Prevalence of extended-spectrum β-lactamase and carbapenem-resistant *Klebsiella pneumoniae* in clinical samples

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ABSTRACT

الأهداف: لتقييم انتشار هذه السلالات المقاومة في العزلات الكلية لـ K. pneumoniae في المستشفيات. يمثل امتداد نطاق β-lactamase المنتجة لـ(ESBL) و *Klebsiella pneumoniae* المعروف بـ(*K. pneumoniae*) المقاومة للكاربابينيم (CRKP) خطرًا كبيرًا على المرضى ونظام الرعاية الصحية.

المنهجية: أجريت هذه الدراسة بأثر رجعي من نوفمبر 2020 إلى نوفمبر 2021. تم إجراء تحديد الحساسية واختبار الحساسية للمضادات الحيوية باستخدام طرق معملية قياسية وفقًا لمعايير EUCAST. تم إجراء الكشف عن إنتاج ESBL و carbapenemase باستخدام طرق النمط الظاهري مثل الاختبار الإلكتروني، واختبار القرص المدمج مع مثبطات مختلفة (ROSCO Diagnostica A/S)، ووسط الكروموجينيك للكشف عن عزلات ESBL/carbapenemase المنتجة للبكتيريا المعوية (CPE)، ونظام VITEK 2 المضغوط (BioMerieux).

النتائج: تم الكشف عن 944 عزلة من *K. pneumoniae* في عينات سريرية مختلفة. من بين هؤلاء، تم الكشف عن السلالات المنتجة لـ ESBL في 944/349 (37٪)، بينما تم الكشف عن السلالات المقاومة للكاربابينيم في 944/188 (20٪) من العزلات. العزلات المتبقية (944/407 [43٪]) تنتمي إلى النوع البري. كانت عزلات ESBL الأكثر شيوعًا في مسحات الجروح (138 [39.5٪])، بينما عزلات CRKP في عينات الفرز (110 [58.5٪]). تم الكشف عن غالبية عزلات ESBL في أقسام الجراحة (105 [30.1٪])، بينما عزلات CRKP في أقسام وحدة العناية المركزة للبالغين (79 [42.٪]).

الخلاصة: أظهرت نتائجنا زيادة وتيرة سلالات CRKP. يمثل هذا مشكلة مهمة من حيث الوقاية من العدوى والسيطرة عليها في المستشفيات.

Objectives: To assess the prevalence of these resistant strains in the overall isolates of *Klebsiella pneumoniae* (*K. pneumoniae*) in hospital settings.

Methods: This retrospective study was conducted from November 2020 to November 2021. The identification and antibiotic susceptibility testing were performed using standard laboratory methods according to the EUCAST standards. The detection of ESBL and carbapenemase production was performed using phenotypic methods such as E-test, combined-disk test with various inhibitors (ROSCO Diagnostica A/S), chromogenic medium for the detection of ESBL/carbapenemase-producing Enterobacteriaceae (CPE) isolates, and the VITEK 2 Compact system (BioMerieux).

Results: 944 isolates of *K. pneumoniae* were detected in various clinical specimens. Among these, ESBL-producing strains were detected in 349/944 (37%), whereas carbapenem- resistant strains in 188/944 (20%) of the isolates. The remaining isolates (407/944 [43%]) belonged to the wild type. ESBL isolates were the most common in wound swabs (138 [39.5%]), whereas CRKP isolates in screening samples (110 [58.5%]). The majority of ESBL isolates were detected in surgical departments (105 [30.1%]), whereas CRKP isolates in adult intensive care unit departments (79 [42.%]).

Conclusion: Our results show an increasing frequency of CRKP strains. This presents a significant issue in terms of infection prevention and control in hospital settings.

Keywords: *Klebsiella pneumoniae*, ESBL, carbapenem resistance, infection prevention and control

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Klebsiella pneumoniae (*K. pneumoniae*) is a gram-negative, encapsulated bacterium that typically inhabits the human intestine but can also be encountered in a variety of environmental niches (soil, water, and so on).[1] This pathogen is associated with severe infections in hospitalized patients, primarily those with severe underlying diseases.

The increasing prevalence of infections caused by multidrug-resistant (MDR) strains is becoming a significant clinical and public health problem. These strains can be difficult to treat, especially in the elderly, immunosuppressed individuals, or infants with immature immunity.[2]

Transmission can occur through the patient's gastrointestinal tract and hands of hospital personnel. The presence of invasive devices, respiratory support equipment, the use of urinary catheters, and administration of antibiotics are risk factors for *Klebsiella species* nosocomial infections.

