'escape' the biocidal effect of antibiotics.[1] The ESKAPE pathogens and *Escherichia coli* (hereafter referred to as ESKAPEEc) are the emerging cause of healthcare-associated infections, especially in critically ill patients admitted to intensive-care units (ICU).[2] The antimicrobial resistance (AMR) of ESKAPEEc negatively affects clinical outcomes, such as length of stay (LoS), morbidity, and mortality, and increases overall costs. Nowadays, the ESKAPEEc AMR represents a threat to the public health. Few highly expensive antimicrobial agents are available, and there is no effective therapy for some multidrug-resistant (MDR) pathogens.[3]

The World Health Organization (WHO) has listed the ESKAPEEc among bacteria needing urgent investigation to develop and test new antibiotics (available at https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf). Notably, carbapenem-resistant *A. baumannii* and *P. aeruginosa*, carbapenem-resistant and/or third generation cephalosporin-resistant *Enterobacterales* are in the critical priority group. Furthermore, vancomycin-resistant *E. faecium* and methicillin-resistant and vancomycin-intermediate/ -resistant *S. aureus* are in the high-priority sub-group. In this scenario, prevention and program for infection control are pivotal.

The prevalence of ESKAPEEc infections among surgical ICU patients has not been fully investigated. During the coronavirus disease (COVID-19) pandemic, protective measures usually adopted in ICU have been further enhanced to prevent the spreading of SARS-CoV-2. For example, the use of personal protective equipment (PPE) has become mandatory even in COVID-19–free departments. More meticulous hand hygiene with alcoholic solutions has been adopted before and after patient contact, and the regular use of vinyl and nitrile gloves and water-repellent gowns has been enforced. Moreover, it has been implemented the use of headgears, face surgical masks, and filtering face piece (FFP) masks.[4] Finally, the contacts of both staff and patients within the department and into the hospital have been highly restricted.[4]

This study retrospectively investigates whether all behavioral precautions adopted during the COVID-19 period might have modified the spreading of ESKAPEEc infections, too. Therefore, we revised the data on ESKAPEEc infections recorded during the pandemic at our COVID-19-free surgical ICU in comparison with those relative to the same period before the pandemic.

2 | PATIENTS AND METHODS

We retrospectively evaluated the records of patients admitted to the surgical ICU of our academic hospital between 1 June 2020 and 28 February 2021 (COVID-19 period) and between 1 June 2019 and 28 February 2020 (pre–COVID-19 period). The study inclusion criteria were LoS in ICU longer than 48 h and absence of ESKAPEEc infection and gut colonization at ICU admission. All patients included in the study tested negative for SARS-CoV-2 via nasopharyngeal swab real-time polymerase chain reaction assay.

The primary study outcome was the rate of patients with ESKAPEEc infections. Secondarily, we evaluated the proportion of multidrug-resistant (MDR) ESKAPEEc isolates, the duration of mechanical ventilation, ICU LoS, ICU mortality, and the day-28 mortality (i.e. mortality in ICU or after ICU discharge). The study was approved by the Institutional Review Board, namely Ethics Committee, of Fondazione Policlinico Agostino Gemelli, IRCCS, Rome, Italy (approval # 3146).

The data recorded are listed in Table 1. Site of infections were defined as previously reported.[5,6] Surveillance rectal swabs (RS, Copan) for detection of carbapenemase-producing *Enterobacterales* (CPE) were performed at ICU admission using the bioMérieux chromID Carba (Marcy l'Étoile). Species identification was performed using the MALDI Biotyper® system (Bruker Daltonik), and antimicrobial susceptibility testing was performed using Vitek®2 (bioMérieux, Marcy-l'Étoile). Antibiotic resistance phenotypes were confirmed by retesting bacterial isolates with commercial broth-microdilution antimicrobial susceptibility testing panels (Merlin DiagnostiKa GmbH). Susceptibility findings were interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (https://eucast.org.). Isolates were defined as MDR,

TABLE 1 Demographics and clinical characteristics of patients with documented infection by ESKAPEEc pathogens

