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Colonização por *Enterobacteriaceae* resistente a carbapenêmicos em pacientes internados no pronto-socorro e em unidades de terapia intensiva: epidemiologia, prevalência e fatores de risco

Tese apresentada à Faculdade de Medicina da Universidade de São Paulo para obtenção do título de Doutor em Ciências

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Orientadora: Profa. Dra. Anna Sara Shafferman Levin

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Carbapenem resistant *Enterobacteriaceae* colonization in patients admitted to the emergency department and in intensive care units: epidemiology, prevalence and risk factors

Thesis submitted to "Faculdade de Medicina da Universidade de São Paulo according to requirements for the degree of Doctor in Science

Program of Doenças Infecciosas e Parasitárias

Advisor: Profa. Dra. Anna Sara Shafferman Levin

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“Eu acho que nós, bois, - Dançador diz, com baba - assim como os cachorros, as pedras, as árvores, somos pessoas soltas, com beiradas, começo e fim: o homem não: o homem pode se ajuntar com as coisas, se encostar nelas, crescer, mudar de forma e jeito..."

Guimarães Rosa

DEDICATÓRIA

À minha esposa **Nayra**, o amor da minha vida.

Ao meu filho **Flávio**, que ainda é pequeno para entender, mas sabe o amor que eu sinto por ele.

Aos meus pais, **Reinaldo** e **Regina**, que me guiam desde o nascimento até agora e sempre.

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NORMALIZAÇÃO

Esta tese foi confeccionada no formato que enfatiza a produção de artigos científicos e está de acordo com as seguintes normas, em vigor no momento desta publicação:

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Esta tese foi redigida em inglês: a Universidade de São Paulo e o programa de pós-graduação de Doenças Infecciosas estimulam a redação neste idioma. Os métodos estão apresentados em português, conforme determinado pelo programa de pós-graduação, e estão traduzidos e apresentados em inglês nos apêndices ao final da tese.

SUMÁRIO

SUMMARY

LISTAS

ABREVIATURAS, SIGLAS E SÍMBOLOS

BHU	-	*Basic Health Units*
BKC	-	*Brazilian Klebsiella Carbapenemase*
CCIH	-	Comissão de Controle de Infecção Hospitalar
CDC	-	*Centers for Disease Control and Prevention*
CLABSI	-	*Central Line–Associated BloodStream Infections*
CLSI	-	*Clinical and Laboratory Standards Institute*
CRE	-	*Carbapenem Resistant Enterobacteriaceae*
CROSS	-	Central de Regulação de Ofertas de Serviços de Saúde
CVE	-	Centro de Vigilância Epidemiológica - Alexandre Vranjak
DLC	-	Divisão de Laboratórios Central
ED	-	*Emergency Department*
ERC	-	Enterobactéria Resistente a Carbapenêmico
ESBL	-	Extended spectrum beta-lactamase
EUCAST	-	*European Committee on Antimicrobial Susceptibility Testing*
GDP	-	G*ross domestic product*
GIM	-	German imipenemase
HCA-MRSA	-	Hospital community-acquired *Methicillin Resistant Staphylococcus aureus*
HCFMUSP	-	Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo
ICHC	-	Instituto Central do Hospital das Clínicas
ICU	-	Intensive Care Unit
IMP	-	Imipenemase
LTCF	-	long-term care facilities

Maldi ToF	-	Matrix Assisted Laser Desorption Ionization Time Of Flight Mass Spectrometry
MCR-1	-	mobile colistin resistance-1
MDRO	-	multi-drug resistant organisms
MHT	-	Modified Hodge Test
MIC	-	*Minimal Inhibitory Concentration*
MRSA	-	*Methicillin Resistant Staphylococcus aureus*
NDM	-	*New Delhi*-metallo-betalactamase
PCR	-	*Polymerase Chain Reaction*
PS	-	Pronto Socorro
RT-PCR	-	Reação em Cadeia de Polimerase em tempo real (*Real-time Polymerase Chain Reaction*)
SAPS 3	-	*Simplified Acute Physiology Score 3*
SCCIH	-	Subcomissão de Controle de Infecção Hospitalar
SOFA	-	*Sequential Organ Failure Assessment*
SUS	-	Sistema Único de Saúde
UTI	-	Unidade de Terapia Intensiva
VIM	-	Verona imipenemase
WHO	-	*World Health Organization*

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RESUMO

Salomão MC. *Colonização por Enterobacteriaceae resistente a carbapenêmicos em pacientes internados no pronto-socorro e em unidades de terapia intensiva*: epidemiologia, prevalência e fatores de risco [tese]. São Paulo: Faculdade de Medicina, Universidade de São Paulo; 2020.

Enterobactérias resistentes a carbapenêmicos (ERC) já foram relatadas em todo o mundo e são associadas a altas taxas de mortalidade. A colonização intestinal atua como um reservatório e favorece a transmissão de mecanismos de resistência. O objetivo desta tese foi investigar a prevalência de pacientes portadores de CRE na admissão hospitalar, fatores de risco associados e a taxa de aquisição no Pronto Socorro (PS) e seu impacto na colonização por ERC na admissão em Unidades de Terapia Intensiva (UTI). Realizamos um estudo transversal com 676 pacientes admitidos no pronto-socorro. Foram coletados swabs retais de pacientes na admissão e após uma semana, para cultura e para reação em cadeia da polimerase multiplex em tempo real (RT-PCR). Quarenta e seis pacientes (6,8%) foram colonizados e a taxa de aquisição foi de 18%. A exposição prévia à assistência médica no último ano, hepatopatia e uso de antibióticos no último mês foram fatores de risco para a colonização. Seis pacientes sem exposição prévia aos cuidados de saúde foram colonizados por ERC na admissão, sugerindo transmissão de ERC dentro da comunidade. No outro estudo, avaliamos o impacto de hospitalizações anteriores no setor de emergência na colonização por ERC na admissão na UTI. Neste estudo caso-controle, comparamos pacientes colonizados por ERC com pacientes que não foram colonizados na admissão na UTI. Os fatores de risco encontrados foram: tempo de internação no Pronto Socorro, aumentando a cada dia de internação, porém com maior impacto após o segundo dia, uso de carbapenem, *Simplified Acute Physiology Score 3* (SAPS 3), endoscopia digestiva alta e transferência de outro hospital. O conjunto de achados desta tese demonstra que a internação no PS aumenta o risco de colonização por ERC no PS e leva ao aumento de risco de colonização por ERC na admissão na UTI. Nossos achados indicam que atuar no controle de ERC no PS ajudará a controlar a resistência a carbapenem nas UTIs.

Descritores: Enterobacteriáceas resistentes a carbapenêmicos; Serviço hospitalar de emergência; Unidades de terapia intensiva; Infecção hospitalar; Resistência microbiana a medicamentos; Infecções comunitárias adquiridas

ABSTRACT

Salomão MC. *Carbapenem resistant Enterobacteriaceae colonization in patients admitted to the emergency department and in intensive care units*: epidemiology, prevalence and risk factors [thesis]. São Paulo: “Faculdade de Medicina, Universidade de São Paulo”; 2020.

Carbapenem Resistant *Enterobacteriaceae* (CRE) are worldwide reported and associated with high mortality rates. Intestinal colonization acts as a reservoir and fosters resistance mechanisms exchange. The aim of this thesis was to investigate the prevalence of patients harboring CRE on hospital admission, risk factors associated and the acquisition rate within the emergency department (ED) and its impact on colonization by CRE on admission to the Intensive Care Units (ICU). We conducted a cross-sectional study with 676 patients admitted to the ED. We collected rectal swabs from patients on admission, and after one week, for culture and for multiplex real-time polymerase chain-reaction (RT-PCR). Forty-six patients (6.8%) were colonized and the acquisition rate was 18%. Previous exposure to healthcare in the last year, hepatopathy and use of antibiotics in the last month were risk factors for colonization. Six patients with no previous exposure to healthcare were CRE-colonized on admission, suggesting transmission of CRE within the community. In the other study, we evaluated the impact of previous hospitalization in the emergency department on CRE colonization at ICU admission. In this case–control study we compared patients colonized by CRE to patients that were not colonized on admission to ICU. The risk factors found were emergency-department stay, increasing every day, but more importantly after 2 days of hospitalization in the ED, use of carbapenem, Simplified Acute Physiology Score (SAPS 3), upper digestive endoscopy, and transfer from another hospital. This thesis demonstrates that ED hospitalization increases the risk for CRE colonization and it has impacts on colonization on ICU admission. Our findings indicate that addressing problems in the ED will help to control carbapenem resistance in ICU.

Descriptors: Carbapenem-resistant enterobacteriaceae; Emergency service, hospitals; Intensive care units; Cross infection; Drug resistance, microbial; Community-acquired infections.

Chapter 1 - INTRODUCTION

Chapter 1 - INTRODUCTION

Hospital infections are a major public health issue. The World Health Organization (WHO) highlights their impact on rising costs for health systems, prolonged hospitalization, as well as increased morbidity, mortality, and antimicrobial resistance (WHO, 2014).

In 2013, the Centers for Disease Control and Prevention (CDC), Atlanta, USA, listed carbapenem-resistant Enterobacteriaceae (CRE) among the main threats to human health, held responsible for 9000 infections and 600 deaths per year in the US. They are classified as a threat that needs urgent intervention, with risk of developing resistance to all antibiotics available to human use (CDC, 2013).

Enterobacteria are gram negative bacteria of the *Enterobacteriaceae* family and are important causes of nosocomial infections, such as *Klebsiella pneumoniae, Escherichia coli,* and *Enterobacter sp*.

Since the emergence of extended spectrum betalactamases (ESBL), the bacteria in this family demonstrated great potential for antibiotic resistance. The emergence of enzymes capable of hydrolyzing a wide range of antimicrobials led to an overall increase in antibiotic resistance, either by their direct or indirect participation through selection pressure (Stewardson et al., 2013; Swaminathan et al., 2013).

In 1983, SHV-2, the first beta-lactamase to be considered an ESBL, was described (Kliebe et al., 1985). With the emergence of other ESBLs and

their dissemination in health services, the use of carbapenems increased, leading to the emergence of the carbapenemases.

Less than 15 years after the emergence of SHV-2, *Klebsiella pneumoniae* carbapenemase (KPC) was described in 1996, an enzyme capable of hydrolyzing all known beta-lactams (Yigit et al., 2001). In 2009, New Delhi metallo-betalactamase (NDM) was described. This enzyme, like KPC, is also capable to hydrolyze carbapenems. NDM is another important cause of bacterial resistance around the world, and its global spread is related to medical tourism (Kumarasamy et al., 2010).

Surveillance data from the Intensive Care Units (ICU) of the State of São Paulo demonstrate dissemination of CRE in virtually all municipalities of the state, with carbapenem-resistance rates of *Klebsiella pneumoniae* in bloodstream infections reaching 55% (CVE, 2016).

An important factor for the spread of these enzymes among *Enterobacteriaceae* lies in the fact these genes are located on plasmids, which easily permits their interspecies transmission.

The spread of resistance, coupled with the lack of new antimicrobial drugs, caused the scientific community to question whether we had reached the “End of the Antibiotic Age” (Yoshikawa, 2002; Nordmann et al., 2011), and the WHO classified carbapenem-resistant *Enterobacteriaceae* (CRE) as one of the 3 major threats to human health (WHO, 2014).

Carbapenemases

Carbapenemases can be divided into classes or groups according to 2 classifications: Ambler (1980), and Bush, Jacoby and Medeiros (1995). The most recent was revised by Bush and Jacoby in 2010.

Ambler's classification is based on the molecular structure of enzymes and is divided into four classes: A, B, C, and D. Classes A, C and D have serines at the active site of their enzymes, while class B is composed of enzymes with zinc in its active site. Bush, Jacoby and Medeiros (1995) classified betalactamases according to their substrates and inhibition profiles, in groups from 1 to 4, and subdivisions. Carbapenemases belong to Ambler's classes A, B and D, and to Bush and Jacoby's groups 2df, 2f, 3a and 3b.

Ambler class A carbapenemases are serine carbapenemases and are capable of hydrolyzing penicillins, cephalosporins, carbapenems, and monobactamics; and are not inhibited by EDTA, clavulanic acid, or tazobactam (Ambler, 1980; Bush; Jacoby, 2010).

An important representative of Ambler Class A, KPC has been found in several types of bacteria, such as: *Enterobacteriaceae* (*Klebsiella* spp*., Escherichia coli, Enterobacter* spp*., Citrobacter* spp*., Morganella* spp*., Serratia marcescens, Raoultella* spp*., Kluyvera,* spp and *Salmonella* spp*)*; and non-*Enterobacteriaceae*: (*Aeromonas, Pseudomonas,* and *Acinetobacter baumannii)* (Viau et al., 2016). Its presence is associated with increased mortality, with epidemiological studies suggesting that its presence may also be associated with increased virulence: ST258 isolates carrying the gene

that encodes KPC-2 (*bla*KPC-2) have a more virulent behavior than those carrying the *bla*KPC-3 gene (Marchaim et al., 2008; Ramirez et al., 2014).

