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Multidrug-Resistant *Klebsiella pneumoniae* in a Patient with SARS-Cov-2 Pneumonia in an Intensive Care Unit in Guayaquil, Ecuador: A Case Report

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Patient: Male, 52-year-old
Final Diagnosis: SARS-COV2 pneumonia associated to multiresistant *Klebsiella pneumoniae*
Symptoms: Dyspnea • chest pain • swelling • fever • general malaise • headache • myalgia • polyarthralgia
Medication: —
Clinical Procedure: —
Specialty: General and Internal Medicine

Objective: Unusual clinical course

Background: Multi-resistant microorganisms are a public health problem. Their incidence has risen due to COVID-19, indiscriminate antibiotics use, corticosteroid treatments, and higher admissions to intensive care units (ICUs) of patients requiring invasive mechanical ventilation. These are risk factors for bacterial over-infection. The present case study that is relevant because of the multiple isolated strains with a resistance pattern: *Klebsiella pneumoniae* carbapenemases (KPC), extended-spectrum beta lactamases (ESBL) and New Delhi metallo-β-lactamase (NDM) in a patient without comorbidities.

Case Report: A 53-year-old Ecuadorian man with no past medical history arrived at the Emergency Department (ED) with dyspnea, nasopharyngeal swab with a positive reverse transcription polymerase chain reaction (RT-PCR) test for SARS-CoV2, and a chest computed tomography (CT) scan showing bilateral ground-glass pulmonary infiltrates with 40% involvement. On day 10 in the ICU, the presence of *Klebsiella pneumoniae* KPC strain was reported in an axillary swab culture. Consequently, the antibiotic was rotated to vancomycin 1 g intravenously (i.v.) every 12 h and meropenem 1 g i.v. every 8 h. On day 15 in the ICU, a tracheal secretion culture was reported with the presence of *Klebsiella pneumoniae* ESBL and a blood culture with *Klebsiella pneumoniae* NDM.

Conclusions: The COVID-19 pandemic is a perfect scenario for superinfection with multi-resistant pathogens such as carbapenem-resistant *Klebsiella pneumoniae* (CRKP), due to the increase in patients admitted to ICUs requiring invasive mechanical ventilation, the use of corticosteroids, and empirical broad-spectrum antibiotic management based on guidelines. The emergence of combined multidrug-resistant strains is a challenge for laboratory detection and the selection of antimicrobial treatment.

Keywords: *Klebsiella pneumonia* • COVID-19 • Pandemics • Respiration, Artificial

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Background

The world is going through a public health crisis that took science and research by surprise: the spread of the SARS-CoV-2 virus, which causes COVID-19 disease. Another alarming situation is multi-resistance to antimicrobials, which is responsible for 700 000 deaths per year worldwide. Carbapenem-resistant *Klebsiella pneumoniae* (CRPK) was first identified in the United States in 1996 and is characterized by causing severe infections. It is becoming a global health problem due to its easy transmissibility and limited treatment options [1,2].

Klebsiella pneumoniae triggers urinary tract, respiratory and intra-abdominal infections, and bacteremia. β-lactams are the most widely used antibiotics in the treatment of infections caused by bacteria, and carbapenems are one of the most important antibiotics in this group. In recent years, bacteria have developed multiple mechanisms of resistance against these antimicrobials, such as: alterations in the permeability of the outer membrane due to loss of porins, presence of efflux pumps, hyper-production of cAMP-type β-lactamases, and production of inactivating enzymes. The most significant enzymes are the *Klebsiella pneumoniae* carbapenemases (KPC), New Delhi metallo-β-lactamase (NDM), active-on-imipenem (IMP), Verona integron-mediated metallo-β-lactamase (VIM), and oxacillin-hydrolyzing (OXA-type) carbapenemases, which can hydrolyze carbapenem antibiotics and inactivate their action against microorganisms of the Enterobacteriaceae family [3-6].

A meta-analysis of 16 studies that involved 3627 participants identified the following risk factors associated with CRPK infection: longer length of hospital stay, admission to an ICU, prior hospitalization, more days in an ICU, transplant recipient, steroid use, and exposure to carbapenems, aminoglycosides, glycopeptides, quinolones, and penicillin [7].

