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# Cribado de organismos productores de carbapenemasas: un estudio retrospectivo, epidemiológico, clínico y laboratorial de tres años en un hospital terciario en el norte de Portugal

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*Cribado de organismos productores de carbapenemasas: un estudio retrospectivo, epidemiológico, clínico y laboratorial de tres años en un hospital terciario en el norte de Portugal*

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## ABSTRACT

**Introduction:** carbapenemase-producing organisms (CPO) are a serious public health problem, and colonization screening is crucial to control its

spread in healthcare facilities. We conducted a retrospective clinical and laboratorial study, based on the CPO screening carried out at our hospital. Our main objectives were to determine the prevalence of CPO infections upon admission, and the incidence of CPO nosocomial infections. Secondary objectives were to review local CPO epidemiology, study the impact of modifications made to the laboratory protocol, and perform a clinical evaluation of enrolled patients.

**Material and methods:** this was a three-year study (2019-2021). It is subdivided in two parts: 1) epidemiology analysis and review of laboratory data; and 2) clinical evaluation of selected patients (the ones with a *de novo* positive CPO screening upon admission.

**Results:** 2.28 % of CPO (molecular) screenings were positive and, for these positive samples, 48.11 % had a positive complementary culture. We found differences in the positivity rate of the cultural exam, based on the culture medium used: 31.86 % with MacConkey agar (MAC), and 61.86 % with ChromID® Carba Smart agar (CARB/OXA) and MAC. Most of CPO identified were *Enterobacterales* (73.36 % *Klebsiella pneumoniae*, 19.71 % *Escherichia coli*), and *K. pneumoniae* carbapenemase (KPC) was the most common resistance mechanism (81.48 %). Only 9.05 % of selected patients had a confirmed CPO infection upon admission, while the incidence of nosocomial CPO infection during hospitalization was 4.40 %.

**Conclusions**: although with a low statistical power, we found that a negative culture (using CARB/OXA+MAC) was associated with the absence of CPO infection upon admission.

**Keywords:** CPO screening. Epidemiology. Nosocomial infection.

**RESUMEN**

**Introducción:** realizamos un estudio clínico y laboratorial retrospectivo, basado en la pesquisa de CPO realizada en nuestro hospital. Nuestros objetivos principales fueron determinar la prevalencia de infecciones por CPO al ingreso y la incidencia de infecciones nosocomiales por CPO. Los objetivos secundarios fueron revisar nuestra epidemiología, estudiar el

impacto de las modificaciones realizadas al protocolo laboratorial y realizar una evaluación clínica de los pacientes seleccionados.

**Materiales y métodos:** este es un estudio de tres años (2019-2021), que se subdivide en: 1) análisis epidemiológico y revisión de datos del laboratorio; y 2) evaluación clínica de los pacientes seleccionados (aquellos con una pesquisa de CPO positiva *de novo* al ingreso).

**Resultados:** la tasa de positividad para el cribado CPO (molecular) fue del 2,28 % y, de estas muestras positivas, el 48,11 % tuvo un cultivo positivo. Se encontraron diferencias en la tasa de positividad del examen cultural: 31,86 % con agar MacConkey (MAC) y 61,86 % con MAC y agar ChromID® Carba Smart (CARB/OXA). La mayoría de los CPO identificados fueron *Enterobacterales* (73,36 % *Klebsiella pneumoniae*, 19,71 % *Escherichia coli*), y la *K. pneumoniae carbapenemase* (KPC) fue el mecanismo de resistencia más común (81,48 %). Solo el 9,05 % de los pacientes seleccionados tenía una infección por CPO confirmada al ingreso, mientras que la incidencia de infección nosocomial por CPO fue del 4,40 %.

**Conclusiones:** aunque con un bajo poder estadístico, encontramos que un cultivo negativo (utilizando CARB/OXA+MAC) se asoció con la ausencia de infección por CPO al ingreso.

**Palabras clave:** Cribado CPO. Epidemiología. Infección nosocomial.

## INTRODUCTION

Carbapenemase-producing organisms (CPO) are a serious public health problem (1). CPO produce enzymes capable of hydrolysing most beta-lactams, and which are not inhibited by most beta-lactamase inhibitors (2-4). Two important aspects of CPO are, on the one hand, the limited therapeutic options (5) and, on the other hand, the potential horizontal transmission of the resistance mechanisms through plasmids (which is a known cause for outbreaks) (6). It is well documented that CPO colonization is a risk factor for CPO infections, and it correlates with increased mortality (7-9). As such, epidemiological vigilance is necessary. The crucial measures to control the spread of this type of microorganisms in healthcare facilities are active surveillance through colonization screening, contact isolation precautions, hygienic control, hand washing, training of healthcare personnel, and

antimicrobial stewardship programs (1,5, 7,10,11). Another role of colonization screening is to guide pre-surgical antibiotic prophylaxis and empiric treatment of patients with acute infectious conditions (12).

There are several recommendations advocating screening for CPO, both national (9,10) and international (1). In our hospital, mandatory CPO screening has been carried out since 2016. At the time, only patients with certain criteria were screened. Summarily, patients were screened upon admission if they lived in continuing care units or nursing homes, or if they had a hospitalization in the year before. In addition, CPO screening was routinely performed during hospitalization, but only in a few of our hospital's services. These criteria did not undergo drastic changes until mid-2022. In that year, our hospital had an outbreak of CPO of significant proportions, which demonstrated the necessity to readjust the screening protocol. Subsequently, all patients were screened upon hospital admission, and a weekly systematic screening was implemented for all hospitalized patients. Due to the changes occurred in mid-2022, we limited ourselves to evaluate data from 2019 to 2021, prior to current standards. Another reason for choosing this time block was the desire to evaluate the changes in our laboratory's protocol (Fig. 1), which occurred in September 2020. Our laboratory performs primarily a molecular test, which defines the patients' CPO colonization status, and which is followed by a complementary cultural exam (solely for positive molecular samples). Since 2016, the cultural exam was performed in a non-selective medium for CPO. In September 2020, a selective medium for CPO was introduced, which theoretically should facilitate the isolation of this type of microorganisms and, hypothetically, increase the positivity rate of the cultural exam (compared to the period in which we used a non-selective medium).

Given the importance of regular assessment of this type of data, we conducted a retrospective epidemiological, clinical, and laboratory study, based on the CPO screening carried out at our hospital.