Metallobetalactamases are Ambler class B carbapenemases and are named because they have zinc (Zn + 2) in their active site. They hydrolyze all beta-lactams except monobactams, and are inhibited by EDTA but not by beta-lactamase inhibitors. They may be located on chromosomes or plasmids (Ambler, 1980; Bush; Jacoby, 2010; Martinez-Martinez; Gonzalez-López, 2014). Plasmid metallobetalactamases are more studied due to their potential for dissemination, with emphasis on IMP (imipenemase), VIM (Verona imipenemase), GIM (German imipenemase), and NDM-1.

Class D is represented by OXA type carbapenemases. OXA betalactamases were named after their predilection for oxacillin as a substrate, but have variable beta-lactam hydrolysis capacity. They may even have activity against carbapenems, but with a low hydrolysis rate. OXA-48 was first described in Turkey in 2001 and, despite being located on a plasmid, it only spread in 2008 reaching Western Europe, the Middle East, and North Africa. Other important carbapenemases of clinical relevance are OXA-181, OXA-204, OXA-232, and OXA-162, more commonly found in *Enterobacteriaceae*, while OXA-23 and OXA-24/40, are usually found in *Acinetobacter baumannii* (Poirel et al., 2010).

Table 1 - Carbapenemases according to Ambler classification, site of activity, inhibitors, and spectrum of enzymatic activity

Ambler Classification	Active site	Inhibitors	Most common carbapenemases	Substrate
A	Serin	Avibactam and other carbapenemase inhibitors under study	KPC, GES	Hydrolysis of all beta-lactams with high carbapenem hydrolysis rate
B	Metallo (Zinc)	EDTA	VIM, IMP, NDM	Hydrolysis of all beta-lactams, sparing monobactams
D	Serin	NaCl (*in vitro*)	OXA-48	Variable hydrolysis capacity, may have activity against carbapenems

Screening, prevalence and control strategies

The potential for dissemination of carbapenemases through plasmids, including interspecies dissemination, makes them a major challenge for control in hospitals. In this context, intestinal CRE carriage may act as an important reservoir and potential disseminator of intrahospital resistance mechanisms. This situation demands programs to diagnose and control CRE colonization in order to prevent spread (Calfee; Jenkins, 2008; Kochar et al., 2009; Geffen et al., 2010; Enfield et al., 2014).

Tests focused on detecting carbapenanse genes may be important tools to identify carbapenemase-producing organisms without the need for

cultures and antimicrobial susceptibility tests (TSA), thus reducing the time to obtain results.

Culture automated tests have already been tested for this purpose, but were not considered suitable for epidemiological analysis. In a study by Anderson et al. (2007), almost 100% of the KPC producers were identified by microdilution, but when using the Vitek2 platform, ertapenem resistance failed to be detected in 6% of the isolates.

Tests to detect carbapenemase activity, such as the Modified Hodge Test (MHT), cannot be performed directly on specimens, requiring further growth in culture medium leading to a delay in obtaining results. In the specific case of MHT, it has low specificity, may be positive in the presence of ESBL, and low sensitivity for NDM and OXA. The test with phenyl boronic acid has a better performance, with good sensitivity and specificity, but requires time for cultivation (Viau et al., 2016).

Commercial chromogenic tests, such as Carba NP test, Blue-Carba test, and Rapid Carb test, are able to detect carbapenemase activity, without specifying which enzyme is present. In general, they have good sensitivity (97.9%) and specificity (100%) (CarbaNP), but have worse performance for OXAs, and *Acinetobacter* (Tijet et al., 2013; Osterblad et al., 2014; Dortet et al., 2014a; Dortet et al., 2014b). Other immunochromatographic tests such as NG Carba 5 are capable to define which carbapenemase family is involved (KPC, NDM, VIM, IMP and OXA-48), but also require prior isolation in culture before the test can be used (Hopkins et al., 2018).

Universal screening strategies with RT-PCR methods present as a main obstacle their high cost, but their high sensitivity and specificity, possibility of obtaining results directly from the sample, short turnaround time, and possibility to determine specifically which carbapenemase is involved, make them an attractive screening tool, especially when applied to patients at high risk for CRE colonization (Viau et al., 2016).

Thus, the main challenges for screening for carbapenemase-producing bacteria are: combining rapid detection; detecting strains with low levels of carbapenemase production; and detecting minority strains in the sample. A good screening test is one that minimizes detection time, maximizes sensitivity, preserves specificity, detects multiple types of carbapenemases, and is, above all, cost-effective (Viau et al., 2016).

CRE surveillance is usually performed in intensive care unit (ICU) patients as these are critical areas where early identification and isolation of colonized patients could lead to decreased secondary colonization and possibly translate into lower rates of complication (Calfee, 2008; Kochar et al., 2009. Enfield et al., 2014).

The main classical risk factors for colonization by CRE are: previous colonization by CRE, hospitalization in acute or long-term care facilities, use of antibiotics, and use of invasive devices (Yamamoto et al., 2017. Asai et al., 2018).

There is a paucity of studies evaluating risk factors for CRE colonization in the Emergency Department (ED), which is classically considered a low risk area. Risk factors for CRE colonization in the ED

already described are: age over 70 years, hospitalizations in long-term care facilities, stroke, hospital discharge in the last month, and antibiotic use for more than 48 hours in the last 3 months. These factors were associated with a higher risk of future infection with gram negative bacilli (Tseng et al., 2017).

In Brazil, few studies specifically evaluated colonization by CRE and its risk factors. Colonization by KPC-producing *Enterobacteriaceae* in intensive care units in Brazil can reach up to 30.4% (Perez et al., 2015). At the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC), the average colonization rate on admission to the ICU is approximately 20% (unpublished data), but the exact location of CRE acquisition and risk factors are still unclear.

In HC, a tertiary referral unit for emergency care in the Unified Health System (SUS – *Sistema Único de Saúde*, in Portuguese), around 30% of the patients admitted to the ICUs are admitted via ED. Due to hospital overcrowding, beds cannot be promptly obtained for all patients, causing many patients to be hospitalized for a long period of time on stretchers in the ED, often very close to each other. This could facilitate the spread of CRE among patients. Half of the patients hospitalized via ED are discharged directly from the ED, without being admitted to a hospital inpatient unit. Unfortunately, the detection of carbapenem-resistant agents in patients, who originate from the ED, on admission to the ICU is not uncommon.

Rationale

Understanding the rate of CRE-colonization in the ED and risk factors, as well as the factors associated with CRE colonization on ICU admission, may allow us to early intervene in the chain of transmission, preventing spread throughout the hospital.

Despite the spread of KPC across the country and around the world, the risk factors for colonization by CRE are still unclear. In this context, it is also important to evaluate the impact of hospitalization in the emergency department on the risk of patients colonized by CRE.

Objectives

1. To evaluate the prevalence, acquisition rate, and risk factors for colonization by CRE in patients admitted to the emergency department;
2. To evaluate the factors associated with colonization by CRE upon admission to intensive care, especially whether hospitalization in the emergency room is a risk factor.

Chapter 2 -

Evaluation of the prevalence of colonization and cross-transmission of carbapenem resistant *Enterobacteriaceae* in patients admitted to the emergency department of the Instituto Central do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo

Chapter 2 - EVALUATION OF THE PREVALENCE OF COLONIZATION AND CROSS-TRANSMISSION OF CARBAPENEM RESISTANT *ENTEROBACTERIACEAE* IN PATIENTS ADMITTED TO THE EMERGENCY DEPARTMENT OF THE INSTITUTO CENTRAL DO HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DE SÃO PAULO

The English version of the research project can be seen in Appendix 1.

2.1 PROJETO DE PESQUISA: Avaliação da prevalência de colonização e transmissão cruzada de enterobactérias resistentes a carbapenêmicos em pacientes admitidos no Pronto Socorro do Instituto Central do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo

OBJETIVOS

1. Investigar a prevalência de pacientes portadores de enterobactérias resistentes a carbapenêmicos à admissão no pronto socorro;
2. Investigar os fatores de risco para colonização por enterobactérias resistentes a carbapenêmicos em pacientes admitidos no pronto socorro;
3. Investigar a taxa de transmissão de enterobactérias resistentes a carbapenêmicos no pronto socorro.

MATERIAIS E MÉTODOS

Método

Foi realizado um estudo prospectivo de prevalência com duração de dois meses, 31 de maio a 7 de julho de 2016, no Pronto Socorro do Instituto Central do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (ICHC-FMUSP). Neste período, foram incluídos consecutivamente todos os pacientes internados e admitidos via Pronto Socorro do ICHC-FMUSP, uma média de 20 admissões/dia.

No momento da admissão, foi preenchido um formulário para todos os pacientes com o objetivo de avaliar os fatores de risco para colonização por ERC.

Todos os pacientes admitidos no período de estudo tiveram culturas de vigilância e RT-PCR obtidos em até 24 horas a partir da admissão, à exceção de dois pacientes, que foram excluídos da análise. Para este fim, foram colhidos dois swabs retais e enviados, um, para microbiologia clássica e, outro, para o laboratório de biologia molecular. Os pacientes cujas culturas da admissão resultaram negativas foram seguidos, com o intuito de se realizar coletas semanais de dois swabs de vigilância que foram processados da mesma forma que aqueles da admissão. Estas coletas foram mantidas durante toda a internação no pronto socorro.

A vigilância individual do paciente foi interrompida quando houve identificação de ERC em cultura clínica ou de vigilância, ou no momento da alta/transferência do Pronto Socorro.

Microbiologia clássica

As culturas de vigilância para ERC foram realizadas de acordo com a metodologia do Centers for Disease Control (CDC) (Siegel et al., 2006), adotada pelo laboratório da Divisão de Laboratórios Central (DLC). O swab foi colocado em meio liquido, para enriquecimento, por 24 horas e, posteriormente, semeado em placar de Agar contendo discos de carbapenêmicos. A triagem foi feita por meio de crescimento de colônias suspeitas de enterobactérias dentro do halo de inibição dos antibióticos. A identificação foi realizada por MALDI-TOF (Vitek MS, BioMerieux ®, Marcy L'Étoile, França) e antibiograma foi automatizado (Vitek2), conforme rotina realizada pela DLC.

A identificação e sensibilidade de enterobactérias foram feitas por meio de método automatizado VITEK2 (BioMerieux ®, Marcy L'Étoile, França). As concentrações inibitórias mínimas (CIM) de colistina e carbapenêmicos foram obtidas inicialmente por VITEK2 (microdiluição em caldo Mueller-Hinton ajustado para cátion automatizado) e confirmadas por disco-difusão no caso dos carbapenêmicos e por E-test (BioMérieux®, Marcy L'Étoile, França) em gradiente de difusão (Agar Mueller-Hinton) para colistina de acordo com as recomendações do Clinical and Laboratory Standards Institute (CLSI) 2016. A interpretação do resultado da CIM de colistina seguiu recomendação do European Committee on Antimicrobial Susceptibility Testing (EUCAST) (≤ 2: sensível e >2: Resistente), já a interpretação do antibiograma em relação aos carbapenêmicos seguiu a

recomendação para Enterobacteriaceae do CLSI 2014 (imipenem ou meropenem≤ 2 µg/dl: sensível e >2 µg/dl: resistente).

Método molecular

A detecção de genes de resistência KPC, NDM, VIM, IMP-1, OXA-48, OXA-181 e OXA-232 foi realizada diretamente da amostras clínicas por metodologia de Real-Time PCR (Cepheid® Xpert® Carba-R). O material dos swabs foram eluídos em tampão e processados de acordo com as normas do kit (Cepheid® Xpert® Carba-R).

Desfechos analisados

1. Prevalência de pacientes portadores de ERC à admissão

A prevalência de pacientes portadores de ERC foi definida pelo número de pacientes com swab de vigilância positivo por meio de cultura aeróbia ou RT-PCR dividido pelo número total de admissões no período.

Prevalência = Número de pacientes com ERC à admissão/número de admissões.

2. Fatores de risco para colonização por ERC

Foram avaliados à admissão as seguintes variáveis: idade, sexo, endereço da residência do paciente, doença de base, motivo da internação, procedência domiciliar ou associada a assistência à saúde (hospitalar ou instituição de longa permanência, sendo considerado somente o período do último ano), se frequentou hospital-dia no último ano, ou hemodiálise no

último mês, insuficiência renal aguda ou crônica, hepatopatia, transplante de órgão sólido, transplante de célula tronco hematopoiética, infecção pelo vírus HIV, AIDS, imunossupressão por drogas, neutropenia, presença de dispositivo invasivo (cateter de longa permanência, dreno, sondas, estomias) e uso de antimicrobianos (> de 72 horas de uso nos últimos 30 dias).

3. Taxa de transmissão de ERC no pronto socorro

A taxa de transmissão no pronto-socorro foi obtida dividindo-se o número de pacientes com swab positivo nas coletas semanais durante a internação do pronto socorro pelo número de pacientes com swab negativo à admissão. Somente fizeram parte do denominador os pacientes que tiverem dois ou mais swabs colhidos durante sua permanência no pronto socorro.