It is challenging to differentiate between SARS-CoV-2 and bacterial pneumonia based on clinical signs and symptoms. An accurate microbiological diagnosis is essential to identify bacterial superinfection pneumonia in patients with severe COVID-19 on mechanical ventilation. Bronchoalveolar lavage (BAL) samples are the criterion standard for detecting respiratory pathogens by bronchoscopy and confirming their superiority over nasopharyngeal swabs or endotracheal aspirates [8,9]. However, in many hospitals, BAL procedures were avoided in patients with SARS-CoV-2 pneumonia, as this is an invasive procedure with high risk for the operator [10].

Case Report

A 53-year-old Ecuadorian man with no past medical history presented with 9 days of myalgia, headache, and general malaise. On day 3 after the onset of his symptoms, he had a fever of 38.2°C and polyarthralgia and was given a RT-PCR SARS-CoV-2 test, with a negative result. For symptom management, he received acetaminophen, antihistamines, hydration, and vitamin C. He self-medicated with two 6 mg doses of ivermectin. On day 7, he presented anosmia, dysgeusia, and dyspnea, so he took a second RT-PCR SARS-CoV-2 test, with a positive result. He arrived at the Emergency Department (ED) with a chest computed tomography (CT) scan showing bilateral ground-glass pulmonary infiltrates, with 40% involvement (**Figure 1**).

His vital signs in the ED showed a respiratory rate of 36 breaths per min, and SaO_2 of 90% on room air. His heart rate was 80 beats per minute, his blood pressure was 135/85 mmHg, and his weight 100 kg. A high-flow nasal cannula was placed at 30 L/min with 50% FiO_2 with a subsequent respiratory rate of 26 breaths per min and SaO_2 of 99%. He received treatment based on dexamethasone, enoxaparin, acetaminophen,

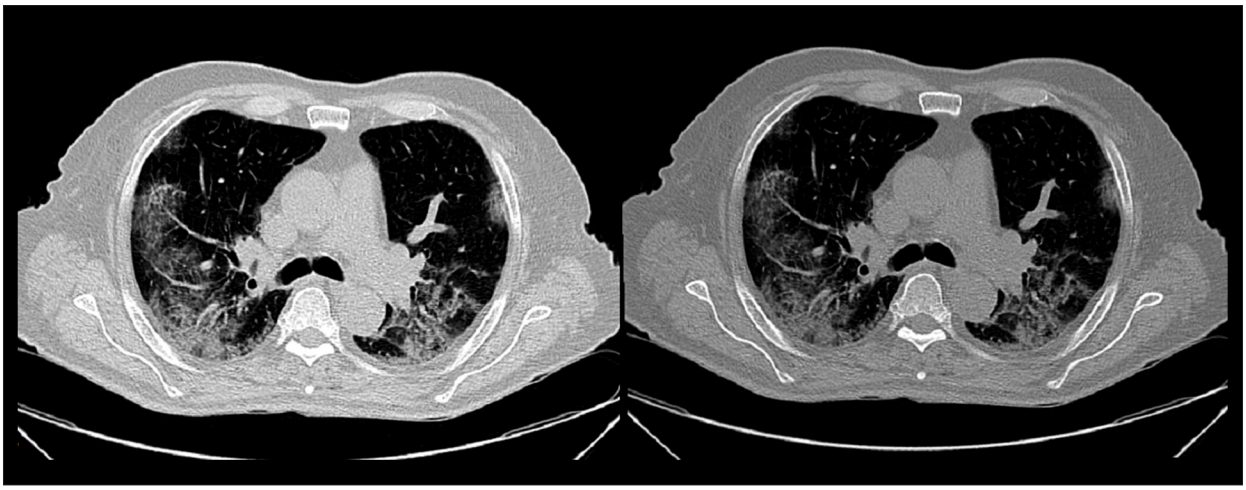

Figure 1. Chest computed tomography scan showing bilateral ground-glass pulmonary infiltrates.