**Objectives**

Our main objectives were to determine the prevalence of CPO infections upon admission, and to determine the nosocomial incidence of such infections during hospitalization, among patients colonized *de novo* by CPO.

Secondary objectives were to review our local CPO epidemiology, study the impact of modifications made to the laboratory protocol, and perform a clinical evaluation of enrolled patients.

## METHODS

We conducted a retrospective study, based on the CPO screening carried out at Centro Hospitalar Universitário São João (CHUSJ), a tertiary-care center in Porto, Northern Portugal. CHUSJ is one of the largest hospitals in the country, with a capacity of around 1,100 beds, 68 of which are in Intensive Care Units.

This study reports to the period between January 2019 and December 2021 (3 years). Methodologically it is subdivided into two parts: 1) epidemiology analysis and review of laboratory data; and 2) clinical evaluation of selected patients (the ones with a *de novo* positive molecular CPO screening upon admission).

### CPO screening criteria

We collected and analyzed data from all screenings carried out in our hospital between 2019 and 2021. During that time, admitted patients were screened for CPO colonization according to the following criteria, which were defined by the infection control group and the management of our hospital: 1) patients living in continuing care units or nursing homes; 2) previous hospitalization (> 48 hours in the previous 12 months) in another hospital (any service), or at our hospital's Infectious Diseases Service or General Surgery Service; and 3) admission to any of our hospital's services with CPO patient cohorts. Also, for non-colonized patients hospitalized in services with CPO patient cohorts, screening was performed every two weeks and at the time of medical release.

Patients who met the referred criteria were screened according to the protocol exemplified in figure 1. For each patient, two rectal swabs were collected (at the same time): 1) Transystem™ (dry) swab, used for the CPO (molecular) screening; and 2) Deltalab® swab (with Amies transport medium), used for the complementary cultural exam. In case of a positive molecular screening, patients were not re-screened for 6 months (neither by molecular nor cultural methods), as CPO colonization was assumed for that

period. After those 6 months, in case of new hospitalization, the standard protocol was followed. Still, those patients were only considered "decolonized" after confirmation of two negative molecular tests, carried out on consecutive days. Only after that did the hospital infection control group order isolation measures to be removed.

**Laboratory protocol**

Our laboratory protocol for CPO screening was divided in two parts (Fig. 1): 1) molecular screening, the result of which determined the CPO colonization status; and 2) cultural exam, only for positive molecular samples. Therefore, non-colonization was assumed in all cases of negative molecular samples.

The method used for the molecular screening was Xpert® Carba-R, which was not changed throughout the study period. This point-of-care testing method uses reverse transcription polymerase chain reaction (RT-PCR) to identify the molecular targets of *K. pneumoniae* carbapenemase (KPC), oxacillinase-48 (OXA-48), Verona integron-mediated metallo-β-lactamase (VIM), New Delhi metallo-β-lactamase (NDM), and imipenemase (IMP). No other molecular methods have been used to screen for carbapenemases not detected by Xpert® Carba-R. Also, other mechanisms of resistance to carbapenems have not been screened.

Noteworthy, the cultural exam protocol underwent significant changes during the study period. Between January 2019 and September 2020, rectal swabs were cultivated in MacConkey agar (MAC) (selective and differential medium for gram-negative bacilli). During this time, all colonies that were morphologically distinct from each other were studied. In October 2020 the cultural exam protocol was changed. Thereafter, rectal swabs were cultivated in MAC and ChromID® Carba Smart agar (CARB/OXA) (selective and chromogenic medium for CPO). Subsequently, colonies isolated from CARB/OXA were studied preferably. As such, in the second phase of the study, colonies isolated from MAC were only studied in case of absence of growth in CARB/OXA. The isolated microorganisms were identified with Matrix-Assisted Laser Desorption/Ionization (Biomerieux® Vitek MS). Antibiograms were determined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints valid at the time of the screening. Minimum inhibitory concentration (MIC) for

carbapenems (ertapenem, meropenem, imipenem) and colistin were recorded. Carbapenems' MIC were determined using the E-test method (with strips and *Mueller-Hinton* agar from Biomerieux®). Colistin's MIC was determined using a microdilution method (Micronaut® MIC–Strip Colistin). For the remaining antibiotics, susceptibility profiles were determined by an automated method (Biomerieux® Vitek 2). In addition, isolated carbapenem-resistant microorganisms were tested to confirm the presence of a carbapenemase resistance mechanism. In the first phase of the study, such confirmation was performed with Xpert® Carba-R. While, in the second phase, we used primarily the CORIS BIOCONCEPT® RESIST-5 O.O.K.N.V., an immunochromatographic test which identifies the enzymes of the carbapenemases KPC, OXA-48, VIM, NDM, and IMP. During the second phase of the study, Xpert® Carba-R was used as a backup method to study carbapenem-resistant microorganisms which had a negative result in the immunochromatographic test. If both confirmatory tests for carbapenemases were negative, then those strains were not considered CPO. We did not use any other molecular or phenotypic methods to test isolated carbapenem-resistant microorganisms. Furthermore, it is noteworthy that this protocol to test for carbapenemases in carbapenem-resistant microorganisms was a standard of our laboratory, and did not only apply to CPO screening samples, i.e. also applied to samples isolated from sites of infection.

**Clinical evaluation (of selected patients)**

We performed a clinical evaluation of selected cases in order to determine the prevalence of CPO infection upon admission, as well as the incidence of nosocomial CPO infection during hospitalization.

Patients were selected among those with a *de novo* positive CPO screening upon admission. Therefore, the previously mentioned criteria were applied (see "CPO screening criteria"), and, for this particular analysis, patients were excluded if any of the following conditions were met: 1) non-colonized patients (i.e. negative molecular CPO screening); 2) previously known colonized patients; 3) "admission CPO screening" performed after the third day of hospitalization; 4) hospital transfer (in or out) before medical discharge; 5) insufficient medical records; 6) incomplete CPO screening data (i.e. both molecular and cultural exams must have been performed, and the

laboratory protocol must have been fully followed); and 7) pediatric population. Patients who met these conditions are from here on referred to as “selected patients”.