Consideramos positivos, para ERC, os pacientes com isolado em cultura de vigilância cujos testes de sensibilidade descritos acima resultaram em CIM de imipenem ou meropenem maiores que 2 µg/dl, ou que tiveram RT-PCR positivo para qualquer carbapenemase.

Consideramos negativos, para ERC, os pacientes sem isolamento anterior em material clínico e, com pelo menos, um swab de vigilância negativo por meio de microbiologia clássica que tenha sido colhido no mesmo dia que o teste molecular.

Aspectos Éticos

Este estudo foi submetido e aprovado pela Comissão de Ética e Pesquisa do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo sob o número CAAE: 83262417.4.0000.0068. A coleta de culturas de vigilância é uma estratégia utilizada em outras áreas assistenciais do hospital, desta forma, foi aprovada dispensa da aplicação do termo de consentimento livre e esclarecido. Os dados da pesquisa serão apresentados somente em conjunto, sem nenhum tipo de identificação.

2.2 ARTICLE

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Article title: Carbapenem-resistant *Enterobacteriaceae* in patients admitted to the Emergency Department: new risk factors and occurrence in patients coming directly from the community

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Abstract:

Background: Carbapenem Resistant *Enterobacteriaceae* (CRE) are worldwide reported and associated with high mortality rates. Intestinal colonization acts as a reservoir and fosters resistance mechanisms exchange. Aim: The aim of this study was to investigate the prevalence of patients harboring CRE on hospital admission, risk factors associated and the acquisition rate within the emergency department (ED). Methods: We conducted a cross-sectional study with 676 patients consecutively admitted to the ED during the months of May to July 2016. A questionnaire was performed and rectal swabs were collected from patients on admission, for culture and for multiplex real-time polymerase chain-reaction (RT-PCR). If the patient was hospitalized for more than one week in the ED, samples were taken again to determine the acquisition rate of CRE. Findings: Forty-six patients were colonized, all positive RT-PCR were KPC. The acquisition rate

was 18%. Patients CRE colonized presented a higher mortality rate. Previous exposure to healthcare in the last year, hepatopathy and use of antibiotics in the last month were risk factors for colonization. Six patients with no previous exposure to healthcare were CRE-colonized on admission, suggesting transmission of CRE within the community. Conclusions: Screening of high-risk patients on admission to the ED is a strategy to early identify CRE carriage and may be a strategy to control CRE dissemination.

Keywords:

Carbapenem Resistant *Enterobacteriaceae*

Antimicrobial Resistance

Hospital epidemiology

Introduction

Carbapenemases became a great concern in the last 15 years since the first description of *Klebsiella pneumoniae* carbapenemase (KPC) [1]. *Enterobacteriaceae* used to be overall sensitive to antibiotics, but since the advent of extended-spectrum betalactamase (ESBL) and then KPC, they have been increasingly resistant to most of the available drugs to treat bacterial infections, leading to great economic and human costs [2].

KPC was first described in 1996 in North Carolina, and since then, it has been reported all over the world, in *Enterobacteriaceae* and even in non-*Enterobacteriaceae* [2, 3]. KPC has been associated with high mortality rates and poor outcomes [4], and epidemiological studies demonstrate that *K.*

pneumoniae isolates belonging to sequence type 258 (ST258) are even more virulent when bearing *bla*$_{KPC}$-2 [5].

The emergence of new determinants of antimicrobial resistance, their spread, plus the lack of new drugs pose a challenge pointing to a potential end of the “Antibiotic Era”. This triggered an alarm by the World Health Organization that declared Carbapenem-Resistant *Enterobacteriaceae* (CRE) as one of three greatest menaces to human health [6].

KPC and other carbapenemases genes, such as the *New Delhi*-metallo-betalactamase (NDM) and other metallobetalactameses such as VIM and IMP family enzymes, are usually harboured in plasmids, which enable them to spread to other bacteria. In this context, intestinal carriage is a major concern to the infection control practitioner, due to the guts potential to act as a reservoir for multi-drug resistant organisms (MDRO), and a niche in which mechanisms of resistance may be exchanged between bacteria. These factors may lead nosocomial dissemination of CRE and possible outbreaks [7].

Many hospitals consider screening for CRE carriage as one of the strategies to control cross transmission. Usually tested in patients in intensive care units (ICU), screening can allow early identification of colonized patients, who are put under contact precautions to prevent the transmission to other patients [8, 9, 10].

In Brazil, colonization with CRE was reported in 30.4% of patients of an ICU [11]. In our hospital, the prevalence of CRE carriage on admission to the ICU is approximately 20% (unpublished data), but the exact time and place in which colonization takes place is not yet clear.

Almost 30% of our patients are admitted *via* the emergency department, which is a referral unit in our public health system. Patients come from all parts of the country. Overcrowded wards and ICUs lead to a delay in transfers from emergency department to other units. Thus patients experience long periods of care in the overcrowded emergency department (ED). This probably facilitates cross transmission. The contribution of MDRO colonization on admission to this panorama is not clear. Moreover, we do not know to what extent patients admitted to ED coming from the community act as reservoirs of CRE.

In this study, our goal is to describe the prevalence of CRE carriage among patients on admission to the ED and risk factors associated with this colonization. We also aim to determine the incidence of acquisition of CRE during hospital stay in the ED.

Methods

We conducted a prevalence study at the Emergency Department (ED) of Hospital das Clínicas, a 1000-bed teaching hospital affiliated to the University of São Paulo, Brazil. The ED is responsible for 30 hospital admissions per day, with a capacity of 50 beds. Generally, its capacity is exceeded and it is not uncommon to have more than 90 patients hospitalized in this area.

During the period from May 31st to July 7th, 2016, 676 patients were consecutively admitted to the ED and prospectively included in this study. All but two patients were screened for CRE colonization in the first 24 hours

after admission with two rectal swabs: one sent for classical culture and the other for real-time polymerase chain-reaction (RT-PCR).

On admission, information regarding clinical data, demographics and possible risk factors for CRE colonization were collected.

Previous exposure to healthcare was defined as previous hospitalization, surgery or treatment in a day-hospital setting within the last year and/or if a patient had been under haemodialysis or received any cancer treatment within the last month.

Comorbidities such as hepatopathy, diabetes mellitus, acute kidney injury, chronic kidney disease, stem cell transplantation, solid organ transplantation, solid organ malignancies, haematological malignancies, HIV infection, neutropenia, and use of immunosuppressive drugs were evaluated. Previous use of antibiotics within the last month was also registered.

Six patients with no previous exposure to healthcare that were colonized by CRE on admission were investigated for other possible factors associated to CRE carriage through an interview over the telephone. The aforementioned possible risk factors were reassessed and the following possible risk factors were also investigated: contact with animals or pets, travel to other countries, contact with people under healthcare or with patients from long term care facilities.

On the seventh day after admission, and then weekly, patients who were still hospitalized in the ED were eligible for re-screening for CRE colonization with rectal culture and RT-PCR, until a CRE was identified or the patient was discharged, died or transferred to another ward in the hospital. If

the patient was transferred to an Intensive Care Unit (ICU), screening for CRE colonization with rectal swab culture was performed on admission to the ICU.

Microbiology

CRE cultures were obtained with swabs and enriched in liquid media for 24 hours, and then they were cultured in an Agar plate with carbapenem disks according to the CDC methodology [12]. Resistant isolates were then identified with MALDI-TOF and automatized antimicrobial susceptibility testing was performed with Vitek2® (BioMerieux ®, MarcyL'Étoile, France).

Minimal inhibitory concentration of carbapenems and colistin were then determined by disk-diffusion and E-test (BioMérieux®, MarcyL'Étoile, France), respectively, according to the Clinical and Laboratory Standards Institute (CLSI) 2016 recommendations [13].

Molecular biology

The detection of resistance genes was done directly from the rectal swabs. The samples were evaluated by RT-PCR for KPC, NDM, VIM, IMP-1 and OXA-48 with Cepheid® Xpert® Carba-R (Cepheid, Sunnyvale, USA) according to manufacturer's recommendations [14].

Data analysis

All the data obtained regarding the clinical data, demographics and potential risk factors for CRE colonization were evaluated. CRE colonized

patients on admission were compared with non-colonized patients. Covariates associated with CRE colonization on bivariate analysis were submitted to a logistic regression model. CRE colonization as risk factor for death was adjusted for age and comorbidities using logistic regression. A p-value of <0.05 was considered significant. Statistical analyses were performed using EPI Info v3.5.1 (Center of Disease Control and Prevention, Atlanta, Georgia, USA).

Results

Six hundred and seventy six patients were included in this study, 52% (353 patients) were male, with a mean age of 57 years (range 2-94 years, median 60 years). Two culture-negative patients were excluded because they did not collect samples for PCR.

On admission, 46 (6.8%) patients were colonized by CRE based on at least one laboratory method, culture was positive for 38 (5.6%) patients, all *Klebsiella pneumoniae*; and RT-PCR was positive for 36 (5.3%), all KPC. (Table I) The mean age of the colonized patients was 63 years (range 9-93 years, median 67 years) and of the non-colonized patients 57 years (range 2-94 years, median 60 years).

The mean length of stay in the ED was 11.55 days (range: <1-105 days). Three hundred and two patients who were not colonized for CRE on admission were hospitalized for more than one week in the ED and 50 of these were re-screened for CRE (17%). Nine (18%) became colonized by CRE during their stay in the ED. The remaining 252 patients were compared

with the 50 re-screened patients demonstrating differences between those two groups regarding chronic kidney disease (14% vs 29%, p: 0.01), and solid organ transplantation (15% vs 42%, p: 0.01).

The in-hospital mortality for all patients was 14% (93/668), with a greater mortality among the patients already colonized on admission, 27% for the colonized on admission and 13% for the non-colonized (p: 0.04) in the bivariate analysis. There was no difference when adjusted for comorbidities and age.

We could determine the origin of 535 patients, most of them, 317 (59%), came from the community, and 218 (31%) had a previous exposure to healthcare within the last year, 3 of which had come from long-term care facilities (LTCF), and 3 from the prison healthcare system.

Six patients (13%) of the 46 patients colonized on admission had no previous exposure to healthcare in the last year: 3 had been hospitalized at least once during their lifetime, but not during the last year; 5 had only been to consultations at outpatient clinics during the last year; and one, previously healthy, was admitted due to polytrauma.

Three of them had used antibiotics, but none had used a regimen with a carbapenem. None of the patients had had close contact with animals or pets, travelled to other countries, had close contact with people who are users of the healthcare system or with patients from long term care facilities (Table II).

Bivariate analysis

The analysis of factors associated with CRE-carriage on admission to the ED can be seen in Table III. In the bivariate analysis the following variables were associated with CRE-carriage: previous exposure to healthcare, the presence of at least one comorbidity; hepatopathy; acute kidney injury; and use of antibiotics in the last month (Table III).

Multivariate analysis

The following variables were evaluated in the multivariate analysis: previous exposure to healthcare, the presence of at least one comorbidity; hepatopathy; acute kidney injury, chronic kidney disease; solid organ transplantation; solid organ malignancies; neutropenia; and use of antibiotics in the last month.

The final model can be seen in Table IV. Previous exposure to healthcare; hepatopathy; and use of antibiotics in the last month were factors significantly associated with colonization by CRE on admission to the ED.

Discussion

We found in our study a prevalence of CRE colonization on admission to the Emergency Department (ED) of 6.8%, with an acquisition rate of 18%. We report CRE colonization in six patients with no previous exposure to healthcare within the previous year. The stay of patients in the ED is long, as 45% of the patients were hospitalized for at least one week. Our study also describes hepatopathy as a new risk factor for CRE colonization, and

confirms known risk factors such as previous exposure to healthcare and previous use of antibiotics.

KPC is the most frequent carbapenemase in Brazil, responsible for 96.2% of all carbapenem resistance in *K. pneumoniae*. [15] Surprisingly, we found 10 carbapenem-resistant *K. pneumoniae* that were negative for KPC, NDM, VIM, IMP-1 and OXA-48. This may be have occurred due to another enzyme, such as the recently described BKC-1 (*Brazillian Klebsiella Carbapenemase)* [16], or due to a combination of other mechanisms such as overexpression of efflux pumps or loss of porins.

PCR in rectal swabs is effective in detecting CRE-carriage, being a very sensitive and specific method, but its expensive costs could limit its application as a screening method. It has been suggested that in high prevalence areas, universal screening should be performed, and in low prevalence areas, only patients with risk factors should be screened [17].

Colonization by ESBL has been studied in patients with liver disease, but hepatopathy had never been described as a risk factor for CRE colonization [18, 19, 20]. We hypothesize that liver disease may act as disrupter of the normal functioning of the gut, possibly causing an unbalance that facilitates colonization by other bacteria than the regular microbiota. Patients with liver disease are more prone to hospitalization or to be frequent users of outpatient clinics, thus may be more exposed to CRE and vulnerable to CRE acquisition and carriage. Further studies are needed.