Klebsiella pneumoniae is a notorious "collector" of MDR plasmids, despite the fact that it is not intrinsically resistant to antibiotics, as it generates only a moderate quantity of chromosomal penicillinases.[3] It has various mechanisms that have led to the development of MDR strains. The production of the broad-spectrum β-lactamase SHV-2 causes ampicillin-intrinsic resistance. Some strains produce β-lactamases with very low carbapenemase activity; however, when combined with permeability deformities, they can exert a greater effect on decreased carbapenem susceptibility. Additionally, *K. pneumoniae* produces enzymes that hydrolyze carbapenems and induce resistance in the absence of permeability defects.[4]

Extensive use of broad-spectrum antibiotics in hospitalized patients has led to an increase in *Klebsiella species* carriage and the subsequent emergence of MDR strains that produce extended-spectrum beta-lactamase (ESBL) and carbapenemase-producing *K. pneumoniae* (KPC).[5]

Bacterial resistance to extended-spectrum penicillin, cephalosporins, and aztreonam is conferred by ESBLs, which are inhibited by β-lactamase inhibitors such as clavulanic acid but not cephamycins or carbapenems. In contrast, carbapenemases are enzymes that degrade penicillin, cephalosporine, aztreonam, and broad-spectrum β-lactams such as carbapenems.[5]

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Carbapenem-resistant *K. pneumoniae* (CRKP) and ESBL-producing *K. pneumoniae* represent serious threats to human health and pose a serious challenge to clinicians. It is often resistant to several antimicrobial agents usually used to treat infections caused by gram-negative bacteria. Consequently, severe infections caused by CRKP are associated with high morbidity and mortality rates, and carbapenems are the β-lactams of choice for the treatment of infections caused by ESBL-producing *K. pneumoniae*.[5,6]

Carbapenemase genes are mainly found on plasmids and are shared by Enterobacteriaceae, including *K. pneumoniae*, as well as other gram-negative bacteria.[4]

The aim of this study was to determine the prevalence and distribution of ESBL producing *K. pneumoniae* and CRKP in clinical samples from the Clinical Center University of Sarajevo, Bosnia and Herzegovina.

Methods. From November 2020 to November 2021, researchers at the Department of Clinical Microbiology, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina carried out this retrospective study. Isolates have been detected from various clinical specimens, including wound swabs, urine, blood culture samples, specimens of the lower respiratory tract, and screening samples (nose, axilla, inguinal, and anal swabs), and from patients admitted to various clinics at the Clinical Center University of Sarajevo.

All specimens were cultured onto standard (blood agar) and differential culture media, such as chrom agar CPSE (CHROMID CPS Elite Agar, BioMerieux, France), and incubated at 37°C for 24 hours (h). Screened samples from the ICU were cultured on chrom agar for the detection of ESBL (CHROMagar, England) and Carbapenem-resistant *Enterobacteriaceae* (CRE) (mSuperCARBA, CHROMAgar, England). Further examination of the cultures was carried out using standard microbiological methods based on the characteristic colony appearance. *K. pneumoniae* isolates were identified by morphological, cultural, and biochemical characterisation using the VITEK 2 Compact system (BioMerieux, Marcy l'E´ toile, France).[7]

The antibiotic susceptibility was evaluated on Mueller-Hinton agar using the Kirby-Bauer disk diffusion technique with the EUCAST standard as follows: ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), piperacillin/tazobactam (30/6 μg), cefazolin (30 μg), cefuroxime (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), cefepime (30 μg), amikacin (30 μg), gentamicin (30 μg), tobramycin (10 μg),

imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), and trimethoprim–sulfamethoxazole (1.25/23.75 µg). The outcomes were interpreted using EUCAST breakpoints.