Relevant data recorded were demography (sex, age), comorbidities, risk factors for CPO colonization (i.e. hospitalizations in the 3 months prior to admission, living in nursing homes or equivalent places, regular contact with hospital-like centers, antibiotic treatments < 6 months prior to admission, chronic proton pump inhibitors treatment, chronic wounds, and chronic medical devices such as central venous catheter or chronic bladder catheter), previous CPO screening results, duration of hospitalization, clinical history and diagnosis, deaths, microbiological findings during hospitalization, and antibiotic treatments in the 6 months prior to admission and during hospitalization.

We emphasize that the category “selected patients” includes patients colonized by CPO upon admission, regardless of neither the diagnosis nor the infection/no-infection status. Such status was defined through a case-by-case analysis of clinical and microbiological records, and it was used to subclassify selected patients in four groups, according to the etiology of hospitalization, and which served to determine the prevalence of CPO infection upon admission (Fig. 2): 1) “No infection”; 2) “Non-CPO infection”; 3) “CPO infection”; and 4) “Infection + Insufficient data”. As such, if the diagnosis was not infection, patients were subcategorized into the “No infection” subgroup. On the counterpart, the subcategorization of patients diagnosed with infection was based on microbiological findings, or lack of them. In other words, in the absence of collection of microbiological samples from the infected organs or systems, or in the face of inconclusive microbiological results (i.e. no causative microorganisms were identified, in spite of documented infection), patients were subcategorized as "Infection + Insufficient data". Whenever a causative microorganism was identified, in at least one representative sample, patients were subcategorized, respectively, into the “Non-CPO Infection” or “CPO Infection” subgroups. As described in “Laboratory protocol”, whenever a carbapenem-resistant microorganism was isolated, it was standard protocol to test for carbapenemases with CORIS BIOCONCEPT® RESIST-5 O.O.K.N.V. and/or Xpert® Carba-R, regardless of the sample type. Thus, cases of “CPO infection” were defined by the

identification of the same microorganism with the same type of carbapenemase, both in the CPO screening cultural exam and in a sample from the site of infection. For this analysis of the infection/no-infection status upon admission, we only considered biological samples collected up to the third day of hospitalization. We did not exclude any types of infections, but rather categorized them into the following groups: 1) pulmonary infections; 2) urinary tract infections; 3) bloodstream infections; 4) infected abscess; 5) infected open wounds; and 6) other focus of infection.

In order to determine the incidence of CPO infection during hospitalization, we only studied patients from the subgroups “No infection” and “Non-CPO infection”. The designation of nosocomial CPO infection was only assigned when a compatible microorganism was identified after the third day of hospitalization. The remaining cases were considered negative.

### Ethical considerations

This project obtained the necessary authorizations from the data protection officer, the ethics committee and the board of directors. Informed consent was not obtained, given that this was a retrospective study and measures were taken to guarantee the anonymity of the patients involved.

### Statistical analysis

The data were analysed using Microsoft® Excel® version 2405 and IBM® SPSS® Statistics version 26. Descriptive measures were used.

## RESULTS

### Epidemiology and laboratory data

Figure 3 summarizes the epidemiology and laboratory data analysis. Between 2019 and 2021, 26035 CPO (molecular) screenings were performed at our hospital. Overall, 2.28 % ($n$ = 594 / 26035 total) of screenings were positive, among which the carbapenemase mechanisms detected were as follows: 81.48 % KPC ($n$ = 484 / 594 total); 10.27 % VIM ($n$ = 61 / 594 total); 4.71 % OXA-48 ($n$ = 28 / 594 total); 1.68 % NDM ($n$ = 10 / 594 total). In eleven cases (1.85 %), two carbapenemase mechanisms were simultaneously detected: 6 cases of KPC+VIM, 3 cases of KPC+OXA-48, and 2 cases of KPC+NDM.

Regarding the cultural exam (of positive molecular samples), 66 cases had insufficient data to be analysed; either because the cultural exam was not carried out at all, or because only partial information was available. Of the remaining 528 cases, 48.11 % ($n = 254$) had a positive cultural exam. Among the CPO strains isolated ($n = 274$), 73.36 % ($n = 210$) were *K. pneumoniae*, 19.71 % ($n = 54$) were *E. coli*, 2.55 % ($n = 7$) were *P. aeruginosa*, 1.46 % ($n = 4$) were *E. aerogenes*, 1.46 % ($n = 4$) were *E. cloacae*, and 1.46 % ($n = 4$) were *C. freundii*. Regarding the resistance mechanism, 96.02 % ($n = 193$ / 201 total) of the isolated *K. pneumoniae* were KPC, while among *E. coli* 83.33 % ($n = 45$ / 54 total) were KPC and 11.11 % ($n = 6$/ 54 total) were OXA-48. All seven *P. aeruginosa* identified were VIM.

We analysed the antibiogram profiles for *K. pneumoniae* and *E. coli* KPC strains (Table I). Resistance percentages for the following antibiotics were (*K. pneumoniae*/*E. coli*): ampicillin 100 % (intrinsic resistance) / 100 %, amoxicillin-clavulanic acid 100 % / 96,15 %, piperacillin-tazobactam 100 % / 88,89 %, cefuroxime 99.49 % / 96,08 %, ceftazidime 98.49 % / 83.33 %, cefotaxime 97.99 % / 71.70 %, cefepime 48.74 % / 31.48 %, ertapenem 98.98 % / 86.54 %, meropenem 52.55 % / 22.00 %, imipenem 82.65 % / 45.10 %, amikacin 3.02 % / 5.56 %, gentamicin 17.59 % / 25.93 %, ciprofloxacin 78.89 % / 48.08 %, trimethoprim-sulfamethoxazole 49.25 % / 53.70 %, colistin 1.55 % / 4.17 %.