Despite the lack of studies, the emergency department is thought to be a low prevalence area for multidrug resistance in the hospital, since the

majority of patients come from the community [21, 22]. Prolonged carriage of CRE is not common in patients not exposed to healthcare, with only a few studies demonstrating persistence of colonization for over 6 months and even domestic transmission in patients returning from endemic countries [23]. On the other hand, most studies that demonstrate carbapenemase-producer organisms in the community usually relates them to a previous healthcare exposure [24]. Our study found a high prevalence of CRE carriage on admission: six patients had no previous exposure to healthcare in the last year, except for outpatient consultations, nor did they have a history of travel or contact with animals or pets. We also found a high acquisition rate in the ED, demonstrating that this population is not at low risk for colonization in our hospital and may have an important role in the transmission and perpetuation of CRE in the entire hospital. These unique data also demonstrate that CRE can be found in the community, in patients whose risk factors for colonization were not clear, something alarming and never described before.

This scenario suggests that screening for CRE carriage in the ED may be an essential procedure to control spread. Due to cost constraints, screening could be limited to high-risk patients for CRE-carriage. The factors associated with CRE-carriage in our study will allow us to determine which population to screen on admission and to put under contact precautions. The use of a molecular method would probably limit the duration of empirical contact precautions in high-risk patients, with results in a much shorter time, when compared with conventional cultures. The early isolation of patients may impact the acquisition rate of CRE in the ED and even in the hospital.

This intervention still requires further studies in our setting to evaluate its impact and cost. The conditions of our ED and hospital, especially overcrowding, probably facilitate transmission and may require an aggressive control strategy.

Our study had some limitations. We did not perform molecular typing or other clonality testing to confirm that the transmission of CRE occurred in the ED. It was not possible to assure that the CRE acquisition occurred in the ED, nor differentiate acquisition from a preliminary carriage that reactivated. We had a considerable proportion of patients lost to follow-up during their stay at the ED, making it impossible to study factors associated with the acquisition of CRE, or the exact acquisition rate during hospitalization in the ED.

Conclusions

In conclusion, we found a high prevalence of CRE-colonization on admission to the ED. Factors associated with colonization on admission were: previous exposure to healthcare; previous use of antibiotics and hepatopathy. The use of screening of the high risk patients on admission to the ED may be a strategy that, coupled with contact precautions, will control CRE spread in the entire hospital. This requires further study. We found a relatively high proportion of CRE-colonized patients with no previous exposure to healthcare that suggests that the dynamics of transmission of CRE within community still requires understanding.

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Tables

Table I - Prevalence on admission of Carbapenem-resistant *Enterobacteriaceae* carriage detected rectal swab cultures and real-time polymerase chain reaction (RT-PCR). Emergency Department, Hospital das Clínicas, University of São Paulo (31 May- 7 July, 2016)

Test	Positive PCR	Negative PCR	Total
Positive culture	28	10	38
Negative culture	8	628	636
Total	36	638	674

Table II - Characteristics of patients colonized by carbapenem-resistant *Klebsiella pneumoniae* on admission to the Emergency Department who had no previous exposure to healthcare in the last year. Hospital das Clínicas, University of São Paulo (31 May- 7 July, 2016)

Patient	Diagnosis on admission	Underlying conditions	Antibiotics used in the last month	Previous hospitalization
65 yo male	Complicated urinary tract infection (UTI)	Prostate hyperplasia	Levofloxacin	None
85 yo male	UTI	Alzheimer's disease Diabetes mellitus	Levofloxacin	26 months before the study
42 yo male	Febrile neutropenia	Systemic lupus erythematosus	None	17 months before the study
26 yo female	Cholangitis	Chronic portal vein thrombosis	None	14 months before the study
69 yo female	Acute aortic aneurism dissection	Parkinson's Disease	Antibiotic for UTI	None
56 yo male	Polytrauma	No known diseases	None	None

yo: years old

Table III - Bivariate analysis of potential factors associated with colonization with carbapenem resistant *Enterobacteriaceae* (CRE) on admission to the Emergency Department. Hospital das Clínicas, University of São Paulo (31 May- 7 July, 2016)

Variables	Number of patients colonized by CRE / patients evaluated (%)	Number of patients not-colonized by CRE / patients evaluated (%)	OR	95% CI	P-value
Previous exposure to healthcare	30/38 (79%)	182/497 (37%)	6.49	2.91 – 14.46	<0.0001
Presence of at least one comorbidity	33/40 (83%)	333/526 (63%)	2.73	1.18 – 6.30	0.014
Hepatopathy	12/37 (32%)	55/495 (11%)	3.84	1.82 – 8.07	0.0002
Diabetes	13/37 (35%)	140/495 (28%)	1.37	0.68 – 2.77	0.37
Acute kidney injury	10/37 (27%)	63/495 (13%)	2.54	1.17 – 5.50	0.015
Chronic kidney disease	12/37 (32%)	95/495 (19%)	2.02	0.98 – 4.17	0.052
Solid organ transplantation	7/37 (19%)	32/495 (6%)	3.38	1.37 – 8.28	0.005
Solid organ malignancies	2/37 (5%)	58/495 (12%)	0.43	0.10 – 1.84	0.18
Haematological malignancies	0/37 (0%)	17/495 (3%)	0.00	Undefined	0.29
HIV infection	2/37 (5%)	14/495 (3%)	1.96	0.43 – 8.99	0.30
Neutropenia	2/37 (5%)	10/495 (2%)	2.77	0.58 – 13.14	0.20
Use of immunosuppressive drugs	2/37 (5%)	44/495 (9%)	0.59	0.14 – 2.52	0.42
Haemodialysis	2/36 (6%)	40/481 (8%)	0.65	0.15 - 2.80	0.43
Surgery in the last year	5/36 (14%)	76/484 (16%)	0.86	0.33 – 2.30	0.50
Healthcare at day hospital	9/36 (25%)	82/480 (17%)	1.62	0.73 – 3.57	0.23
Use of antibiotics in the last month	25/36 (69%)	139/481 (29%)	5.59	2.68 – 11.67	<0.0001

OR: odds ratio; 95%CI: 95% confidence interval

Table IV - Multivariate analysis of potential factors associated with carbapenem-resistant carriage of *Klebsiella pneumoniae* on admission in the Emergency Department. Hospital das Clínicas, University of São Paulo (31 May- 7 July, 2016)

Variables	OR	95% CI	p
Previous exposure to healthcare	4.98	2.07 – 11.97	0.0003
Comorbidities	1.43	0.39 – 5.21	0.58
Hepatopathy	3.95	1.55 – 10.09	0.004
Acute kidney injury	2.31	0.86 – 6.23	0.10
Chronic kidney disease	1.20	0.46 – 3.14	0.72
Solid organ transplantation	2.11	0.74 – 6.03	0.16
Solid organ malignancies	0.44	0.09 – 2.13	0.31
Neutropenia	6.08	0.92 – 40.27	0.06
Use of antibiotics in the last month	5.32	2.32 – 12.17	0.0001

OR: odds ratio; 95%CI: 95% confidence interval

Chapter 3

COLONIZATION BY CARBAPENEM RESISTANT *ENTEROBACTERIACEAE* ON ADMISSION TO INTENSIVE CARE: HOSPITALIZATION IN THE EMERGENCY DEPARTMENT AND OTHER RISK FACTORS FOR COLONIZATION

Chapter 3: COLONIZATION BY CARBAPENEM RESISTANT *ENTEROBACTERIACEAE* ON ADMISSION TO INTENSIVE CARE: HOSPITALIZATION IN THE EMERGENCY DEPARTMENT AND OTHER RISK FACTORS FOR COLONIZATION *(in Portuguese)*

The English version of the research project can be seen in Appendix 2:

3.1 PROJETO DE PESQUISA: Colonização por enterobactérias resistentes a carbapenêmicos à admissão na terapia intensiva: internação no pronto-socorro e outros possíveis fatores de risco para colonização, um estudo de caso controle.

OBJETIVOS

1. Avaliar o impacto que a internação no pronto-socorro exerce para a colonização pelas enterobactérias resistentes a carbapenêmicos na admissão na UTI

Hipótese nula

A internação em Pronto-Socorro precedendo a internação na UTI apresenta risco semelhante à internação em outras unidades de internação hospitalar para colonização por ERC.

Hipótese alternativa

A internação em Pronto-Socorro precedendo a internação na UTI é fator associado à colonização por ERC.

MATERIAL E MÉTODOS

Foi realizado um estudo caso-controle retrospectivo com os pacientes internados nas UTIs do HCFMUSP no período de setembro de 2015 a julho de 2017.

O HCFMUSP é um hospital terciário e o Instituto Central (ICHC) é local no qual se concentram a maior parte dos leitos de UTI da instituição, contando com 10 UTIs, com 109 leitos de terapia intensiva.

A Comissão de Controle de Infecção Hospitalar (CCIH) realiza continuamente a vigilância de colonização e infecção por agentes multirresistentes nessas unidades, inclusive ERC. Para tal, todos os pacientes admitidos nas UTIs do ICHC são submetidos rotineiramente à coleta de uma cultura de swab retal de vigilância para ERC no momento da admissão na unidade e colocados em precaução de contato até o resultado final, sendo retirados da precaução caso negativos ou mantidos em isolamento caso positivos.

A precaução de contato consiste em uso de aventais descartáveis e luvas por parte dos funcionários de saúde, além de uso de material exclusivo para o paciente como termômetro, esfigmomanômetro e estetoscópio.

As culturas de vigilância são realizadas pelo laboratório de microbiologia do ICHC, seguindo metodologia padronizada no local, com incubação “overnight” dos swabs em caldo de tioglicolato, seguido, em caso de turvação do meio, de semeadura em ágar Macconkey com disco de Ertapenem, Imipenem e Meropenem. Caso haja crescimento de colônias sugestivas de enterobactérias dentro do halo do disco de Ertapenem, Imipenem e Meropenem, conforme valores recomendados pelo *Clinical and Laboratory Standards Institute* (CLSI) 2016, essas colônias são isoladas e identificadas por Maldi-ToF.

Os casos foram obtidos retrospectivamente do banco de dados da CCIH, que compila todos os casos de culturas de vigilância positivas do ICHC. Este estudo utilizou 2 controles para cada caso. Pacientes que foram internados mais de uma vez nas UTIs com coleta de cultura de vigilância foram considerados somente uma vez, na primeira internação.

Foi realizado cálculo de tamanho amostral, sendo definido o número de 99 casos e 198 controles, considerando-se 35% dos pacientes das UTIs com internação maior que 2 dias no PS, para um poder estatístico de 80%, com Odds Ratio de 2,0, intervalo de confiança de 95% e p 0,05.

Definição de caso

Os casos foram definidos como pacientes que tiverem resultado de cultura de swab de vigilância para ERC positivos coletados em até 2 dias após a admissão em uma das UTIs do ICHC, nos anos de 2015 a 2017. A

colonização ou infecção prévia por ERC nesta internação foi fator de exclusão.

Definição de controle

Os controles foram pacientes internados com até uma semana de intervalo dos casos, cujas culturas de vigilância admissionais na unidade (até 2 dias) eram negativas. O pareamento dos pacientes ocorreu de forma que dois controles foram pareados a um caso, pertencendo à mesma unidade do caso, com o período de internação do caso e do controle não apresentando intervalo maior do que 7 dias entre a admissão de ambos na unidade. Caso houvesse mais de dois pacientes elegíveis como controles para o caso, estes foram selecionados através de sorteio.

Variáveis de estudo

Os casos foram comparados aos controles utilizando-se das variáveis a seguir:

Variáveis demográficas:

- Idade (em anos);
- Sexo (masculino ou feminino);

Variáveis de procedência do paciente:

- Unidade de internação onde foi coletada a cultura de vigilância;
- Unidade de internação de onde o paciente foi imediatamente transferido;

- Tempo de internação hospitalar antes da cultura de vigilância (em dias);
- Tempo de internação no PS;
- Internação no PS > 2 dias;
- Paciente admitido diretamente na UTI;
- Paciente vindo de transferência de outro serviço (sim ou não);
- Paciente com internação hospitalar prévia (sim ou não, e o número de meses antecedendo a internação atual).

Variáveis da admissão na UTI e escores de gravidade:

- Doença que motivou a internação em UTI
- Gravidade da doença que motivou a internação em UTI (definida pelo score de SAPS - *Simplified Acute Physiology* Score II); (LeGall et al., 1993)
- Escore SOFA (*Sequential Organ Failure Assessment*) no dia da coleta de cultura de vigilância. (Vincent et al., 1996)
- Pós-operatório de risco (sim ou não);
- Politrauma (sim ou não);
- Acidente vascular cerebral (sim ou não).

Variáveis de infecção:

- Infecção no momento da coleta da cultura de admissão (sim ou não);

- Sepse (SEPSIS 3, utilizando-se o critério do qSOFA>2, ou aumento de SOFA>=2) no dia de coleta da cultura de vigilância (Singer et al., 2016),
- Foco da infecção: pneumonia (sim ou não), infecção urinária (sim ou não), infecção de corrente sanguínea (sim ou não, utilizando-se dos critérios de Centers for Disease Control and Prevention, 2018), diarreia (sim ou não), meningite (sim ou não), febre amarela (sim ou não);
- Colonização ou infecção prévia por ERC em outra internação (sim ou não).