The isolates were screened for possible ESBL production using ceftazidime (30 µg) and cefotaxime (30 µg). Isolates that showed reduced susceptibility were suspected to be ESBL-producing strains and confirmed for ESBL production using combination disk test (CDT) with ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10 µg) discs and cefotaxime (30 µg) and cefotaxime/clavulanic acid discs (30/10 µg). The inhibition zone around the cephalosporin disk or tablet combined with clavulanic acid was compared to the zone around the disk or tablet with cephalosporin alone. If the inhibition zone diameter was 5 mm greater with clavulanic acid than without, the test was judged positive.[7] The isolates were tested for carbapenemase production using a combined-disk test containing meropenem and inhibitors (ROSCO Diagnostica A/S, Denmark).[8] Boronic acid inhibits class A carbapenemases, whereas dipicolinic acid and ethylenediaminetetraacetic acid (EDTA) inhibit class B carbapenemases. OXA-48 is inhibited by temocillin, with minimum inhibitory concentration (MIC) >128 mg/L as a phenotypic marker. Cloxacillin, which inhibits AmpC β-lactamases, was added to the assay to distinguish between AmpC hyperproduction, porin loss, and carbapenemase production.[7]

Statistical analysis. Statistical procedures used include Chi-square test which is comparison of observed and expected frequencies as part of the test. *P*-value for Chi-square test is significant with <0.05.

Results. A total of 944 *K. pneumoniae* isolates were retrieved from clinical samples, including primary sterile body fluids (blood and urine samples), wound swabs, lower respiratory tract samples, and screening ICU samples. Carbapenem-resistant *K. pneumoniae* was detected in 20% (n=188), ESBL in 37% (n=349), and wild type in 43% (n=407) of the isolates (**Figure 1**).

Among the 349 ESBL isolates, wound swabs accounted for 138 (39.5%), urine samples for 121 (34.7%), screening samples for 49 (14%), blood samples for 30 (8.6%), and lower respiratory tract specimens for 11 (3.6%) of the isolates (**Table 1, Figure 2**).

The majority of ESBL isolates were detected in surgical departments (105 [30.1%]), followed by internal departments (79 [22.6%]), adult ICU department (68 [19.5%]), paediatric ICU (37 [10.6%]), other paediatric departments (29 [8.3%]), and infectious disease clinics (31 [8.9%]) of the isolates (**Table 1**).

There was a statistically significant difference in distribution of ESBL strains in relation to sample and clinic, with ESBL strains in wound swabs and screening swabs mostly distributed in the surgical departments, and urine samples in the adult internal unit.

Among the 188 CRKP isolates, screening samples accounted for 110 (58.5%), wound swabs for 45 (23.93%), blood samples for 12 (6.4%), urine samples for 11 (5.9%), and lower respiratory tract specimens for 10 (5.3%) of the isolates (**Table 2, Figure 2**).

Among the 188 CRKP isolates, 79 (42.%) were detected in adult ICU departments, 69 (36.7%) in surgical departments, and 19 (10.1%) in infectious disease clinics. Internal departments accounted for 15 (8%) isolates. Paediatric ICU and other paediatric departments accounted 3 (1.6%) isolates (**Table 2**).

There was a statistically significant difference in the representation of CRKP strains in relation to sample and clinic, with CRKP strains in screening swabs most

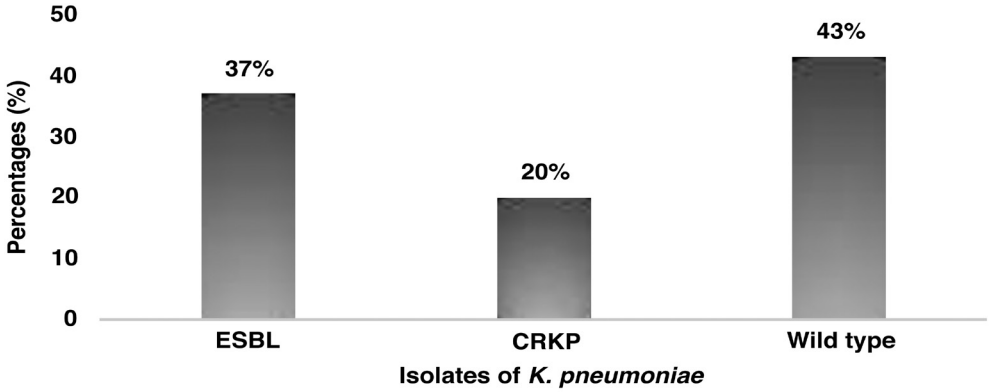

Figure 1 - Distribution of extended-spectrum beta-lactamase, Carbapenem-resistant *Klebsiella pneumonia (K. pneumoniae)*, and wild type of *K. pneumoniae* in clinical samples.

Table 1 - Distribution of *Klebsiella pneumoniae* ESBL isolates according to the specimen type and clinics.