PARAMETER	COVID−19 *N* (%)	Pre-COVID−19 *N* (%)	57	*p*-value
Age, median (IQR), y	70 (55–73)	68 (53–79)		0.726
Male	17 (73.9)	25 (71.4)		1.000
Body mass index, median (IQR), Kg/m^2	25.4 (23.7–29.4)	26.6 (21.9–29.4)		0.930
Indication for surgical ICU admission				
Major abdominal surgery including liver/kidney transplant	18 (78.2)	27 (77.1)		0.431
Vascular surgery	3 (13.0)	3 (8.6)		
Trauma surgery	2 (8.7)	1 (2.3)		
Cerebral Hemorrhage	0.0	3 (8.6)		
Orthopedic surgery	0.0	1 (2.9)		
Most relevant comorbidities				
Chronic lung disease	8 (34.8)	11 (31.4)		1.000
Type 2 diabetes	4 (17.4)	7 (20.0)		1.000
Hypertension	15 (56.5)	16 (45.7)		0.591
Ischemic heart disease	5 (26.7)	8 (22.9)		1.000
Cancer	11 (47.8)	14 (40.0)		0.596
ASA class				
2	4 (17.4)	4 (11.4)		0.281
3	5 (21.7)	17 (48.6)		
4	12 (52.2)	9 (25.7)		
5	2 (8.7)	5 (14.3)		
Surgical complexity				
3	5 (21.7)	7 (20.0)		0.353
4	18 (78.3)	25 (71.4)		
Hospital LoS pre-ICU, median (IQR), d	2 (0–8)	2 (1–11)		0.711
SAPS II, median (IQR)	39 (33–48)	46 (36–58)		0.143
Elective surgery	6 (26.1)	11 (31.4)		0.772
Surgical time (min)	191 (140–383)	175 (70–430)		0.599
Mechanical ventilation (hours)	96 (24–214)	73 (36–200)		0.886
Tracheotomy	6 (26.0)	5 (14.3)		0.315
ICU LoS, median (IQR), d	11 (7–18)	8 (4–16)		0.225
ICU death	4 (17.4)	13 (37.1)		0.144
28 days hospital mortality	6 (26.1)	17 (48.6)		0.106

Abbreviations: ASA, American Society of Anesthesiologists; ICU, intensive-care unit; IQR, Interquartile range; LoS, length of stay; SAPS II, Simplified Acute Physiology Score II.

extensively drug-resistant (XDR) and pandrug-resistant (PDR) according to definitions of Magiorakas et al.[7]

2.1 | Interventions

Usual care before and during the COVID-19 pandemic included hand hygiene with 70% denatured ethyl alcohol provided by dispensers placed at each patients' room entrance. Cleaning and disinfection of patients' room surfaces and furnishings was carried out with sodium hypochlorite 350 ppm for floor and walls, 1050 ppm spray for high-touch surfaces, and 5000 ppm for toilets. During the pandemic the use of PPE was further enforced, together with a stronger adherence to protocols for handwashing. All operators wore filtering face FFP-2 covered by the surgical mask, and the frequency and duration of cleaning of patient rooms and inanimate surfaces were intensified. Moreover, during the COVID-19 period, the access to ICU was restricted to healthcare professionals only, and visits of patients' relatives were not allowed. During the investigated periods, in ICU operated the same medical and nurse teams. Moreover, the policy for the antibiotic use remained unchanged throughout the entire study. Reporting of the study conforms to broad EQUATOR guidelines.[8]

2.2 | Statistical analysis

Continuous variables were expressed as median and interquartile range (IQR) and categorical variables as number (%). Univariate analysis was performed by Mann-Whitney *U* test or Fisher's exact test, as appropriate. An interrupted time series analysis was performed. Several hospital-related, ICU-related, and patient-related variables were investigated to exclude their effect on the reduction in MDR during the COVID-19 pandemic. All tests were two-sided, and a *p*-value <0.05 was considered statistically significant. Analyses were performed using the NCSS 10 v 10.0.19 and SPSS 25.0. The data are available from the corresponding author upon reasonable request.

3 | RESULTS

An initial total population of 2650 patients admitted at ICU after elective or emergency surgical procedures was evaluated. Among them, we excluded 2321 patients for LoS in ICU ≤48 h and 24 patients for positive ESKAPEEc tests at ICU admission (Figure S1). A total of 305 patients (173 in the COVID-19 period and 132 in the pre-pandemic period) were included in the analyses. ESKAPEEc infections were documented in 23 (13.3%) patients in the COVID-19 period in comparison to 35 (26.5%) in the pre–COVID-19 period ($p = 0.005$). Demographics, diagnosis, comorbidities, American Society of Anesthesiologists (ASA) score, type and duration of surgery, Simplified Acute Physiology (SAPS) II score, duration of mechanical ventilation, rate of tracheostomy, LoS before ICU admission, LoS in ICU, ICU death rate, and 28 days hospital mortality did not differ between these groups (Table 1).