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acetylcysteine, and bronchodilators. During his second day of hospitalization, the fever persisted with a temperature of 39°C, a respiratory rate of 38 rpm, SpO_2 92% with high-flow parameters at 50 L/min and FiO_2 of 100%. Therefore, he was admitted to the ICU and endotracheal intubation was carried out. His laboratory findings were leukocytes 10 500/mm^3, neutrophils 73.4%, lymphocytes 19.2%, hemoglobin 13.3 g/dl, hematocrit 39.4%, and platelets 196 000/mm^3. The first blood, urine, and sputum samples were taken for culture with subsequent initiation of broad-spectrum antibiotics based on piperacillin-tazobactam 4.5 g i.v. every 6 h. Subsequently, the cultures were reported negative. On day 9, axillary, inguinal, and rectal swab samples were taken for the rescue of multi-resistant microorganisms as part of the ICU's hospital protocol. On day 10 of his stay in the ICU, the presence of *Klebsiella pneumoniae* KPC strain was reported in an axillary swab culture, while inguinal and rectal swab cultures were negative. The antibiotic was rotated to vancomycin 1 g i.v. every 12 h and meropenem 1 g i.v. every 8 h.

On day 11 in the ICU, due to an increase in leukocytes and inflammatory markers plus lung involvement in a control chest CT scan and isolation of *Klebsiella pneumoniae* KPC strain in the axillary swab sample, the infectious disease service indicated tracheal aspirate collection for Gram, culture, and KOH, to confirm that the patient had gone from colonization to infection by a multi-resistant pathogen. Subsequently, colistin 100 mg i.v. was added every 8 h.

On day 15 of his stay in the ICU, a tracheal secretion culture was reported with the presence of 10 000 000 CFU/ml *Klebsiella pneumoniae* ESBL strain, with minimum inhibitory concentrations (MIC) for ampicillin-sulbactam >32, amikacin <2, ertapenem <0.5, imipenem <0.25, meropenem <0.25, ciprofloxacin >4, gentamicin <1, and piperacillin-tazobactam >128. The lower respiratory track sample was taken using a non-invasive technique for quantitative culture analysis.

On day 16 of his stay in the ICU, a blood culture was reported with the presence of *Klebsiella pneumoniae* strain NDM. These samples were sent to the National Institute of Public Health Research (INSPI) for confirmation of bacterial resistance. An NDM-type carbapenemase-producing microorganism, resistant to cefotaxime, ciprofloxacin, meropenem, cefepime, ertapenem, ceftazidime, imipene, and aztreonam, and sensitive to gentamicin and amikacin, was obtained by molecular biology. On day 20 of his ICU stay, the patient was successfully extubated. On day 34, he was discharged in good general condition.

Samples of blood cultures, axillary swabs, and tracheal secretions were taken from a patient from Guayaquil, Ecuador. They were inoculated in selective enriched culture media of blood agar, MacConkey, and supplemented chocolate, and placed

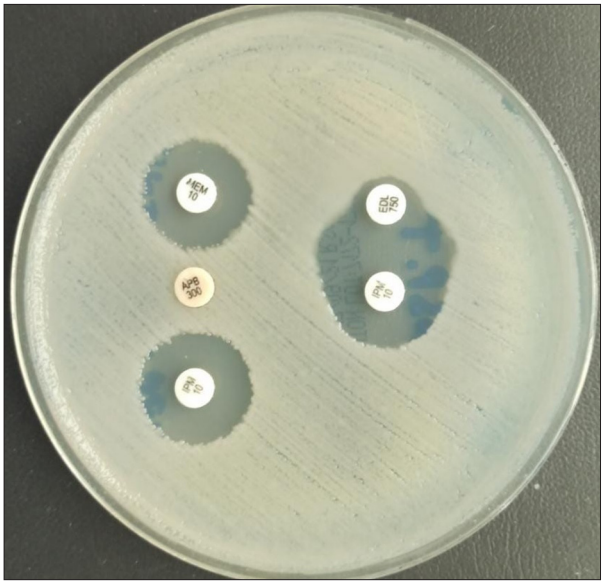

Figure 2. Induction method with EDTA and APB disk to presumptively detect resistance mechanisms, showing a reaction with the EDTA disk (NDM).