K. pneumoniae ESBL	LRT specimens	Screening swabs	Wound swabs	Blood	Urine	Total
Adult ICU	5 (1.4)	16 (4.6)	28 (8.0)	4 (1.1)	15 (4.9)	68 (19.5)
Pediatric ICU	2 (0.6)	3 (0.9)	8 (2.3)	8 (2.3)	16 (4.6)	37 (10.6)
Pediatric internal unit	2 (0.6)	1 (0.3)	1 (0.3)	3 (0.9)	22 (6.3)	29 (8.3)
Infectious disease clinic	0 (0)	0 (0)	15 (4.3)	3 (0.9)	13 (3.7)	31 (8.9)
Surgical department	1 (0.3)	24 (6.9)	71 (20.3)	4 (1.1)	5 (1.43)	105 (30.1)
Adult internal unit	1 (0.3)	5 (1.4)	15 (4.3)	8 (2.3)	50 (14.3)	79 (22.6)
Total	11 (3.1)	49 (14.0)	138 (39.5)	30 (8.6)	121 (34.7)	349 (100)
P-value	0.297	<0.001	<0.001	0.347	<0.001	<0.001

Values are presented as number and percentages (%). ICU: intensive care unit, LRT: lower respiratorytract, ESBL: extended-spectrum beta-lactamase.

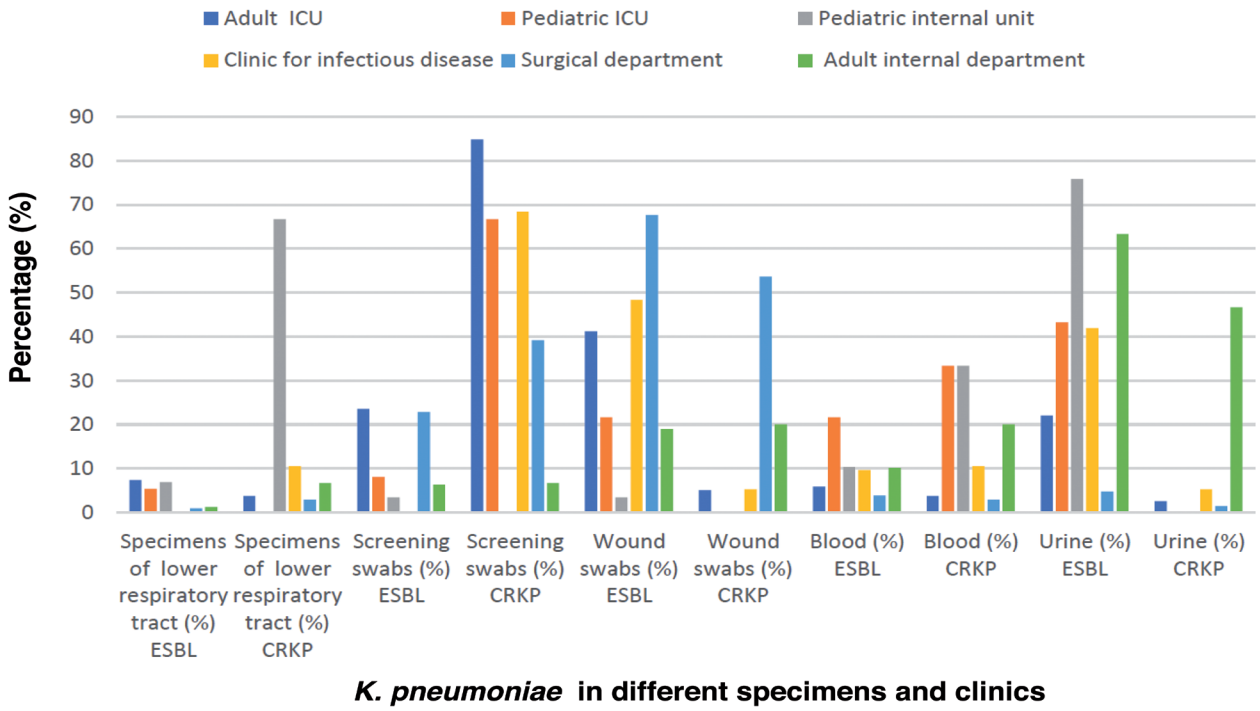

Figure 2 - The overall distribution of isolates according to the specimens and hospital departments. *Klebsiella pneumoniae: K. pneumoniae*

Table 2 - Distribution of CRKP isolates according to the specimen type and clinics.