Table 2 shows the identified pathogens and their antibiotic resistance. In total, ESKAPEEc were identified in 31 cultures in the COVID-19 period and 60 in the pre–COVID-19 period (Table 2). During the pandemic, no infection due to *A. baumannii* was recorded ($p = 0.017$), and extended-spectrum beta-lactamase (ESBL)–producing *E. coli* infections significantly decreased ($p = 0.017$). These results were confirmed using an interrupted time series analysis (Table S1). In particular, ASA classes, comorbidities, pre-ICU LoS in hospital, ICU. LoS, the number of ICU admissions, or hospital admissions did not affect the trend for the reduced infections rate of XDR *A. baumannii* and ESBL-producing *E.coli*.

Overall, nine out of the 31 isolates in the COVID-19 period and 35 out of 60 isolates in the pre-COVID-19 period exhibited MDR (58.3% vs. 29.0%, $p = 0.014$). MDR ESKAPEEc positive rectal swabs were recorded in one patient during the COVID-19 period (KPC-producing *K. pneumoniae*) and in four patients during the pre–COVID-19 period (*A. baumannii* in 2 patients and KPC-producing *K. pneumoniae* in 2 patients). The distribution of infection sites did not differ between the study groups.

4 | DISCUSSION

This study shows that during the pandemic the ESKAPEEc infections in our surgical COVID-free ICU have been decreasing in comparison with the same pre-pandemic period. Overall, during the pandemic, the proportion of MDR pathogens among all ESKAPEEc isolates was significantly lower than before. In particular, ESBL-producing *E. coli* infections were significantly reduced and *A. baumannii* infections disappeared. Notably, this trend was not affected by other variables related to patient comorbidities, surgery complexity, or rate of hospital admissions.

The ESKAPEEc pathogens are usually isolated on surfaces with which patients or ICU operators came in contact, such as bed rails, high-touch surfaces, and room equipment (stethoscopes, ultrasound scanners, medical records).[9] Hence, it is plausible that our findings may be ascribed to those measures implemented to counteract the spread of SARS-CoV-2. In fact, during the study period, there was a strong adherence to WHO guidelines stressing the importance of using PPE for the prevention of SARS-CoV-2 diffusion among healthcare operators.[4] From 8 March 2020, starting the lockdown in Italy, most efforts in our hospital focused on using masks 24 h a day, wearing at the same time a FFP2 filter mask covered by a surgical mask and water-repellent gowns. Moreover, the meticulous cleaning of hands and environments was intensified, and paths rigorously dedicated to SARS-CoV-2 patients were created. In addition, there were restrictions on the movement of staff and patients within the hospital, whilst relative visits were absolutely prevented. Notably, many of preventive measures had been already implemented before pandemic. Nevertheless, during pandemic, the hand hygiene before and after patient contact become more scrupulous, and it was associated to the continual use of gloves.

Contaminated surfaces are potential reservoirs of pathogens, and several studies show that inanimate surfaces and equipment contamination contribute significantly to the transmission of pathogens in healthcare facilities, including ICUs.[9,10] It has been reported that Gram-positive and Gram-negative bacteria are able to

TABLE 2 Epidemiology and infection site of ESKAPEEc pathogens

PATHOGEN, RESISTANCE, and INFECTION SITE	COVID−19 *N* (%)	Pre-COVID−19 *N* (%)	Total	*p*-value
Microorganisms				
Enterococcus faecium	5 (21.7)	8 (22.9)	13	1.000
VRE	0	5 (14.3)	5	0.145
Staphylococcus aureus	4 (17.4)	9 (25.6)	13	0.533
MRSA	3 (13.0)	6 (17.1)	9	1.000
Klebsiella pneumoniae	6 (26.1)	8 (22.9)	14	1.000
Carbapenemase-producing	3 (60.0)	5 (62.5)	8	1.000
Acinetobacter baumannii	0	8 (22.9)	8	0.017
XDR	0	8 (22.9)	8	0.017
Pseudomonas aeruginosa	2 (8.7)	5 (14.3)	7	0.691
MDR	2 (8.7)	2 (5.7)	4	1.000
Enterobacter cloacae	8 (34.8)	5 (14.3)	13	0.106
MDR	1 (4.3)	1 (2.9)	2	1.000
Escherichia coli	6 (26.1)	17 (48.6)	23	0.106
ESBL-producing	0	8 (22.9)	8	0.017
Infection Site				
Ventilator-acquired pneumonia	11 (47.8)	16 (45.7)	27	1.000
Hospital-acquired pneumonia	2 (8.6)	2 (5.7)	4	1000
Skin and aoft-tissue infection	1 (4.3)	6 (17.0)	7	0.225
Intra-abdominal infection	8 (34.8)	7 (20.0)	15	0.234
Urinary tract infection	1 (4.3)	4 (11.4)	5	0.638
Bloodstream infection	4 (17.4)	7 (20.0)	11	1.000
Rectal swab	1(4.3)	4 (11.4)	5	0.638