in an incubator at 37°C for 48 h. Bacterial colonies were recovered, on which the Gram stain and oxidase test were performed. For the sensitivity test, the automated platform BD Phoenix 100 was used. A result was shown in 18 to 24 h with the identification and MIC of the isolate. As complementary tests, an assay was performed of the induction method with ethylenediaminetetraacetic acid (EDTA) and boronic acid (APB) disk to presumptively detect resistance mechanisms, showing a reaction with the EDTA disk (NDM) (**Figure 2**). The IMP and VIM enzymes have a hydrolytic profile that includes all beta-lactam antibiotics except aztreonam and they are not inhibited by clavulanic acid, sulbactam, or tazobactam. However, they are inhibited by divalent cation chelating agents such as EDTA and thiol compounds such as 2-mercaptopropionic acid or dipicolinic acid. Recently, the NDM-1 enzyme has affected the world due to the multi-resistant or broad-resistant profile generated by these microorganisms.

A rapid test was carried out to detect OXA-48, KPC and NDM in bacterial isolates (RESISTET-3 O.K.N. K-Set/Coris BioConcept). Positive feedback was shown for KPC (**Figure 3**). As an additional test, a rapid test was performed to detect OXA-48, KPC, and NDM in bacterial isolates (RESISTET-3 O.K.N. K-Set/Coris BioConcept). A positive reaction for NDM (**Figure 4**) was shown, which was then sent to the reference laboratory, CRN-RAM of the National Institute of Public Health Research (INSPI), to confirm the diagnosis.

Tracheal aspirate showed *Klebsiella pneumoniae*, KPC strain. This strain is sensitive to carbapenems (imipenem, ≤0.25, ertapenem ≤0.5, meropenem ≤0.25) and aminoglycosides

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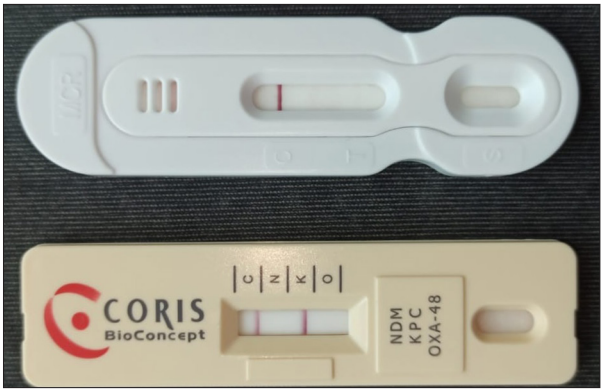

Figure 3. Rapid test for the detection of OXA-48, KPC, and NDM in bacterial isolates (RESISTET-3 O.K.N. K-Set/Coris BioConcept), showing positive reaction for KPC.

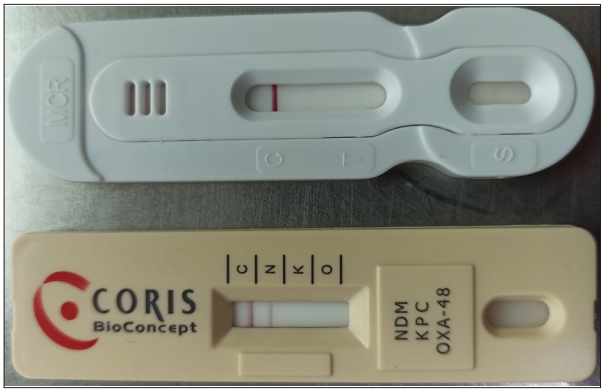

Figure 4. Rapid test for the detection of OXA-48, KPC, and NDM in bacterial isolates (RESISTET-3 O.K.N. K-Set/Coris BioConcept), showing a positive reaction for NDM.

Table 1. Tracheal aspirate MIC *Klebsiella pneumoniae*, ESBL strain.

Klebsiella pneumoniae, ESBL strain		
Antibiotics	**Interpretation**	**MIC**
Ampicillin+sulbactam	R	≥32
Amikacin	S	≤2
Ertapenem	S	≤0.5
Imipenem	S	≤0.25
Meropenem	S	≤0.25
Ciprofloxacin	R	≥4
Gentamicin	S	≤1
Piperacillin+tazobactam	R	128

Antimicrobial susceptibility interpretation of tracheal aspirate.