CRKP	LRT specimens	Screening swabs	Wound swabs	Blood	Urine	Total
Adult ICU	3 (1.6)	67 (35.6)	4 (2.1)	3 (1.60)	2 (1.1)	79 (42.0)
Pediatric ICU	0 (0)	2 (1.1)	0 (0)	1 (0.5)	0 (0)	3 (1.6)
Pediatric internal unit	2 (1.1)	0 (0)	0 (0)	1 (0.5)	0 (0)	3 (1.6)
Infectious disease clinic	2 (1.1)	13 (6.9)	1 (0.5)	2 (1.1)	1 (0.5)	19 (10.1)
Surgical department	2 (1.1)	27 (14.4)	37 (19.7)	2 (1.1)	1 (0.5)	69 (36.7)
Adult internal unit	1 (0.6)	1 (0.5)	3 (1.6)	3 (1.6)	7 (3.7)	15 (8.0)
Total	10 (5.3)	110 (58.5)	46 (23.9)	12 (6.4)	11 (5.8)	188 (100)
P-value	0.910	<0.001	<0.001	0.849	0.029	<0.001

Values are presented as number and percentages (%). CRKP: carbapenem-resistant *Klebsiella pneumoniae*, LRT: lower respiratorytract, ESBL: extended-spectrum beta-lactamase.

represented in the adult ICU departments, and wound swabs in surgical department.

Discussion. Multidrug-resistant bacteria are increasingly being isolated from clinical samples, especially from patients who are on long-term antibiotic therapy and hospitalization and often undergo invasive procedures.[9,10] Among them, MDR *K. pneumoniae* is the predominant cause of nosocomial infections and may harbour a wide range of antibiotic-resistance genes, including ESBLs and/or carbapenemases.[11]

Higher rates of resistance to third-generation cephalosporins and carbapenems in *K. pneumoniae* are worrisome; they suggest the spread of resistant clones in healthcare settings and indicate that treatment options for patients with infections caused by this pathogen are severely limited in many countries. These strains are primarily transmitted from patient to patient in a hospital setting, either directly by healthcare employees' hands or indirectly through the environment; however, the gastrointestinal tract of colonized patients is the primary source of hospital outbreaks.[12]

ESBL- and CRKP-producing *K. pneumoniae* are increasingly responsible for invasive infections. Resistance to carbapenems is usually accompanied by resistance to several important antimicrobial groups, resulting in a severely limited spectrum of treatment options for serious infections and high mortality rates.[13]

Due to previous exposure to multiple antibiotics, limited treatment options frequently pose additional treatment challenges. Therefore, CRKP and ESBL-producing *K. pneumoniae* are prioritized pathogens for the development of new antibiotics by the WHO.[14]

Furthermore, the Centers for Disease Control and Prevention have assigned carbapenem resistant Enterobacterales the highest threat level and declared that they require immediate public health attention.[15]

In this study, we determined the prevalence of ESBL-producing *K. pneumoniae* and CRKP in clinical samples by observing 944 isolates of *K. pneumoniae* for one year.

Our results revealed that of the total number of K. pneumoniae isolates, MDR accounted for 57% (ESBL-producing strains: 37%, carbapenem-resistant strains: 20%).

The prevalence of ESBL is clearly increasing and varies across different geographical regions, with low rates of 3-8% reported in Sweden, Japan, and Singapore, compared with much higher prevalence rates documented in studies from Portugal (34%), Italy (37%), Latin American countries (30-60%), and Turkey (58%).[16]

According to data published by the European Antimicrobial Resistance Surveillance Network (EARS-Net) and Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) between 2016 and 2020, there was a significant increasing trend in ESBL in countries of the European Union and European Economic Area (EU/EEA; excluding the United Kingdom). In 2020, 18 (44%) countries of 44, particularly in the southern and eastern parts of the region, reported ESBL percentages of ≥50%.[12]

The prevalence of ESBL-producing *K. pneumoniae* in a study carried out by Pathak et al[17] was 90.9%. According to data published in 2017, the percentage of ESBL-producing *K. pneumoniae* isolates in our hospital was 37.8%, and compared with the latest study, there were no significant changes in their prevalence.[18]

However, the first outbreak of CRKP in our hospital occurred in September 2017, with 6 isolates. The latest results of this study with 188 CRKP isolates over a 1-year period indicates a significant increase in carbapenem resistance.[7] Possible causes include the horizontal spread and excessive use of antibiotics caused by the emergence of the new coronavirus, which necessitated modifications to prevent the spread of SARS-CoV-2.