**Clinical evaluation (of selected patients)**

Only 210 patients met the selection criteria for *de novo* positive CPO screening upon admission (Table II). 56.67 % ($n = 119$ / 210 total) of selected patients were male (average 70.34 years old [y.o.]; range: 22-100 y.o). Women's mean age was 79.53 y.o. (range 37-101 y.o.). Regarding the patients' comorbidities: 62.38 % ($n = 131$ / 210 total) had arterial hypertension, 42.38 % ($n = 89$ / 210 total) had dyslipidemia, 33.81 % ($n = 71$ / 210 total) had diabetes *mellitus*, 25.71 % ($n = 54$ / 210 total) had chronic kidney disease, 21.43 % ($n = 45$ / 210 total) had neoplasia (any type), 20 % ($n = 42$ / 210 total) had cerebrovascular disease, 18.10 % ($n = 38$ / 210 total) had atrial fibrillation (AF), 4.29 % ($n = 9$ / 210 total) had other arrhythmias (non-AF), 17.14 % ($n = 36$ / 210 total) were smokers / ex-

smokers, 16.67 % ($n$ = 35 / 210 total) had heart failure, 14.29 % ($n$ = 30 / 210 total) were obese, 13.81 % ($n$ = 29 / 210 total) had ischemic heart disease, 9.05 % ($n$ = 19 / 210 total) had chronic obstructive pulmonary disease/bronchiectasis, 7.62 % ($n$ = 16 / 210 total) had peripheral vascular disease, 7.14 % ($n$ = 15 / 210 total) suffered from alcoholism, 6.19 % ($n$ = 13 / 210 total) had obstructive sleep apnea syndrome, 0.95 % ($n$ = 2 / 210 total) had asthma, and 55.71 % ($n$ = 117 / 210 total) were dependent for activities of daily living (ADL) (26.19 % [$n$ = 55 / 210 total] were partially dependent, while 29.52 % [$n$ = 62 / 210 total] were totally dependent for ADL). Median number of comorbidities was four (average 3.77 / 18 total number of comorbidities listed). Regarding the risk factors for CPO colonization: a) 78.57 % ($n$ = 165 / 210 total) of selected patients had a significant risk factor for CPO contact transmission; a1) 51.43 % ($n$ = 108 / 210 total) were hospitalized in the 3 months prior to admission; a2) 35.24 % ($n$ = 74 / 210 total) lived in nursing homes or equivalent places; a3) 4.76 % ($n$ = 10 / 210 total) had regular contact with hospital-like centers (8 haemodialysis-dependent patients, and 2 patients with hematological conditions); b) 70 % ($n$ = 147 / 210 total) of selected patients had antibiotic treatments <6 months prior to admission; c) 43.81 % ($n$ = 92 / 210 total) had chronic proton pump inhibitors treatment; d) 10.48 % ($n$ = 22 / 210 total) had chronic wounds; and e) 6.67 % ($n$ = 14 / 210 total) had medical devices (central venous catheter or chronic bladder catheter). Median number of risk factors was two (average 2.10 / 5 total number of risk factors listed). Only eleven (5.24 %) of selected patients did not have at least one of the risk factors aforementioned.

Regarding the motive for hospital admission, 78.57 % ($n$ = 165 / 210 total) were due to a medical problem, 12.38 % ($n$ = 26 / 210 total) due to an acute surgical problem, and 9.05 % ($n$ = 19 / 210 total) due to a scheduled surgery. Upon admission, 55.71 % ($n$ = 117 / 210 total) of selected patients had diagnosis of infection. According to medical records, 39.42 % ($n$ = 46 / 117 total) had pulmonary infection, 21.37 % ($n$ = 25 / 117 total) had urinary tract infection, 11.11 % ($n$ = 13 / 117 total) had bloodstream infection, 11.11 % ($n$ = 13 / 117 total) had an infected abscess, 5.13 % ($n$ = 6 / 117 total) had an infected open wound, and 11.97 % ($n$ = 14 / 117 total) had other focus of infection. Merely six of selected patients (2.86 %) received

medical discharge on the same day of evaluation in our hospital's emergency department. The remaining were admitted for hospitalization, with an average duration of 14.92 days (deaths excluded). Unfortunately, 16.19 % ($n = 34$ / 210 total) of selected patients died. Considering deaths matched by subgroups, 17.65 % ($n = 6$ / 34 total) had "No infection", 20.59 % ($n = 7$ / 34 total) had a "Non-CPO infection", and 14.71 % ($n = 5$ / 34 total) had a "CPO infection". Among patients with "Infection + Insufficient data" (47.06 % [$n = 16$ / 34 total]), half of them died under empirical treatment with a beta-lactam typically ineffective against CPO (i.e. aminopenicillins, with or without a beta-lactamases inhibitor, or cephalosporins up to the third generation), while the other half did not receive antibiotic treatment, due to high deterioration of the patient's general condition (end-of-life palliative care).

Overall, 44.29 % ($n = 93$ / 210 total) of selected patients had "No infection", 34.29 % ($n = 72$ / 210 total) had a "Non-CPO infection", and 9.52 % ($n = 20$ / 210 total) had a "CPO infection" (Fig, 2). In 11.90 % ($n = 25$ / 210 total) of cases there was "Infection + Insufficient data" to identify the causative agent of the infection. Thus, considering both conclusive and inconclusive cases, the prevalence of CPO infection upon admission was 9.52 %-21.42 %.

As our laboratory's protocol was changed during the study period, we paired the four clinical subgroups according to the culture medium used (Table III). Notably, there was a marked difference in the positivity rate between the periods in which only MAC was used (31.86 %) and in which CARB/OXA+MAC were used (61.86 %). Also, all cases with a negative culture and "CPO infection" were recorded while using MAC alone. After the implementation of CARB/OXA+MAC, we did not record any more of such cases.

In order to determine the incidence of CPO nosocomial infection, we analysed the two subgroups "No infection" and "Non-CPO infection". Of these patients, six were excluded, as they were discharged on the same day of evaluation. Of the remaining 159 patients, only seven (average of 38.71 days of hospitalization) presented a microbiological isolate compatible with nosocomial CPO infection. Five of those patients had a negative cultural exam on the admission CPO screening (4 of them with MAC and 1 of them with CARB/OXA+MAC). The remaining two patients had a nosocomial CPO infection caused by the same microorganism identified on the cultural

screening exam. The average length of stay for patients who did not have nosocomial CPO infection was only 12.48 days of hospitalization. In sum, the incidence of CPO nosocomial infection was 4.40 %.

## RESULTS AND DISCUSSION

Regarding our local epidemiology, KPC (81.48 %) was by far the most common carbapenemase identified, followed by VIM (10.27 %). *K. pneumoniae* (73.36 %) and *E. coli* (19.71 %) were the most common CPO isolated. From a statistical point of view, we only had enough cases to evaluate the antibiograms of the *K. pneumoniae* and *E. coli* KPC strains. Such results were as expected, that is, resistance to beta-lactams was very high (except for meropenem and cefepime). Resistance to quinolones was markedly high, and most strains were susceptible to aminoglycosides and colistin.