Table 2. Blood culture and axillar swab MIC *Klebsiella pneumoniae*, NDM strain.

Klebsiella pneumoniae, NDM strain		
Antibiotics	**Interpretation**	**MIC**
Colistin	S	≤0.5
Tigecycline	R	≥8
Aerobic blood culture: positive and anaerobic blood culture: positive		
Aerobic axillar swab: positive and anaerobic axillar swab positive		

Antimicrobial susceptibility interpretation of blood culture and axillar swab; aerobic and anaerobic.

(amikacin ≤2.0, gentamicin ≤1.0), and resistant to antibiotics with beta lactamase inhibitors (ampicillin+sulbactam ≥32, piperacillin+tazobactam 128) and first-generation quinolones (ciprofloxacin ≥4) (**Table 1**).

Aerobic and anaerobic blood culture showed *Klebsiella pneumoniae*, CEPA NDM. This is colistin-sensitive ≤0.5 and tigecycline-resistant ≥8.0 (**Table 2**). An axillary swab contained *Klebsiella pneumoniae*, NDM strain. This is colistin-sensitive ≤0.5 and tigecycline-resistant ≥8.0 (**Table 2**).

A blood culture confirmed at the National Institute of Public Health was performed using the Kirby Bauer manual identification method. The following sensitivity was obtained: sensitive to amikacin and gentamicin and resistant to ceftazidime, cefotaxime, cefepime, ciprofloxacin, aztreonam, meropenem, imipenem, ertapenem, and intermediate colistin MDC 0.5 ug/ml. CIM-BMD+DD susceptibility tests were carried out and CLSI interpretation standards were met. The results showed a carbapenemase-positive, ABP-IMI synergy-negative, EDTA-IMI synergy-positive, MDM-type carbapenemase-producing microorganism (**Table 3**).

Discussion

We presented the case of a patient without comorbidities with severe pneumonia due to COVID-19. The case was complicated by superinfection with CRKP associated with invasive mechanical ventilation in an ICU in Guayaquil, Ecuador.

Two other case studies have been presented of patients in the ICU of a tertiary hospital in Spain. The patients had COVID-19 and developed superinfection by multi-resistant microorganisms associated with risk factors such as the use of mechanical ventilation, corticosteroids, broad-spectrum antibiotic treatment, and increased length of hospital stay [11,12].

An outbreak of multi-resistant *Klebsiella pneumoniae* occurred in an ICU that was full with patients with COVID-19.

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Table 3. Blood culture confirmation *Klebsiella pneumoniae*, NDM strain (confirmed at the INSPI Guayaquil, Ecuador). Microbiological findings.
Klebsiella pneumoniae ss. Pneumonia.

Cefotaxime	**R**	6 mm	Ceftazidime	**R**	6 mm
Ciprofloxacin	**R**	6 mm	Gentamicin	**S**	20 mm
Meropenem	**R**	12 mm	Imipenem	**R**	10 mm
Cefepime	**R**	6 mm	Amikacin	**S**	19 mm
Ertapenem	**R**	10 mm	Aztreonam	**R**	12 mm
Colistin_MDC			0.5 ug/Ml (I)		
Identification method			Manual		
Sensitivity test			CIM-BMD + DD		
Interpretation rule			CLSI		
Carbapenemase			Positive		
Inactivation Carbapenemes mCIM			Positive		
Inactivation Carbapenemes eCIM			Positive		
Synergy ABP-IMI			Negative		
Synergy EDTA-IMI			Positive		
Molecular biology research			Yes		
bla-KPC			Negative		
bla-VIM			Negative		
bla-IMP			Negative		
bla-NDM			Positive		
bla-OXA48			Negative		
Comments			NDM-type carbapenemase-producing microorganism Consider use of combination therapy Please maintain vigilance of the microorganism		

Antimicrobial susceptibility interpretation of blood culture confirmed at the INSPI.