Karruli et al[19] pointed out that during COVID-19, MDR infection was a common complication in ICU patients, and Tiri et al[20] reported an increase from 6.7% to 50% in CPE in ICUs between 2019 and April 2020.

Other investigations conducted in New York and Italy medical centers also found an increase in the detection of CPE in COVID-19 patients. The extensive use of broad-spectrum antibiotics among COVID-19 patients is an essential aspect of bacterial spread.[21-23]

Studies showed that 72-74% of COVID-19 patients received broad-spectrum antibiotics, whereas only 8–17.6% had bacterial or fungal co-infection identified.[24-26]

These results raise concerns about the overuse of antibiotic therapy and its consequent contribution to the development of bacterial resistance.[27]

Antimicrobial resistance monitoring data in Europe 2022–2020 showed the highest percentages of carbapenem-resistant Enterobacteriaceae in southern and southeastern Europe, similar to EURGen-Net data. Carbapenem resistance percentages are generally low in the northern and western parts of the WHO European region; 16 (39%) of 41 countries/areas reported antimicrobial resistance (AMR) percentages <1%. Twelve (30%) countries reported percentages ≥25%, six of which (15% of 41 countries/areas) reported AMR percentages ≥50%.

As a result, multiple EU/EEA countries have prepared and implemented carbapenem-resistant *Enterobacteriaceae* recommendations and guidance papers, demonstrating a trend toward nationally coordinated responses to this public health problem.

Carbapenem-resistant *K. pneumoniae* may be resistant to carbapenems by a variety of mechanisms, the most common of which being the development of carbapenemase enzymes. Because certain carbapenemases do not produce a completely carbapenem-resistant phenotype, it is not feasible to estimate the total prevalence and spread of carbapenemase-producing Enterobacterales. Because of its inability to hydrolyze carbapenems, OXA-48 is particularly difficult to detect in the laboratory.[12]

In our study, ESBL isolates were mostly isolated from wounds (39.5%), urine specimens (34.7%), and mainly in the surgical units (30.1%). Wound infection is most often connected with an extended hospital stay, which increases the chance of contracting various resistant organisms through medical equipment and the hospital environment.

Paterson and Bonomo reported that most patients with ESBL producers may colonise the gastrointestinal tract.[28] Unlike ESBL isolates, CRKP was mostly isolated from screening swabs (58.5%) and mainly in the adult ICU (42%).

Yan et al[29] reported that the rate of rectal CPE colonisation in high-risk patients from the ICU was 16.7%. However, some studies showed that for CRE-colonised patients, CRE infection rates can vary from 7.6% to 44.4%.[29,30]

Multiple procedures, the use of invasive devices, and frequent administration of antimicrobials make ICU patients highly susceptible to infection.

Sarowska et al[31] reported that the largest number of CRKP isolates came from the anesthesiology and intensive care unit, which cares for people with difficult clinical conditions, where hospitalization is statistically the longest.

Therefore, active screening for colonization using rectal surveillance cultures and implementation of contact precautions are highly effective and can reduce the transmission of CRKP among patients.[32]

Considering the prevalence of MDR *K. pneumoniae* genotypes, our hospital is implementing infection control measures to prevent patient-to-patient transmission. Measures are limited to standard precautions supplemented by additional precautions, contact, and patient screening.

However, given the high incidence of MDR *K. pneumoniae* strains found in this investigation, the practical use of third-generation cephalosporins and carbapenems in critically sick patients has to be reconsidered immediately.

Study's limitation. The absence of other clinical data (such as, data on past hospitalization or antibiotic therapy) that might assist identifying individuals at high risk of ESBL and CRKP carriage.

In conclusion, among *K. pneumoniae* strains isolated in diverse clinical samples, we discovered a significant incidence of ESBL-producing and carbapenem-resistant isolates, with the surgical and ICU departments being the most typically afflicted. This presents a significant issue in terms of infection prevention and control in hospital settings. Successful containment of these isolates can be achieved through a set of measures, such as active surveillance cultures, isolation of carriers, allocation of dedicated medical staff, and rational use of antimicrobials. Microbiological laboratories' involvement in the monitoring and management of MDR bacteria epidemics is critical for a prompt and effective reaction, as well as awareness of the epidemiological condition in various units. Finally, the significance of excellent communication mechanisms for the transmission of findings and decision-making on the treatment of patients infected with these microorganisms should not be underestimated.

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