The fact that our laboratory's cultural exam protocol was changed during the study period could represent a bias. However, as we took this into account, we believe that not only does the study remain valid, but it becomes even more relevant. As expected, we confirmed that CARB/OXA is better than MAC for isolating CPO. An enlightening fact is the striking difference in the positivity rate of the cultural exam between the MAC and CARB/OXA+MAC periods (32.46 % *versus* 61.46 %). Considering the characteristics of both culture mediums, the greater ease in isolating CPO in CARB/OXA is understandable. Moreover, theoretical knowledge of this fact was the main reason for the changes in the cultural exam protocol.

Another aspect to address regarding the cultural exam is the fact that the positivity rate is far from 100 %. If the difference recorded between the two-cultural medium used is comprehensible, it is less understandable that, when using CARB/OXA+MAC, almost 40 % of patients with a positive molecular screening had a complementary negative cultural exam. Although we do not have concrete data to explain these findings, one could speculate that these cases may perhaps portray genotypic colonizations, which would justify its identification by molecular methods, but not by phenotypic ones. Other possible explanations would be the low inoculum (in the collected swabs) or a possible delay in laboratory processing. However, the samples for cultural

exam were collected using a dedicated swab with a transport medium, which should reduce the likelihood of this type of interference. Nevertheless, we do not have data on the volume of inoculum, nor the time elapsed between collection and laboratory processing.

Regarding the clinical evaluation, we necessarily had to select patients. Our hospital's screening criteria are too broad, which picks patients at very different stages of hospitalization. To be able to draw conclusions, and considering our objectives, we only selected patients with a confirmed *de novo* positive CPO screening upon admission. As the pediatric population was a negligible minority, it was excluded. We also excluded patients who underwent "admission CPO screening" after the third day of hospitalization, because such cases could reflect in-hospital transmission, which would distort the conclusions about the prevalence of CPO infection upon admission.

Our selected patients' population consisted mostly of dependent elderly and presented a median of four comorbidities. As expected, most of the selected patients had a significant risk factor for CPO contact transmission or had antibiotic therapy <6 months prior to admission. Only a minority (5.24 % [$n$ = 11 / 210 total]) did not have at least one risk factor for CPO colonization.

In order to determine the prevalence of CPO infection upon admission, selected patients were categorized into four subgroups. We could have simply excluded patients from the subgroup "Infection + Insufficient data", but we believe that enrolling them allowed us to portray the results more realistically. Only 9.52 % of selected patients had a confirmed "CPO infection", while 78.58 % definitely did not have such type of infection. Thus, considering the subgroup "Infection + Insufficient data", the prevalence of CPO infection upon admission was 9.52 %-21.42 %.

We found more cases of "CPO infection" associated with a positive culture than with a negative one. Furthermore, in our selected patient population, and since we began using CARB/OXA, all culture-negative cases have been associated with the absence of CPO infection upon admission. Although one must weigh the low statistical power, this last single finding is very relevant, as it reinforces the superiority of CARB/OXA over MAC. Additionally, it provides supplementary clinical information to the CPO cultural exam, adding to its epidemiological role.

We used a simple definition for nosocomial CPO infections, which considered doubtful cases as negative. Therefore, we may have an underestimation bias. According to our criteria, the incidence of nosocomial CPO infection was 4.40 %, which is lower than that described in other publications.

**Other publications**

We found other studies that focused on the topic of CPO epidemiology. Even tough, and notably, we did not find any Portuguese study with a methodology similar to ours, that is, based on a systematic CPO screening carried at a hospital level. Still, these smaller studies performed a more in-depth study of the carbapenemase subtypes and are therefore worth mentioning. Regarding to non-Portuguese articles, we found some which studied the incidence of nosocomial CPO infection, although with some methodological differences.

One of the oldest Portuguese studies regarding CPO epidemiology was carried out between 2006 and 2013 (13). The authors collected samples from 13 hospitals in various regions of Portugal, in the context of a national surveillance network, and found a predominance of KPC-3 (85.71 %), in a total of 35 isolated CPO. Guiana extended-spectrum-5 (GES) (11.43 %) and VIM-2 (2.86 %) were the other types of carbapenemases identified. The calculated prevalence of CPO was 1.7 % ($n$ = 35 / 2105 total samples).

A subsequent study (2013-2018) carried out in a hospital in Lisbon (Central-South region of Portugal), involving a total of 46 carbapenemase-producing *K. pneumoniae*, also demonstrated a predominance of KPC-3 (78 %), followed by OXA-181 (20 %) and GES-5 (17 %) (14). More recently, a study was published that evaluated 106 carbapenemase-producing *K. pneumoniae* collected between 2018 and 2019 at the *Centro Hospitalar de Trás-os-Montes e Alto Douro*, located in Vila Real, Northern Portugal (15). The authors found a predominance of KPC-2 (91 %), followed by OXA-48 (9 %), which contrast with previous publications. Also, they found an isolate with co-production of KPC-2 and GES-5. Although we must emphasize the temporal, geographical, and methodological differences between these other Portuguese studies and ours, we highlight that the predominance of KPC is unequivocal in all of them. We also highlight the presence of 11.95 % metallo-β-lactamases in our hospital, something that was not recorded in

any of those studies. Possibly, such difference can be partially explained by the fact that our study included strains of carbapenemase-producing *P. aeruginosa*, which was not found in any of the others.

Regarding to non-Portuguese articles, in 2017 a systematic review was published, which included ten retrospective studies about CPO epidemiology (9). These articles reported to the following locations: Canada, Germany, Greece, Israel, Republic of Korea and United States of America. Only one of them was based on CPO screening carried out at hospital level, and, in all of them, the screening method used was the cultural exam. Also, most of those studies only used MAC as the culture medium. In the systematic review, authors found an average nosocomial CPO infection of 16.5 %, although reporting a wide range of values (7.6-44.4 %). The hypotheses suggested for this wide range were the distinct epidemiological characteristics of the studies, whether in terms of types of microorganisms, patient population or clinical environment. A subsequent prospective study, carried out in Thailand, pointed to rates of the same order of magnitude (20 %) (16). Although our main objectives were very similar to those articles', we found differences in methodology and in terms of secondary objectives. Namely, as clinical pathologists, one of our goals was to evaluate and improve the performance of the laboratory protocol. Also, the aforementioned studies used cultural exam as a screening method, which was not our case. Therefore, the results presented may highlight differences inherent to the use of molecular methods in CPO screening, the use of a selective and chromogenic medium (CARB/OXA), as well as due to the epidemiological particularities of our hospital.