Abbreviations: ESBL, Extended spectrum beta-lactamase; MDR, Multidrug-resistant; MRSA, Methicillin-resistant *Staphylococcus aureus*; VRE, Vancomycin-resistant enterococcus; XDR, Extensively drug-resistant.

survive for months on inanimate dry surfaces, with longer persistence in humid conditions and low temperatures. Nevertheless, it has been also demonstrated that surfaces' contamination with epidemiologically important pathogens more frequently depends on the inadequate cleaning and disinfection rather than a faulty product or procedure.[11] Indeed, according to our finding, the use of PPE, together with more scrupulous surface cleaning and hand hygiene, may have limited the dissemination of ESKAPEEc into the surrounding environment, thus preventing many patients from their exposure.

Our results are in agreement with those of a recent study by Losurdo et al. showing that the containment measures implemented during the COVID-19 emergency, such as the mandatory use of the surgical mask and the absence of visitors, were associated with a reduction in surgical site infections in a COVID-19 free surgical ward.[12] In contrast, other studies in COVID-19 patients reported an increased incidence of secondary infections by pathogenic bacteria as compared to patients without SARS-CoV-2 infection. In a recent study conducted in our hospital, De Pascale et al. observed that patients with SARS-CoV-2 infection were more likely to develop ventilator-acquired pneumonia due to methicillin-resistant *S. aureus* than patients admitted to the same ICU before the pandemic.[13] Fan et al. investigated that lung tissue microbiota in 20 patients deceased from COVID-19 who were mechanically ventilated.[14] The authors found that bacterial community was enriched with Acinetobacter species, including carbapenem-resistant *A. baumannii*.[14] Moreover, Bardi et al. reported a high incidence of nosocomial, bacterial, and/or fungal infections among COVID-19 patients admitted to the ICU, and in particular of infections due to MDR pathogens.[15] These findings highlight that in these settings factors specifically related to SARS-CoV2 infected patients may play a role. Possible explanations include that SARS-CoV-2 patients are prone to develop an impaired lung immune response, or that SARS-CoV-2 infection alters the dynamics of intermicrobial interactions, leading to increased species growth pathogenic. On the other hand, COVID-19 patients exhibit predisposing factors such as concomitant immunomodulatory therapies or early administration of broad-spectrum antimicrobials, with consequent development of bacterial resistance. Finally, in a context of critical COVID-19 patients, also the reduced attention to control measures for MDR pathogens by the overwhelmed staff can be implicated.[13]

The present study suffers from some limitations. First, it is a monocentric experience including a relatively small number of patients in an ICU specifically dedicated to surgical patients. This prevents the translation of our findings to medical ICU settings or non-ICU settings. Finally, due to the retrospective design, the molecular typing of isolated strains was not performed, hampering to trace the source of infections.

In conclusion, this study suggests that a stronger adherence to hygiene measures, the continual use of PPE in a COVID-19 free pathway, together with high social distancing, may restrain the transmission of ESKAPEEc pathogens in the surgical ICU.

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AUTHOR CONTRIBUTIONS

Concept and design: Rita Gaspari, Luciana Teofili, Alfonso W Avolio. Data recruitment: Giorgia Spinazzola, GianMarco Maresca, Barbara Fiori, Teresa Spanu. Statistical analysis: Luciana Teofili, Alfonso W Avolio, Nicola Nicolotti. Supervision: Rita Gaspari, Gennaro De Pascale, Massimo Antonelli. All authors reviewed and approved the manuscript. Rita Gaspari had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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