Comorbidades:

- Escore de Charlson (Charlson et al., 1987)
- Presença de hepatopatia crônica com cirrose no dia de coleta da cultura de vigilância (CHILD A ou maior, classificado em sim ou não);
- Insuficiência renal crônica dialítica (sim ou não);
- Doença reumatológica ou auto-imune (sim ou não);
- Infecção pelo HIV (positivo ou negativo);
- Neoplasia maligna de órgão sólido (sim ou não);
- Neoplasia hematológica (sim ou não);
- Transplante de órgãos sólidos (sim ou não);
- Transplante de medula óssea (sim ou não).

Disfunções orgânicas no dia da coleta da cultura admissional:

- Insuficiência hepática aguda (sim ou não, classificado conforme surgimento de encefalopatia hepática dentro de 8 semanas após o início dos sinais e sintomas de hepatite aguda, com evidências de hepatite aguda grave: icterícia progressiva; coagulopatia com INR >1,5; ou Fator V <50%);
- Insuficiência respiratória (sim ou não, se apresentar ao menos um dos critérios a seguir: PaO2 < 60 mmHg, ou PaCo2 > 45 mmHg, ou frequência respiratória > 30 incursões por minuto, ou ventilação mecânica);
- Insuficiência renal aguda [sim ou não, segundo critério "F –*Failure*" dos critérios RIFLE (Bellomo et al., 2004), creatinina sérica aumentada três vezes ou débito urinário abaixo de 0.3 ml/kg, em vinte e quatro horas];
- Choque (sim ou não, conforme a presença de ao menos um dos seguintes fatores: Pressão Arterial <90x60 mmHg, ou Pressão arterial média < 65 mmHg, não responsiva a volume, ou uso de drogas vasoativas para manter níveis pressóricos, como noradrenalina, adrenalina, dobutamina ou dopamina).

Presença de invasões por mais de 1 dia calendário:

- Traqueostomia (sim ou não), gastrostomia (sim ou não), ou colostomia (sim ou não);
- Uso de ventilador mecânico (sim ou não);

- Cateter venoso central (sim ou não).

    Caso o dispositivo tivesse sido retirado há menos de 2 dias calendário, o mesmo foi considerado como presente.

Realização de procedimentos na internação antes da admissão na UTI: hemodiálise, endoscopia digestiva alta, colonoscopia, ou uso de nutrição parenteral.

Antibioticoterapia

- Uso de antibiótico no momento da internação na UTI ou prévio (até 3 meses)

Desfechos hospitalares

- Infecção por ERC após cultura de vigilância (sim ou não);
- Tempo de internaçãoo total;
- Uso de antibioticoterapia adequada (sim ou não, definida pelo uso de ao menos 1 droga sensível em antibiograma) e tempo de introdução (dias entre início de sintomas até início de antibioticoterapia adequada)
- Óbito nesta internação (sim ou não).

Um instrumento de coleta e banco de dados foi criado utilizando o programa REDCap (Research Electronic Data Capture) (Harris et al., 2009).

Análise estatística

A análise estatística foi realizada com o programa *Statistical Package for the Social Sciences* (SPSS) 11.5 (SPSS Inc., Chicago, IL, Estados Unidos). Os casos foram comparados aos controles, utilizando-se o teste de qui-quadrado ou testes exatos ou corrigidos quando apropriado. Um intervalo de confiança de 95% foi utilizado, com significância estatística se $p<0,05$.

Variáveis com significância estatística na análise bivariada $p<0,05$ foram submetidas a análise multivariada com outras variáveis de confusão em modelo de regressão logística. O modelo de regressão logística foi realizado excluindo variáveis redundantes ou variáveis com baixa representatividade na amostra.

Aspectos Éticos

Este estudo foi submetido e aprovado pela Comissão de Ética e Pesquisa do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo sob o número CAAE: 91604518.9.0000.0068. Por se tratar de um estudo retrospectivo, sem coleta de amostras, somente com coleta de dados de prontuário, foi aprovada dispensa da aplicação do termo de consentimento livre e esclarecido. Os dados da pesquisa serão apresentados somente em conjunto, sem nenhum tipo de identificação.

3.2 ARTICLE

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Article title: Increased Risk for Carbapenem-Resistant Enterobacteriaceae Colonization in Intensive Care Units after Hospitalization in Emergency Department.

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Summary

Carbapenem-resistant *Enterobacteriaceae* (CRE) colonization is common in hospital patients admitted to intensive care units (ICU) from the emergency department. We evaluated the impact of previous hospitalization in the emergency department on CRE colonization at ICU admission. Our case–control study included 103 cases and 201 controls; cases were patients colonized by CRE on admission to ICU and controls were patients admitted to ICU and not colonized. Risk factors were emergency-department stay, use of carbapenem, Simplified Acute Physiology Score (SAPS 3), upper digestive endoscopy, and transfer from another hospital. Our study

demonstrates that ED stay before ICU admission was associated with CRE colonization on admission to the ICU. Our findings indicate that addressing problems in the ED will help to control carbapenem resistance in ICU.

Introduction

Klebsiella pneumoniae carbapenemase (KPC) described in 1996, is an enzyme capable of hydrolyzing all β-lactam antimicrobial drugs known at the time [(1)]. Since then, other carbapenemases have been described in *Enterobacteriaceae* all over the world, leading to a substantial increase in resistance to antimicrobial drugs [(2, 3)].

Surveillance data from central line–associated bloodstream infections (CLABSI) in intensive care units (ICUs) of the State of São Paulo, Brazil, demonstrated an increase of carbapenem-resistant *Klebsiella pneumoniae*, from 14% in 2011 to 55% in 2017 [(4)]. In 2017, *Klebsiella pneumoniae* was the most frequent species causing CLABSI (20%) in Sao Paulo.

Hospital das Clínicas of the University of São Paulo, routinely performed CRE screening for patients admitted to ICU since January 2014. Early identification and isolation of colonized patients was implemented to decrease secondary colonization. Concomitant training sessions for hand hygiene and contact precautions took place during this period. Despite all efforts, ICUs had a high colonization pressure (17% - 29%, mean 21%) due to admission of colonized patients, mainly from ED (I. Boszczowski, unpub. data).

In 2016, we found that 7% of patients admitted to the ED were positive for CRE. However, among those who were negative on admission, 18% became colonized during their stay in the ED. These findings led us to hypothesize that hospitalization in the ED may be a risk factor for CRE colonization in other units of the hospital [5]; ≈60% of the patients admitted to ICUs come from hospitalizations in the ED.

In our study we evaluated the impact of hospitalization in the ED on CRE-colonization on admission to an Intensive Care Unit (ICU).

Methods

Setting

Hospital das Clinicas is a 2,200-bed public tertiary-care hospital in São Paulo and is the largest hospital complex in Latin America. The main building has ≈1,000 beds and is the location of ED and most of the hospital's ICU beds (10 ICUs and 109 intensive care beds).

The ED is a very busy unit. In 2018, 69,000 emergency consultations were performed. The average hospitalization rate in the ED is 150 patients/week and median length of stay is 6 days. It has 50 beds for hospitalization, but occupation often exceeds 90 beds, with patients on stretchers and often in corridors (Figure 1).

Figure 1 - A corridor in the emergency department of Hospital das Clínicas, Sao Paulo, Brazil, showing patients on stretchers, December 2016

Approximately 60% of ICU patients are admitted from the ED. To monitor and control CRE colonization, CRE surveillance cultures are performed on all patients admitted to ICUs at the time of admission and placed under contact precautions until the return of results. Colonized patients with CRE remain under contact precautions for their entire stay in the unit.

Microbiology

Surveillance cultures are performed at the clinical microbiology laboratory in accordance with the institution's standard methodology. Rectal swabs from patients are incubated overnight in thioglycolate broth. Positive

growth samples are plated on MacConkey agar with ertapenem, imipenem, and meropenem discs. If there are colonies suggestive of *Enterobacteriaceae* growth within the carbapenems' disk halo, these colonies are isolated and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, as recommended by Clinical and Laboratory Standards Institute 2016 [(6)].

Study Design

We conducted a retrospective case–control study with patients hospitalized in ICUs at HC during September 2015–July 2017. This study used 2 controls for each case. We obtained cases from the infection control department database, which compiles all cases of positive surveillance cultures. Patients who were hospitalized more than once in ICUs were considered only once, during their first hospitalization.

We defined a case as a patient admitted to one of the ICUs during 2015–2017 who had a positive CRE surveillance culture collected within 2 days of admission. We defined a control as a patient admitted to the ICU whose surveillance cultures collected within the first 2 days of admission were negative. Colonization or prior infection with CRE on admission were excluding criteria. We paired controls by ICU and hospitalization period, with a maximum interval of 1 week from the admission of the cases. When >2 patients were eligible as controls for a case, we randomly chose 2 from all the potential controls. The proportion of controls admitted in the ICUs from the ED was similar to the proportion of patients coming from the ED found in

our historical series. CRE screening methodologies were the same for all patients in the study period, whether they were cases or controls.

We collected data from medical records for demographic variables; hospitalization records before ICU admission; clinical characteristics at time of ICU admission; severity scores and organ failures; indwelling devices; clinical procedures before ICU admission; concurrent conditions; use of antimicrobial drugs (for ≥48 hours before ICU admission) and infection before ICU admission; previous colonization; infection by CRE; length of hospital stay; and death. We defined central line–associated bloodstream infections (CLABSI) accordingly to the 2018 CDC definition [7].

We used REDCap (Research Electronic Data Capture) program (*8*) to create a data collection tool and database.

Statistical Analysis

We calculated sample size, and determined the numbers of 99 cases and 198 controls. We assumed that 35% of the cases had an ED stay >2 days; with a power of 80%; odds ratio was 2.0; 95% CI, p = 0.05.

We performed statistical analysis using Stata version 16 (StataCorp, https://www.stata.com) and Statistical Package for the Social Sciences (SPSS) version 11.5 (SPSS Inc., http://www.spss.com). We compared cases with controls using Wilcoxon or McNemar test when appropriate. All tests were 2-tailed, with 95% CI; $p<0.05$ was considered statistically significant. For variables with $p<0.05$ in the bivariate analysis, we conducted multivariate analysis with other confounding variables in a conditional logistic regression

model. Length of ED stay was a continuous variable and was transformed into a dichotomic variable using SPSS decision tree tool, and for the final model we chose the one with a better fit. We used stepwise backward modeling for the conditional logistic regression and kept the most significant variables were kept in the final model. We used 2 models, one using length of ED stay as a continuous variable and the other as a dichotomous variable. Smoking and sepsis variables comprised more than 40% of missing data (Tables 1 and 2) and were dropped out.

The Ethics and Research Committee of Hospital das Clinicas da Faculdade de Medicina da Universidade de Sao Paulo approved this study (number CAAE: 91604518.9.0000.0068).

Results

We included 304 patients in this study, 103 cases and 201 controls, and collected surveillance cultures for all patients. Of the 103 case-patients, 99 were colonized by *Klebsiella pneumoniae*, 2 by *Enterobacter cloacae,* and 2 by *Escherichia coli*. One hundred and eighty-eight patients (62%) were admitted to medical ICUs and 116 patients (38%) to surgical ICUs. Sixty-five patients were admitted directly to the ICU: 38 transferred from another hospital, 17 from the operating room and for 10 this information was not available. Eighty-six patients were transferred from another ward and 152 from the ED; information was not available for 1 patient. Sixty percent of cases and controls stayed in the ED for some time during their hospitalization.

Bivariate analysis demonstrated the 11 characteristics that were factors associated with CRE colonization on ICU admission (Table 1). The most common infections on ICU admission were pneumonia (37%), skin and soft-tissue infection (14%), and CLABSI (10%).