**Limitations**

Our study has several limitations. This should be considered when comparing our results with other similar studies. First, we only studied patients who were screened for CPO colonization. Secondly, our hospital's CPO screening criteria in 2019-2021 were not broad enough, focusing on screening patients with risk factors for CPO colonization. As mentioned, those criteria have since been significantly changed, and that is one of the reasons we only analysed this three-year period. Furthermore, the cultural exam was only performed for positive molecular samples, and non-colonization was assumed in all

cases of negative molecular samples, which prevents the calculation of sensitivity and specificity of the screening protocol. Also, two different samples were used for the (molecular) screening and the cultural exam. Even though the samples were collected at the same time, a small bias should be considered. Another important limitation is related to the molecular method Xpert® Carba-R, which only identifies five types of carbapanemases. Although these are the most frequent ones in Portugal according to the literature (13-15), it represents an important bias. Namely, because Xpert® Carba-R does not identifies neither GES nor OXA-carbapenemases besides OXA-48. As this is retrospective study, we are limited to the available data. This molecular method was selected by our laboratory for its applicability in screening, as it is a point-of-care method, which combines rapid response with the reduced need for specialized technical processing. Unfortunately, it is not the best method for an extensive epidemiological analysis. As such, our study does not evaluate the entire epidemiology of CPO colonization in our hospital. However, despite all these limitations, we believe that our results are sufficiently representative.

We recognize that this study would have been more complete if we had also enrolled the subgroup of patients with a negative CPO screening upon admission. Unfortunately, during the study period, screenings during hospitalization were not carried out systematically. Therefore, it would be very difficult to establish standardization criteria and draw conclusions. Furthermore, we found many flaws in admission screenings. For example, we registered many cases of patients who screened positive during hospitalization (i.e. CPO screening performed after the third day), but who had not been screened upon admission. That is the main reason why, from a pool of 528 patients, 318 (60.23 %) were excluded from the “selected patients” category.

Lastly, it is a universal concept that local epidemiology is always changing. Consequently, it is necessary to carry out epidemiological surveillance on a recurrent basis and adjust prevention measures accordingly. Our hospital is a paradigmatic example, given that after an outbreak of CPO was recorded in mid-2022, several readjustments were made to the screening protocol. Currently, admission CPO screening criteria are more wide-ranging, and all admitted patients are screened once a week. Although this change is

welcome, unfortunately, it makes our study partially obsolete, as it no longer reflects our screening reality. Therefore, it would be opportune to reproduce this study in the future, in order to evaluate possible epidemiological changes.

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REVISTA DE MEDICINA DE LABORATORIO

Table I. Antibiogram profiles for *K. pneumoniae* and *E. coli* KPC strains

| | ***K. pneumoniae* KPC strains (*n* = 201)** | | | ***E. coli* KPC strains (*n* = 54)** | | |
|---|---|---|---|---|---|---|
| **Antibiotics profile** | **S** | **I** | **R** | **S** | **I** | **R** |
| Ampicillin | | | *intrinsic resistance | 0 | 0 | 53 (100 %) |
| Amoxicillin-clavulanic acid | 0 | 0 | 196 (100 %) | 2 | 0 | 50 (96.15 %) |
| Piperacillin-tazobactam | 0 | 0 | 199 (100 %) | 6 | 0 | 48 (88,89 %) |
| Cefuroxime | 1 | 0 | 194 (99.49 %) | 1 | 1 | 49 (96,08 %) |
| Ceftazidime | 3 | 0 | 196 (98.49 %) | 6 | 3 | 45 (83.33 %) |
| Cefotaxime | 3 | 1 | 195 (97.99 %) | 10 | 5 | 38 (71.70 %) |
| Cefepime | 12 | 90 | 97 (48.74 %) | 15 | 22 | 17 (31.48 %) |
| Ertapenem | 2 | 0 | 195 (98.98 %) | 7 | 0 | 45 (86.54 %) |
| Meropenem | 34 | 59 | 103 (52.55 %) | 32 | 7 | 11 (22.00 %) |
| Imipenem | 25 | 9 | 163 (82.65 %) | 18 | 10 | 23 (45.10 %) |
| Amikacin | 186 | 7 | 6 (3.02 %) | 50 | 1 | 3 (5.56 %) |
| Gentamicin | 164 | 0 | 35 (17.59 %) | 40 | 0 | 14 (25.93 %) |
| Ciprofloxacin | 32 | 10 | 157 (78.89 %) | 23 | 4 | 25 (48.08 %) |
| Trimethoprim-sulfamethoxazole | 101 | 0 | 98 (49.25 %) | 25 | 0 | 29 (53.70 %) |
| Colistin | 190 | 0 | 3 (1.55 %) | 46 | 0 | 2 (4.17 %) |

KPC: *K. pneumoniae* carbapenemase.

Table II. Characterization of the selected patient population

| **Selected patients (*n* = 210)** | | |
|---|---|---|
| **Age** | **average** | |
| Male (range: 22-100 y.o.) | 70.34 y.o. | |
| Female (range: 37-101 y.o.) | 79.53 y.o. | |
| **Sex** | ***n*** | **%** |
| Male | 119 | 56.67 % |
| Female | 91 | 43.33 |

| | | % |
|---|---|---|
| **Comorbidities (average 3.77)** | | |
| Arterial hypertension | 131 | 62.38 % |
| Dyslipidemia | 89 | 42.38 % |
| **Diabetes *mellitus*** | 71 | 33.81 % |
| Chronic kidney disease | 54 | 25.71 % |
| Neoplasia (any type) | 45 | 21.43 % |
| Cerebrovascular disease | 42 | 20.00 % |
| Atrial fibrillation (AF) | 38 | 18.10 % |
| Other arrhythmias (non-AF) | 9 | 4.29 % |
| Smoker/ex-smoker | 36 | 17.14 % |
| Heart failure | 35 | 16.67 % |
| Obesity | 30 | 14.29 % |
| Ischemic heart disease | 29 | 13.81 % |
| Chronic obstructive pulmonary disease/bronchiectasis | 19 | 9.05 % |
| Peripheral vascular disease | 16 | 7.62 % |
| Alcoholism | 15 | 7.14 % |
| Obstructive sleep apnea syndrome | 13 | 6.19 % |
| Asthma | 2 | 0.95 |