It is assumed that there was non-compliance with standard and contact biosafety regulations. NDM-1K is thought to have been transmitted in the environment due to invasive procedures and the administration of immunomodulatory therapy in patients with COVID-19. Failure to change gloves between patient check-ups is possibly one of the causes of the propagation of NDM-1K-producing bacteria. Non-compliance with biosafety standards occurred worldwide as ICUs were overwhelmed and understaffed, and the lack of sanitary implements to control these multi-resistance outbreaks aggravated the patients' situations and raised the risk of mortality [13].

In a study, patients with a positive result in the reverse transcriptase polymerase chain reaction (RT-PCR) test for the SARS-CoV-2 virus were included in the COVID-19 group, and more than 1 species of MMR was detected. The positivity of the analyzed samples was made in axillary (2%), pharyngeal (2.4%), nasal (0.9%), and rectal (8.2%) exudates. The latter were the most significant samples for the detection of ESBL-producing Enterobacteriaceae [11].

Clinical teams in Chicago's ICUs routinely obtained BAL fluid from mechanically ventilated patients at the time of intubation and when ventilator-associated pneumonia (VAP) was clinically suspected to administer antibiotics. With the initial surge of COVID-19 in Chicago, the standard bronchoscopy and bronchoalveolar lavage (BAL) technique was modified to minimize operator exposure to infectious aerosols [14]. Based on ICU hospital protocol, axillary, inguinal, and rectal swab samples are obtained for screening multi-resistant microorganisms, similar

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to a studio of optimization of multiple muco-cutaneous site sampling method for screening MRSA colonization in ICUs [15].

In Michigan in 2018, *Klebsiella michiganensis*, a producer of KPC-2, NDM-1, and NDM-5, was identified in a Chinese patient with acute diarrhea. In Switzerland in 2019, a case of KPC-3 was reported, while in South Africa, *Klebsiella michiganensis* was identified that produced OXA-181 and NDM-1 in a stool sample [16].

The INEI-ANLIS antimicrobial service "Dr Carlos Malbrán" (National Reference Laboratory LNR) in Argentina confirmed during the first wave of the COVID-19 pandemic the health emergency and dissemination by combined carbapenemase-producing *Enterobacteriaceae*. During the period from May to November 2020, the LNR isolated 196 gram-negative bacilli for reference studies. A total of 52/196 (27%) isolates were identified as dual carbapenemase producers from 13 hospitals: 31 KPC + NDM, 19 NDM+ OXA-163, and 2 KPC+ IMP. Co-expression of different classes of carbapenemases in *Enterobacterales* was not reported in Argentina prior to the COVID-10 pandemic [17].

In Latin America, 11.6% of hospitalized patients develop nosocomial infections caused by different procedures. In Ecuador, mortality due to multi-resistant microorganisms is approximately 50%. Complete surveillance of CRKP in Ecuador has not been reported previously, although some cases have been described (23 cases reported in 2016) [18,19].

The clinical case described here presents the same risk factors as the studies carried out in Spain on patients with COVID-19. Some case reports of combined multi-resistant strains were published in years prior to the COVID-19 pandemic. In contrast, during the first wave of COVID-19 cases in Argentina, co-expression of classes of carbapenemases in *Enterobacterales* were reported for the first time. In Ecuador, it is the first time that the co-expression of strains of *Klebsiella pneumoniae* (KPC+ESBL and NDM) has been reported. Fortunately, despite the severity of the case, the patient was discharged after being hospitalized for 34 days, with arterial hypertension as a cardiovascular sequelae.

Conclusions

The COVID-19 pandemic generated a perfect scenario for the superinfection of multi-resistant pathogens such as CRKP, due to the increase in patients admitted to ICUs who required invasive mechanical ventilation, the use of corticosteroids, and empirical broad-spectrum antibiotic management based on guidelines. The emergence of combined multidrug-resistant strains constitutes a challenge for laboratory detection and selection of specific antimicrobial treatment.

Biosafety measures and immediate communication with the infectious diseases service must be carried out once a patient with CRKP has been identified for decision making to prevent the spread of life-threatening multi-resistant microorganisms.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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