Table 1 - Characteristics of patients, bivariate analysis, and conditional logistic regression of variables potentially associated with colonization by carbapenem-resistant *Enterobacteriaceae* at ICU admission*

Covariate	Bivariate analysis				Conditional logistic regression	
	Cases	Controls	OR (95% CI)	p value	OR (95% CI)	p value
Female sex	34/103 (33%)	91/201 (45%)	0.58 (0.35–0.95)	0.03		
Mean age, y (range)	50.55 (14–84)	49.78 (4–89)	1.00 (0.99–1.01)	0.62		
Previous hospitalization at ICU admission						
Previous stay in another unit during hospitalization	75/101 (74%)	163/201 (81%)	0.84 (0.44–1.60)	0.60		
Previous stay in the ED during hospitalization	62/103 (60%)	125/201 (62%)	1.07 (0.65–0.77)	0.78		
Length of ED stay, d	2 (0–55)	1 (0–37)	1.08 (1.01–1.15)	0.02	1.10 (1.02–1.19)	0.01
ED stay >2 d	34/103 (33%)	35/201 (17%)	2.45 (1.40–4.32)	0.002		
Days of hospitalization before surveillance culture, median (range)	3 (1–95)	2 (1–37)	0.99 (0.99–0.99)	<0.001		
Transfer from another hospital	43/101 (43%)	51/193 (26%)	2.79 (1.26–3.68)	0.005	2.52 (1.07–5.89)	0.03
Previous hospitalization	52/85 (61%)	63/163 (38%)	2.91 (1.53–5.52)	0.001		
Clinical characteristics at ICU admission						
Infection	63/101 (63%)	82/140 (42%)	2.62 (1.52–4.54)	0.001	1.76 (0.56–5.50)	0.33
Sepsis	46/62 (74%)	54/81 (66%)	1.41 (0.52–3.85)	0.50		
Surgery before ICU admission	53/102 (52%)	106/194 (55%)	0.92 (0.53–1.62)	0.78		
Trauma	8/100 (8%)	25/194 (13%)	0.62 (0.28–1.40)	0.25		
Stroke	5/100 (5%)	17/194 (9%)	0.61 (0.17–2.18)	0.45		

continue

Table 1 - Characteristics of patients, bivariate analysis, and conditional logistic regression of variables potentially associated with colonization by carbapenem-resistant *Enterobacteriaceae* at ICU admission* (continuation)

Covariate	Bivariate analysis				Conditional logistic regression	
	Cases	Controls	OR (95% CI)	p value	OR (95% CI)	p value
Severity scores						
SAPS 3, % median (range)	22 (4–92)	16 (0–98)	1.01 (1.002–1.02)	0.01	1.01 (1.002–1.03)	0.02
SOFA, median (range)	5 (0–19)	5 (0–19)	1.09 (0.95–1.07)	0.77		
Invasive procedures and devices						
Dialysis	14/100 (14%)	11/194 (6%)	2.50 (0.97–6.42)	0.06		
Tracheostomy	2/99 (2%)	1/194 (0%)	4.92 (0.36–44.67)	0.26		
Colostomy	2/99 (2%)	2/194 (1%)	2.00 (0.28–14.34)	0.49		
Upper digestive endoscopy	10/101 (10%)	5/194 (3%)	3.70 (1.11–12.32)	0.003	18.9 (1.83–195.98)	0.01
Colonoscopy	2/101 (2%)	0/194 (0%)				
Parenteral nutrition	2/101 (2%)	1/ 194 (1%)	3.77 (0.19–74.94)	0.38		

ontinue

Table 1 - Characteristics of patients, bivariate analysis, and conditional logistic regression of variables potentially associated with colonization by carbapenem-resistant *Enterobacteriaceae* at ICU admission* (conclusion)

Covariate	Bivariate analysis				Conditional logistic regression	
	Cases	Controls	OR (95% CI)	p value	OR (95% CI)	p value
Underlying conditions						
CCI score, mean (range)	3.10 (0–9)	2.98 (0–11)	0.99 (0.96–1.02)	0.48		
Smoking	25/62 (40%)	46/137 (34%)	1.17 (0.49–2.78)	0.72		
Diabetes mellitus	20/102 (20%)	44/198 (22%)	0.86 (0.46–1.62)	0.65		
Malignant neoplasm	9/102 (9%)	23/198 (12%)	0.77 (0.35–1.70)	0.52		
Rheumatologic or autoimmune disease	11/102 (11%)	16/198 (8%)	1.44 (0.66–3.15)	0.36		
Cirrhosis	15/102 (15%)	11/198 (5%)	2.25 (0.85–5.91)	0.10		
Chronic kidney disease	12/102 (12%)	14/198 (7%)	1.51 (0.56–3.99)	0.40		
Solid organ transplant	8/102 (8%)	16/198 (8%)	0.62 (0.23–1.64)	0.33		
HIV infection	3/100 (3%)	7/198 (4%)	1.13 (0.27–4.76)	0.86		
Hematological malignancy	2/102 (2%)	6/198 (3%)	0.59 (0.13–2.87)	0.52		
Hematopoietic stem cell transplant	1/102 (1%)	1/198 (0%)	2.00 (0.12–32.42)	0.63		
Antimicrobial drug use						
Any drug at ICU admission†	81/99 (81%)	142/193 (71%)	1.56 (0.83–2.91)	0.161		
Carbapenem at ICU admission†	25/80 (31%)	12/141 (9%)	3.92 (1.51–10.21)	0.005	4.62 (1.30–16.40)	0.02
Any drug use in previous 3 mo	50/72 (69%)	48/145 (33%)	5.38 (2.31–12.53)	<0.001		

*CCI, Charlson Comorbidity Index; ED, emergency department; GCS, Glasgow Coma Scale; ICU, intensive care unit; OR, odds ratio; SAPS 3, Simplified Acute Physiology 3, presented as prediction of mortality risk in percentage; SOFA, Sequential Organ Failure Assessment.

†Initiated >48 h before ICU admission.

Table 2 - Multivariate analysis for potential factors associated with colonization by carbapenem-resistant *Enterobacteriaceae* on ICU admission*

Covariate	OR	95% CI	p value
ED stay longer than 2 d	5.85	1.94–17.65	0.002
Transfer from another hospital	2.10	0.95–4.78	0.076
SAPS 3 score	1.02	1.003–1.03	0.02
Carbapenem use on ICU admission (initiated more than 48 h before ICU admission)	4.78	1.31–17.47	0.02
Infection at ICU admission	2.86	1.08–7.55	0.03
Upper digestive endoscopy	16.40	2.16–124.50	0.01

*Model using length of ED stay as dichotomous variable. OR, Odds Ratio; CI, confidence interval; ED, emergency department; ICU, intensive care unit; SAPS 3, Simplified Acute Physiology Score 3.

The median length of stay in the ED was longer for cases (2 days, range 0–55) than controls (1 day, range 0–37; p = 0.02) (Figure 2). We analyzed the length of stay in the ED with the decision tree tool; we selected a stay >2 days as cutoff for this variable (χ^2 = 12.799; p = 0.017). We found that 38/62 (61%) of the patients with CRE colonization at ICU admission were already colonized after 3 days of hospitalization in the ED (Figure 3).

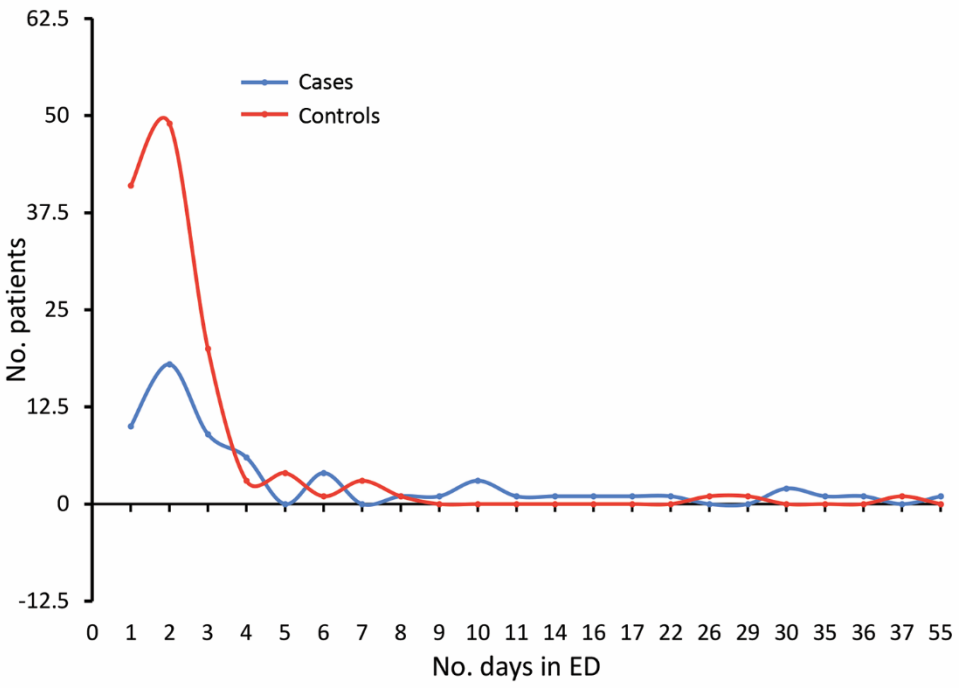

Figure 2 - Distribution of days of stay in the emergency department (ED) comparing patients subsequently admitted to an intensive care unit who had a positive CRE culture within 2 days of admission (cases) and patients whose CRE culture was negative (controls)

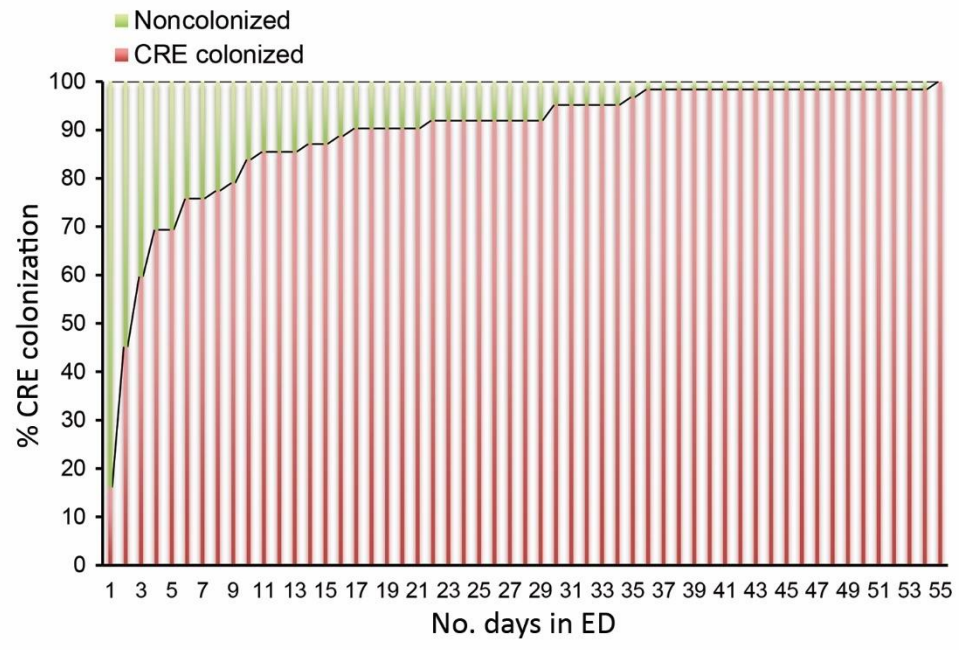

Figure 3 - Distribution of colonization of carbapenem-resistant *Enterobacteriaceae* (CRE) in patients admitted to an intensive care unit after a stay in the emergency department (ED), Hospital das Clínicas, Sao Paulo, Brazil, September 2015–July 2017

We performed the multivariate analysis with 2 models, using ED length of stay as a continuous or a dichotomous variable (>2 days). ED stay was a risk factor for colonization by CRE in both analyses: continuous (per day: OR = 1.10, 95% CI 1.02–1.19, p = 0.01) (Table 1) and >2 days of hospitalization (OR = 5.85, 95% CI 1.94 - 17.65, p = 0.002) (Table 2). Use of carbapenem on ICU admission (initiated >48 hours before ICU admission), Simplified Acute Physiology Score (SAPS 3), transfer from another hospital, and upper digestive endoscopy were risk factors for CRE colonization on ICU admission (Table 1).

Patients colonized by CRE on ICU admission had higher rates of infection by CRE then patients not colonized by CRE when they sought care, 18 (18%) and 11 (6%), p: 0.001; and higher in-hospital mortality rates, 38 (38%) and 48 (24%), p: 0.016, respectively.

Discussion

This study confirms our hypothesis that ED stay is a risk factor for CRE colonization in patients at admission to the ICU. Other risk factors are current use of carbapenem at time of ICU admission (carbapenem use initiated >48 hours before ICU admission), SAPS 3, upper digestive endoscopy, and transfer from another hospital (Table 1).

Including ED stay as a risk factor is a notable new finding. A stay in the ED is usually not considered to be a risk factor for CRE colonization [(9)]. In a previous study, our group demonstrated that patients admitted to the ED

had 6.8% prevalence of CRE colonization at admission to the ED and 18% acquisition rate for patients hospitalized in the ED for longer than 1 week. Six patients that were not treated in a healthcare facility were colonized by CRE at ED admission, implying circulation of this resistance mechanism in the community [5]. Our findings show that ED hospitalization is indeed a risk factor for CRE colonization on ICU admission, whereas a previous stay in another hospital unit was not.

Although it is not common, CRE can be found outside the hospital. CRE has been described in chicken meat in Egypt; in hospital sewage in Brazil, China, and Spain; and in community sources of water in Brazil, Portugal, and Italy. Community-acquired CRE infection is difficult to determine; however, up to 30% of patients with CRE infection on hospital admission have had no previous exposure to the healthcare system [10–15].