| | | |
|---|---|---|
| | | % |
| *Dependence for daily activities* | 117 | 55.71 % |
| • Partial dependence | 55 | 26.19 % |
| • Total dependence | 62 | 29.52 % |
| **Risk factors for CPO colonization (average 2.10)** | | |
| *Risk factor for CPO contact transmission* | 165 | 78.57 % |
| • Hospitalized < 3 months prior to admission | 108 | 51.43 % |
| • Living in nursing homes or equivalent places | 47 | 22.38 % |
| • Regular contact with hospital-like centers | 10 | 4.76 % |
| Antibiotic treatment < 6 months prior to admission | 147 | 70.00 % |
| Chronic proton pump inhibitors treatment | 92 | 43.81 % |
| Chronic wounds | 22 | 10.48 % |
| Medical devices | 14 | 6.67 % |
| **Motive for admission** | ***n*** | **%** |
| Medical problem | 165 | 78.57 % |
| Acute surgical problem | 26 | 12.38 % |
| Scheduled surgery | 19 | 9.05 % |
| **Clinical and microbiological evaluation** | ***n*** | **%** |
| No-infection | 93 | 44.29 % |
| *Infection* | 11 | 55.71 |

| | | |
|---|---|---|
| | 7 | % |
| • Pulmonary infection | 46 | 39.32 % |
| • Urinary tract infection | 25 | 21.37 % |
| • Bloodstream infection | 13 | 11.11 % |
| • Infected abscess | 13 | 11.11 % |
| • Open wounds | 6 | 5.13 % |
| • Other | 14 | 11.97 % |

AF: atrial fibrillation; CPO: carbapenemase-producing organisms; y.o.: years-old.

| **Selected patients (*n* = 210), *de novo* positive molecular screening (2019-2021)** | | | | | |
|---|---|---|---|---|---|
| **Cultural exam/Clinical subgroup** | **No infection** | **Non-CPO infection** | **CPO infection** | **Infection + Insufficient data** | **Total** |
| Negative culture | 55 (48.25 %) | 39 (34.21 %) | 5 (4.39 %) | 15 (13.16 %) | 114 (54.29 %) |
| Positive culture | 37 (38.54 %) | 33 (34.38 %) | 16 (16.67 %) | 10 (10.42 %) | 96 (45.71 %) |
| Total | 93 (44.29 %) | 72 (34.29 %) | 20 (9.52 %) | 25 (11.90 %) | 210 (100 %) |
| **Selected patients, cultural exam with MacConkey agar (2019-September/2020) (*n* = 114)** | | | | | |
| Negative culture | 32 (41.56 %) | 30 (38.96 %) | 5 (6.49 %) | 10 (12.99 %) | 77 (68.14 %) |
| Positive culture | 14 (38.89 %) | 9 (25 %) | 7 (19.44 %) | 6 (16.67 %) | 37 (31.86 %) |
| **Selected patients, cultural exam with ChromID® Carba Smart agar + MacConkey agar (October/2020-2021) (*n* = 96)** | | | | | |
| Negative culture | 23 (62.16 %) | 9 (24.32 %) | 0 (0 %) | 5 (13.51 %) | 37 (38.14 %) |
| Positive culture | 23 (38.33 %) | 24 (40 %) | 9 (15 %) | 4 (6.67 %) | 60 (61.86 %) |

Table III. Analysis of selected patients (*n* = 210), matched by clinical subgroups and the result of the cultural exam. Sub-analysis based on the culture medium used: MacConkey agar (2019-September/2020) *versus* ChromID® Carba Smart agar + MacConkey agar (October/2020-2021)

CPO: carbapenemase-producing organisms.

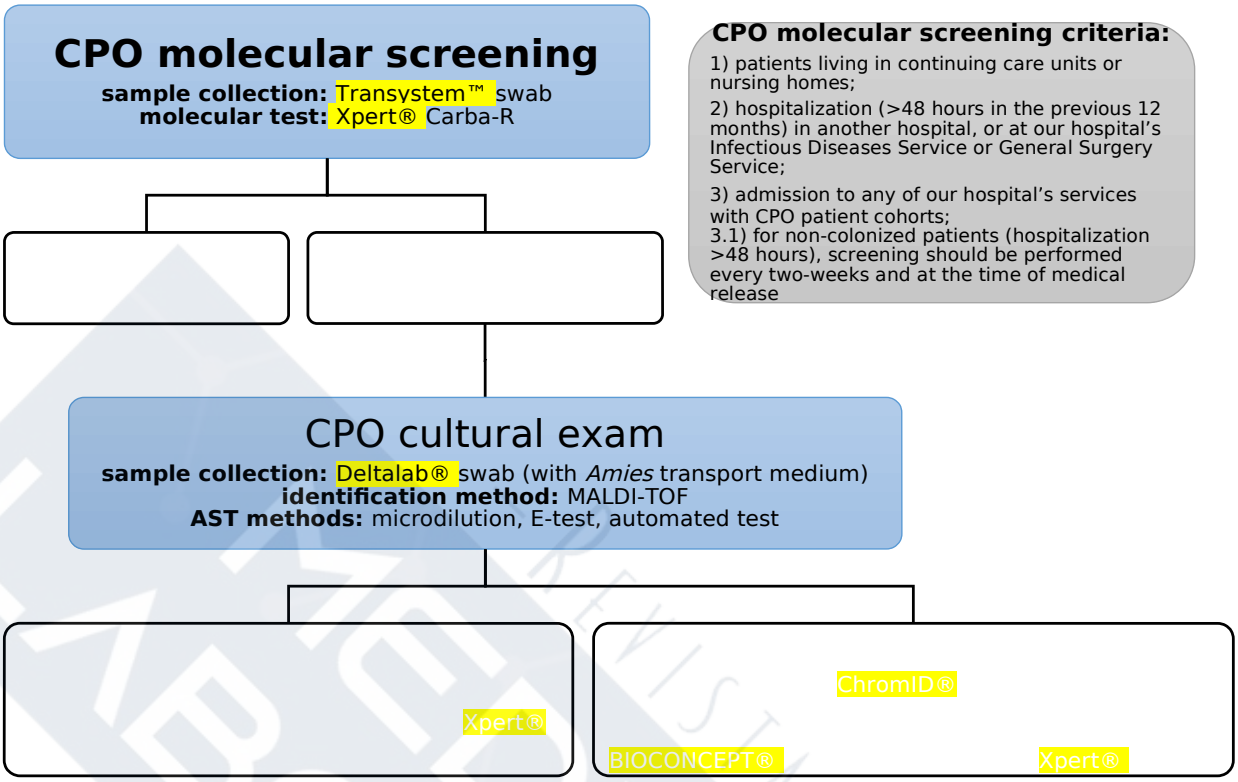


Figure 1. Scheme of the laboratory protocol for CPO screening (AST: antibiotic susceptibility testing; CPO: carbapenemase-producing organisms; MALDI-TOF: Matrix-Assisted Laser Desorption/Ionization.

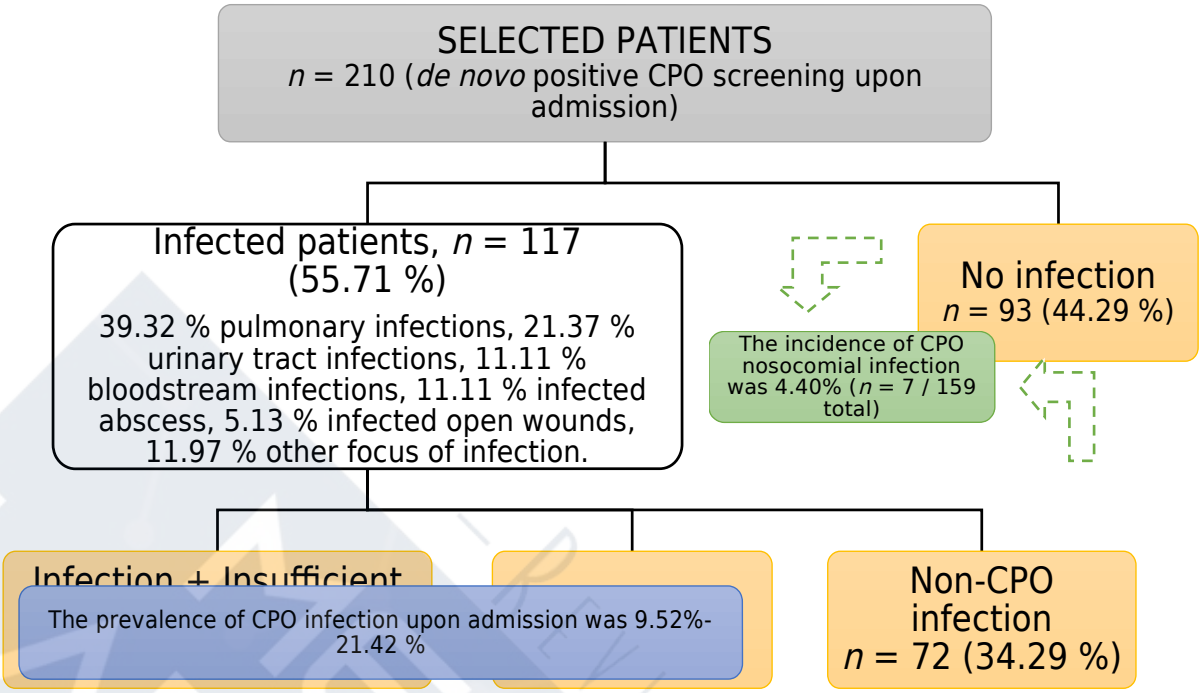


Figure 2. Subcategorization of selected patients in four subgroups (yellow), according to the etiology of hospitalization (CPO: carbapenemase-producing organisms).

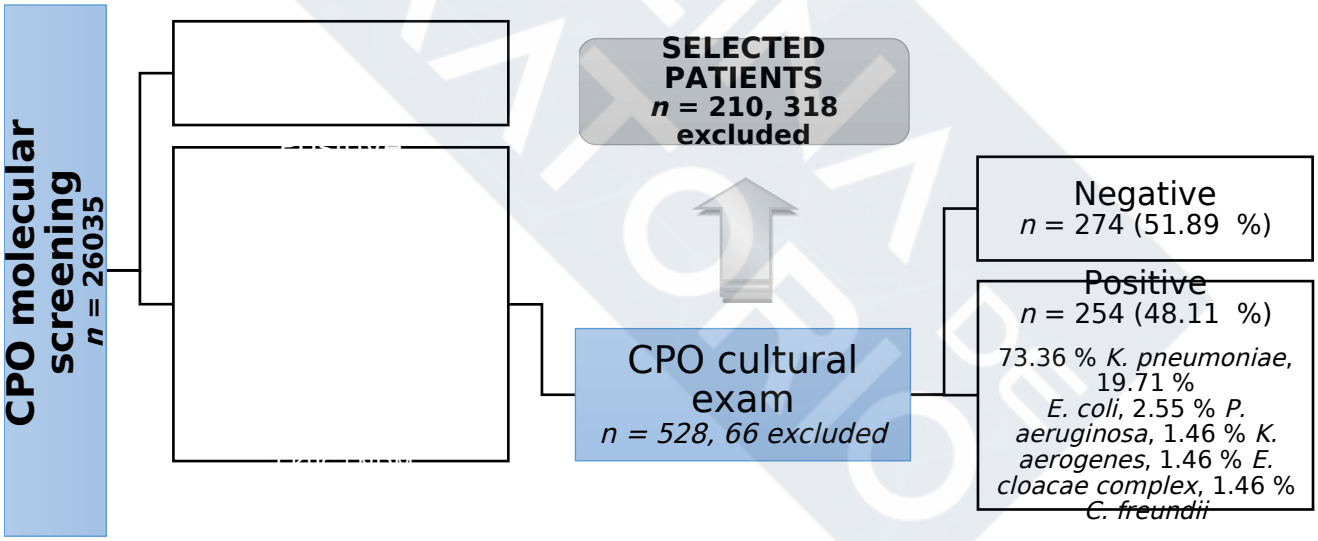


Figure 3. Summary of laboratory protocol data analysis (2019-2021) (CPO: carbapenemase-producing organisms; KPC: *K. pneumoniae* carbapenemase; NDM: New Delhi metallo-β-lactamase; OXA-48: oxacillinase-48; VIM: Verona integron-mediated metallo-β-lactamase.