The acquisition or transmission of CRE in the emergency department may be a result of the work overload in our ED. Ours is a tertiary-care public hospital in Brazil with an overcrowded ED. It is not unusual to have patients with high-complexity illness hospitalized on stretchers for longer than a week because of a shortage of ICU or ward beds to which to transfer patients, or to have a low ratio of healthcare workers per patient. Prolonged ED stays probably facilitate cross-transmission of multidrug-resistant organisms such as CRE. Although on first thought the problem may be considered a local one, specific to our hospital and setting, this problem extends to other Brazil hospitals. Two other hospitals reported long stays in the ED, with 1 hospital reporting a median length of stay of 3 days [16], and another reporting that

21% of patients stayed in the ED for >5 days [17]. Mortality rates in the EDs of these hospitals are high as well, at 7.4% at the first and 3.9% at the second. Furthermore, we expect long ED stay is an enormous problem in other countries, although very seldom reported [18–20]; lack of access to healthcare in developing countries leads to other problems: healthcare-associated infection rates are much higher in developing countries than in high-income countries [21], as are drug resistance rates [22]. In a disadvantaged healthcare system, patients with known risk factors [23–25] are often hospitalized for prolonged periods in the ED and are a potential source of multidrug-resistant bacteria for other patients in the ED and ICUs.

The need to establish strategies to control CRE transmission in EDs and hospitals is urgent; resistance is not an isolated problem in a specific hospital unit or even in a specific hospital. High workload, understaffing, and turnover of healthcare workers make it very difficult to improve adherence to hand hygiene in the ED; additional strategies are needed [26, 27] and interventions must be multimodal. They must include a change in the workflow of the ED and hospital as well as the entire health system to reduce overcrowding [26–28]. The lack of infrastructure in the ED puts patients in stretchers too near to each other, probably facilitating cross-transmission. In this scenario, good hand hygiene may not be achievable. Dividing patients into cohorts and assigning dedicated staff may reduce transmission of CRE [29]. Hospital staff should discuss screening strategies for CRE, and early isolation and contact precautions in the ED [30]. Rising antimicrobial resistance is a substantial threat to global health [31], and prolonged ED

hospitalization may play a major role in hospital-acquired resistance in low- and middle-income countries.

We found other risk factors that have already been associated with CRE colonization, including transfer from another hospital [24, 25], use of carbapenem [23–25], SAPS 3, and upper digestive endoscopy [32]. All of them are associated with previous exposure to healthcare or severity of patients [33].

The previous use of carbapenem is well described as a risk factor for CRE colonization [23–25, 33]. In our study, the patients were using carbapenem for ≥48 hours by the time of surveillance culture. Although this timeframe is very short, it may have been sufficient for selection of carbapenem-resistant bacteria. We must emphasize that, even though carbapenem use was an independent risk factor in multivariate analysis, the attending physicians may have prescribed it because after a certain length of time in the ED, the patient is at risk for infection by antimicrobial-resistant bacteria.

Of interest, although cirrhosis was not associated with CRE colonization, upper digestive endoscopy was, which suggested that the risk for colonization after endoscopy is probably due to the procedure itself and not to the patient's concurrent conditions. There were no clusters of endoscopy-related CRE colonization in the study period, suggesting that it was not an outbreak. Colonization may be a result of improper cleaning procedures. Because this was a retrospective study, we could not test the endoscopes for CRE colonization at the time that colonization occurred. Prospective surveillance for endoscopy-related CRE is underway.

It is difficult to assess the influence of local factors in the hospital ED on colonization by CRE. Factors such as low adherence to hand hygiene and contact precautions, proximity of beds, and others work together to facilitate the transmission of microorganisms. A limitation of this study is that it was not possible to evaluate the effect of each of these variables individually.

Other limitations of our study were the retrospective nature of a case–control study; missing data for some variables; potential bias of retrospectively obtaining data from medical records; the fact that the study was done in only one hospital, requiring confirmation in other centers or a multicenter study.

In conclusion, this study demonstrates that prolonged ED stay is a risk factor for CRE colonization on admission to the ICU. Other risk factors were the use of carbapenem at ICU admission (initiated more than 48 hours before ICU admission), SAPS 3, upper digestive endoscopy, and transfer from another hospital. The implications of these findings should lead to interventions in the ED if we are to control CRE in other hospital units.

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Chapter 4 -

CRITICAL ANALYSIS AND DISCUSSION

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Surveillance strategies for multidrug-resistant organisms (MDR) usually do not consider the ED as a risk site for MDR colonization, and little data are available about the role of the ED in MDR transmission within the hospital (Richter; Marchaim, 2016).

The main results of this thesis are the demonstration of colonization by CRE on admission to the ED of 6.8%; the rate of acquisition of colonization within the ED of 18%; and the increased risk of CRE colonization of patients on ICU admission who remained in the ED. These findings are an important contribution to the understanding of the dynamics of transmission and the role of the ED in CRE colonization in the hospital environment.

Multidrug-resistant bacteria are a local and global problem (WHO, 2014; O'Neill, 2016; CVE, 2018). Pathogen resistant infections are responsible for estimated 700,000 deaths worldwide annually, and by 2050, without control interventions, it would reach 10 million deaths, with a burden of US$ 100 trillion (O'Neill, 2016). Antibiotic resources are scarce and scale actions are needed to reverse the current scenario.

The One Health approach encompasses multidisciplinary cooperation locally, nationally, and globally, to promote the health of people, animals, and the environment (AVMA, 2008). Antimicrobial resistance should be addressed from this perspective.

The overt use of antibiotics in animal production is usually reported as one source of multidrug-resistance (Liu et al., 2016; Wang et al., 2017). Antimicrobial resistance in humans classically is associated with previous hospital exposure (Holmes et al., 2016), and community resistance may be caused by the export of resistant bacteria from the hospital to the out-of-hospital environment (Schwaber et al., 2011). Resistance phenotypes were considered as unique to the hospital. However, more recently the community has been implicated as the source of resistance, as occurred with community-acquired *Staphylococcus aureus* resistant to methicillin (CA-MRSA). In the case of MRSA, things went the other way, with community-acquired resistant strains becoming hospitalar strains, Hospital community-acquired *Staphylococcus aureus* (HCA-MRSA) (Lakhundi; Zhang, 2018). Another antimicrobial resistance mechanism, the mobile colistin resistance-1 (MCR-1), which confers resistance to colistin, also had its origin in the community and was later introduced into the hospital. (Liu et al., 2016; Perdigão Neto et al., 2019).

Community-acquired CRE is unusual, however in our study we found 6 patients who were colonized on admission without having any known risk factors, and who had not been in a hospital within the previous year. Other patients who were admitted to the ED already colonized by CRE had known risk factors for CRE, leading to a high influx of CRE-colonized patients into the ED. Furthermore, 18% of the patients who remained in the ED, and were negative on admission, became colonized, and a stay in the ED longer than 2 days was a risk factor for arriving in the ICU already colonized by CRE.

These data is a strong evidence that the ED is a source of dissemination of resistant bacteria in the hospital, especially in the ICUs.

Since Florence Nightingale, isolation, individualization of care, and reducing the number of beds per ward are recommended (Martins; Benito, 2016), but this is not always done. The workflow of our ED and its structure, which is insufficient for its demand, favor cross-transmission of MDR organisms. Currently the ED receives only patients referred by the governmental central regulation system of health services (Central de Regulação de Ofertas de Serviços de Saúde - CROSS), which refers to our hospital only complex cases that require tertiary care. Consequently, this system favors the entry of patients already colonized by MDR. Due to the lack of beds available in ICUs and other hospital wards, patients must await transfer in the ED until there is a vacancy in other units of the hospital. The ED has only 50 beds for patient observation, but it is common to see 90 patients, distributed also on stretchers in the corridors of the ED. This common scene in Brazilian public hospitals does not allow for adequate adherence to infection prevention strategies especially adherence to hand hygiene, a fundamental measure (Muller et al., 2015; Carter et al., 2016). Internal audit data show that hand hygiene is performed only in 1%-39% of the opportunities observed. The ED team is periodically trained and bedside alcohol gel dispensers are available for inpatient beds and alcohol gel totems for corridors. However, adherence to hand hygiene remains low, mainly due to lack of local structure, and heterogeneous availability of sinks and alcohol hand rub. This explains why compliance with hand hygiene is lower during

care of patients on stretchers in the corridors (1%), while adherence improves where patients are in regular beds and where there is greater availability of structure for hand hygiene. Another reason for poor adherence to hand hygiene is overcrowding. Studies show that poor adherence to hand hygiene is directly related to excess of patients in emergency units (Muller et al., 2015; Carter et al., 2016). Effective measures to reduce overcrowding and improve flow of patients from the ED to the other hospital wards could reduce the local work overload, which could facilitate adherence to hand hygiene in the ED.

Brazil has a universal healthcare system, the Unified Health System (*Sistema Único de* Saúde, in Portuguese, SUS) (Macinko; Harris, 2015). In this system, HC is a reference for complex cases of the state of São Paulo. Despite the fact that the ED does not receive patients who seek the hospital spontaneously, the unit still receives more patients than its capacity. This is due to the large number of complex cases in SUS.

Health financing in Brazil fluctuates around 8% of gross domestic product (GDP), which is lower than countries that offer universal access to high quality healthcare, such as Canada (10.4%), and the United Kingdom (9.9%) (Saldiva; Veras, 2018). However, it is not only lower investment that justifies SUS financing problems. There is also how resources are managed: SUS prioritizes tertiary care and invests little in prevention. An example of this is the annual budget of HC, a tertiary hospital, which in 2017 was approximately R$ 1.85 billion (or approximately US$ 561 million) (Ramos, 2018) while the approved health budget for the entire city of São Paulo

(where HC is located) for 2019 was R$ 8.7 billion (or approximately US$ 2.2 billion) (Globo, 2018). This discrepancy between investments in preventive medicine and primary care, and the investment made in tertiary care leads to the lack of prevention of serious diseases and the consequent overcrowding of high complexity services.

Besides SUS, almost 50% of Brazilian health investment is focused on the private health system, to which only 23% of the population has access. The federal government has encouraged in recent years the creation of popular health plans, with lower coverage for more serious diseases. Thus, health plans end up covering low-cost diseases, leaving the most serious diseases to the SUS, burdening the system and increasing the complexity of cases treated, leading to situations similar to those found in the HC (Saldiva, 2018). The SUS referral and counter-referral system of patients between primary care and higher levels of care is also deficient and contributes to the overload of tertiary hospitals. Frequently, patients are promptly referred from the Basic Health Units (BHU) to more complex services due to lack of staff or infrastructure. Often, this is so constant that the patient himself seeks directly emergency rooms and tertiary hospitals, which contributes to the overcrowding of these services (Saldiva; Veras, 2018). Counter-referral, in turn, does not work and patients are rarely referred to primary care, contributing to overcrowding of specialized care (Saldiva; Veras, 2018).

The patient’s flow from the ED to other wards of the hospital also contributes to its deficient capacity. Bureaucracy and lack of information automation delay transfers. The hospital has electronic medical records, but

some services still require to fulfil paper forms, which generates redundant information and delays in the patient's hospitalization. Terminal cleaning takes longer than necessary, either due to internal communication failures or lack of staff to perform it. This problem is systemic, recurring in several areas of the hospital, such as ICUs and operating rooms, which leads to a cascade effect that results in decreased bed turnover. Strategies for improving communication, terminal cleaning procedures and reducing bureaucracy are key to improve this flow.

The aforementioned measures depend on extensive changes in the health system and in the hospital's administrative structure, which are difficult or time consuming to implement.

More immediately, we can discuss local adoption of CRE transmission control measures. Hand hygiene, as previously mentioned, is deficient. Educational measures are made periodically, but as long as there is no improvement in infrastructure or in the ratio of staff to patient, by either increasing staff or decreasing overcrowding, high levels of compliance with hand hygiene will be difficult to reach.

Another possibility is to focus on interventions based on screening for patients colonized by CRE and early isolation. In this thesis, we demonstrate that it is possible to quickly and accurately identify patients colonized by CRE upon admission to ED. RT-PCR technology is critical for implementing this type of intervention in the emergency, as this is an area of high patient turnover where patients cannot wait for days for the result of a conventional culture. Colonized patients could then be placed under contact precautions

or in cohorts of colonized patients while awaiting transfer to other hospital units. If effective, this intervention may reduce secondary CRE colonization in the ED, which in this thesis was 18% in patients who were hospitalized for more than one week in the ED, and potentially reduce CRE colonization in patients admitted to the ICU and other hospital wards, originating from the ED.

In summary, to control CRE colonization in the ED and in the hospital, a multimodal approach is required, including: interventions in CRE surveillance strategies and screening for colonized patients in the ED; changes in ED's infrastructure; and improvement in patient's flow in ED and hospital. Furthermore, restructuring primary care, and systems of referral and counter-referral of patients in the health system may be necessary as the hospital and the ED are not isolated from the entire healthcare system. Taking this into consideration, this situation is not unique to our ED but reflects a broader situation that affects other EDs in Brazil and other low-middle-income countries with a situation similar to ours.

Chapter 5 - CONCLUSIONS

Chapter 5:

CONCLUSIONS

Colonization by CRE is present at the time of admission to the ED (6.8%). Factors associated with colonization on admission to the ED were: previous exposure to healthcare; previous use of antibiotics and hepatopathy. During hospitalization in the ED, colonization rate is 18% in one week in patients negative on admission. Hospitalization and stay in the ED increases the risk for colonization by CRE on admission to the ICU. These findings are unprecedented and make an important contribution to the understanding of the dynamics of transmission and the role of the ED in CRE colonization in the hospital.

Chapter 6 - REFERENCES

Chapter 6:

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Chapter 7 - APPENDICES

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Appendix 1:

RESEARCH PROJECT 1: Evaluation of the prevalence of colonization and cross-transmission of carbapenem resistant *Enterobacteriaceae* in patients admitted to the emergency department of the Instituto Central do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo

Objectives:

1. To investigate the prevalence of patients colonized by carbapenem resistant *Enterobacteriaceae* (CRE) on admission to the emergency department;
2. To investigate risk factors for colonization by CRE in patients admitted to the emergency room;
3. To investigate the acquisition rate of CRE in the emergency room.

Methods:

A prospective two-month prevalence study was conducted from May 31st to July 7th, 2016, at the Emergency Department of the Instituto Central do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (ICHC-FMUSP). During this period, all hospitalized patients admitted to the ICHC-FMUSP Emergency Department were consecutively included, 20 admissions/day on average.

At the time of admission, a form was completed for all patients to assess risk factors for colonization by CRE.

All patients admitted during the study period had surveillance cultures and RT-PCR obtained within 24 hours of admission, except for two patients, who were excluded from the analysis. To this end, two rectal swabs were collected and sent, one to classical microbiology and other to molecular biology laboratory. Patients whose admission cultures were negative were followed in order to perform weekly screening with two surveillance swabs that were processed in the same way as those described for admission.

These collections were maintained throughout the emergency department stay.
Individual patient surveillance was discontinued when CRE was identified in clinical or surveillance culture, or at the time of discharge/transfer from the Emergency Department.

Microbiology:
Surveillance cultures for CRE were performed according to the Centers for Disease Control (CDC) methodology (Siegel, 2006), adopted by the laboratory of the Central Laboratory Division (DLC). The swab was placed in liquid medium for enrichment for 24 hours and subsequently seeded on an agar plate containing carbapenem discs. Suspected *Enterobacteriaceae* colonies that grew within the antibiotic inhibition zone were selected for identification. Identification was performed with MALDI-TOF (Vitek MS, BioMerieux ®, Marcy L'Étoile, France) and antimicrobial suceptibilty test was permformed with automated methodology (Vitek2) as routinely performed by DLC.
Identification and sensitivity of *Enterobacteriaceae* were done by automated VITEK2 method (BioMerieux ®, Marcy L'Étoile, France). The minimum inhibitory (MIC) concentrations of colistin and carbapenems were initially obtained by VITEK2 (automated cation-adjusted Mueller-Hinton broth microdilution) and confirmed by disk diffusion in the case of carbapenems and by E-test (BioMérieux®, Marcy L ' Etoile, France) on diffusion gradient (Mueller-Hinton Agar) for colistin according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) 2016. The interpretation of the colistin MIC result followed the recommendation of the European Committee on Antimicrobial Susceptibility Testing (≤ 2: sensitive and> 2: resistant), however, the interpretation of the antibiogram in relation to carbapenems followed the CLSI 2014 Enterobacteriaceae recommendation (imipenem or meropenem≤ 2 µg/dl: sensitive and> 2 µg/dl: resistant).

Molecular biology

The resistance genes KPC, NDM, VIM, IMP-1, OXA-48, OXA-181 and OXA-232 were detected directly from clinical samples by Real-Time PCR (Cepheid® Xpert® Carba-R) methodology. Swab material was eluted in buffer and processed according to kit standards (Cepheid® Xpert® Carba-R).

Outcomes

1. Prevalence of patients colonized by CRE on admission

The prevalence of CRE-colonized patients was defined by the number of patients with positive surveillance swab by culture or RT-PCR divided by the total number of admissions in the period.

Prevalence = Number of patients with CRE on admission / number of admissions.

2. Risk factors for CRE colonization

The following variables were assessed at admission: age, gender, patient's address, underlying diseases, reason for admission, community or healthcare-associated origin (hospital or long-term care facility, with only the last year's period being considered).), hemodialysis last month, acute or chronic renal failure, liver disease, solid organ transplantation, hematopoietic stem cell transplantation, HIV infection, AIDS, drug induced immunosuppression, neutropenia, presence of invasive devices (long-term catheter, drain, probes, ostomies) and antimicrobial use (> 72 hours of use in the last 30 days).

3. CRE acquisition rate in the Emergency Department

The acquisition rate in the emergency department was obtained by dividing the number of patients with positive swabs in the weekly collections during hospitalization in the emergency department by the number of patients with negative swabs on admission. The denominator only included patients who had two or more swabs collected during their stay in the emergency room.

We considered positive for CRE patients with surveillance culture isolates whose sensitivity tests described above resulted in imipenem or meropenem MICs greater than 2 µg/dl, or who had positive RT-PCR for any carbapenemase.
Patients with no previous CRE isolation in clinical specimens and with at least one negative surveillance swab were considered negative for CRE.

Ethical aspects:

This study was submitted and approved by the Research Ethics Committee of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo under the number CAAE: 83262417.4.0000.0068. Screening with surveillance cultures is a control measure used in other areas of the hospital, so it was approved exemption from the application of informed consent. The survey data will be presented only together, without any identification.

Appendix 2:

3.1 RESEARCH PROJECT: Colonization by Carbapenem resistant *Enterobacteriaceae* on admission to intensive care: hospitalization in the emergency department and other risk factors for colonization.

Objectives:
1. To evaluate the impact that hospitalization in the emergency department has for colonization by Carbapenem resistant *Enterobacteriaceae* (CRE) on ICU admission

Null hypothesis:

Hospitalization in the emergency department prior to ICU hospitalization presents a similar risk to hospitalization in other hospital units for colonization by CRE.

Alternative hypothesis:

Hospitalization in the emergency department prior to ICU admission is a factor associated with colonization by CRE.

Material and methods:

A retrospective case-control study was conducted with patients admitted to the HCFMUSP ICUs from September 2015 to July 2017.
HCFMUSP is a tertiary hospital and the Central Institute (ICHC) is the location where most of the institution's ICU beds are concentrated, with 10 ICUs and 109 intensive care beds.
The Hospital Infection Control Commission (HICC) continuously monitors colonization and infection by multidrug-resistant agents in these units, including CRE. To this end, all patients admitted to the ICHC ICUs are routinely submitted to surveillance rectal swab cultures upon admission to

the unit and placed in contact precaution until its results, being removed from precaution if negative or kept in precaution if positive.

The contact precaution consists of wearing disposable gowns and gloves by health workers, as well as the use of exclusive patient material such as thermometer, sphygmomanometer and stethoscope.

Surveillance cultures are carried out by the ICHC microbiology laboratory following standardized methodology with overnight incubation of swabs in thioglycolate broth followed by sowing on Macconkey agar with Ertapenem disc, Imipenem and Meropenem. If colonies suggestive of enterobacteria grow within the disk halo of Ertapenem, Imipenem and Meropenem, as recommended by the Clinical and Laboratory Standards Institute (CLSI) 2016, these colonies are isolated and identified by Maldi-ToF.

The cases were retrospectively obtained from the HICC database, which compiles all cases of ICHC positive surveillance cultures. This study used two controls for each case. Patients who were hospitalized more than once in ICUs with surveillance culture collection were considered only once in the first hospitalization.

A sample size calculation was performed, and 99 cases and 198 controls were defined, considering 35% of ICU patients hospitalized for more than 2 days in the ED, for a statistical power of 80%, with an Odds Ratio of 2, 0, 95% confidence interval and 0.05.

Case Definition:

Cases were defined as patients who had a positive CRE surveillance swab culture collected within 2 days of admission to one of the ICHC ICUs from 2015 to 2017. Previous CRE colonization or infection in this hospitalization was exclusion factor.

Control Definition:

Controls were patients admitted up to one week apart, whose admission surveillance cultures at the unit (up to 2 days) were negative. The pairing of the patients occurred so that two controls were matched to one case, belonging to the same unit of the case, with the period of hospitalization of the case and the control showing no interval greater than 7 days between the admission of both in the unit. If there were more than two patients eligible as controls, they were selected by lot.

Study Variables:

Cases were compared to controls using the following variables:

Demographic Variables:
• Age (in years);
• Sex (male or female);

Patient origin variables:
• Inpatient unit where the surveillance culture was collected;
• Inpatient unit from which the patient was immediately transferred;
• Length of hospital stay before surveillance culture (in days);
• Length of stay in the ED;
• ED admission> 2 days;
• Patient admitted directly to the ICU;
• Patient coming from another service transfer (yes or no);
• Patient with previous hospitalization (yes or no, and number of months preceding current hospitalization).

ICU admission variables and severity scores:
• Disease that motivated ICU admission

• Severity of disease that motivated ICU admission (defined by the SAPS score - Simplified Acute Physiology Score II); (LeGall et al., 1993)
• Sequential Organ Failure Assessment (SOFA) score on the day of surveillance culture collection. (Vincent et al., 1996)
• Postoperative (yes or no);
• Polytrauma (yes or no);
• Stroke (yes or no).

Infection Variables:
• Infection at the time of admission culture collection (yes or no);
• Sepsis (SEPSIS 3, using the qSOFA criterion> 2, or SOFA increase> = 2) on the surveillance culture collection day (Singer et al., 2016),
• Infection source: pneumonia (yes or no), urinary tract infection (yes or no), bloodstream infection (yes or no, using the Centers for Disease Control and Prevention, 2018) criteria, diarrhea (yes or no)), meningitis (yes or no), yellow fever (yes or no);
• Colonization or previous infection by CRE in another hospitalization (yes or no).

Comorbidities:
• Charlson Score (Charlson et al., 1987)
• Presence of chronic liver disease with cirrhosis on the day of surveillance culture collection (CHILD A or higher, rated yes or no);
• Dialytic chronic renal failure (yes or no);
• Rheumatological or autoimmune disease (yes or no);
• HIV infection (positive or negative);
• Malignant solid organ neoplasia (yes or no);
• Hematologic malignancy (yes or no);
• Solid organ transplantation (yes or no);
• Bone marrow transplant (yes or no).

Organic Dysfunctions:

• Acute liver failure (yes or no, classified according to onset of hepatic encephalopathy within 8 weeks of onset of acute hepatitis signs and symptoms, with evidence of severe acute hepatitis: progressive jaundice; coagulopathy with INR> 1.5; or Factor) V <50%);

• Respiratory failure (yes or no if it meets at least one of the following criteria: PaO_2 <60 mmHg, or $PaCO_2$> 45 mmHg, or respiratory rate> 30 incursions per minute, or mechanical ventilation);

• Acute renal failure (yes or no, according to RIFLE criteria “F –Failure” (Bellomo, 2004), three-fold increased serum creatinine, or urinary output below 0.3 ml/kg in 24 hours);

• Shock (yes or no, depending on at least one of the following: Blood Pressure <90x60 mmHg, or Mean Blood Pressure <65 mmHg, non responsive to volume, or use of vasoactive drugs to maintain blood pressure levels, such as norepinephrine, adrenaline, dobutamine or dopamine).

Presence of invasions for more than 1 calendar day:

• Tracheostomy (yes or no), gastrostomy (yes or no), or colostomy (yes or no);

• Use of mechanical fan (yes or no);

• Central venous catheter (yes or no).

If the device had been removed less than 2 calendar days before culture, it was considered present.

Performing in-hospital procedures prior to ICU admission:

• Hemodialysis, upper digestive endoscopy, colonoscopy, or use of parenteral nutrition

Antibiotic Therapy:

• Antibiotic use at or prior to ICU stay (up to 3 months)

Hospital Outcomes:

• CRE infection after surveillance culture (yes or no);

• Total hospitalization time;

• Use of appropriate antibiotic therapy (yes or no, defined by the use of at least 1 sensitive drug on antibiogram) and time of introduction (days from symptom onset to initiation of adequate antibiotic therapy)

• Death in this hospitalization (yes or no).

A collection instrument and database was created using the REDCap (Research Electronic Data Capture) program (Harris et al., 2009).

Statistical analysis:

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) 11.5 program (SPSS Inc., Chicago, IL, United States). Cases were compared to controls using the chi-square test or exact or corrected tests when appropriate. A confidence interval of 95% was used, with statistical significance if $p < 0.05$.

Variables with statistical significance in the bivariate analysis $p < 0.05$ were submitted to multivariate analysis with other confounding variables in a logistic regression model. The logistic regression model was performed excluding redundant variables or variables with low representativeness in the sample.

Ethical aspects:

This study was submitted and approved by the Ethics and Research Committee of the University of São Paulo School of Medicine Hospital das Clínicas under the number CAAE: 91604518.9.0000.0068. As this was a retrospective study, with no sample collection, it was approved exemption from the application of informed consent. The survey data will be presented only together, without any identification.