



Temporal pattern of *Klebsiella pneumoniae* carbapenemase (KPC) on surfaces of an intensive care unit of a large hospital

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Abstract

Background: Carbapenem-resistant Enterobacteriaceae are an increasing cause of healthcare-associated infections worldwide. Patients with infections caused by *Klebsiella pneumoniae* resistant to carbapenems (KPC) have significant increases in both all-cause mortality and 30-day mortality. The aims of this study was to investigate the prevalence of KPC on environmental samples collected during and after an outbreak caused by KPC in an intensive care unit (ICU) of a teaching hospital.

Methods: Between 2010 and 2014 we conducted a total of 132 environmental monitoring campaigns from different critical surface of ICU ward in a Teaching Hospital Policlinico Umberto I. Samples were collected on surfaces in patient rooms and health care area. All samples were cultured and *K. pneumoniae* isolates were identified by standard microbiological techniques. The presumptive colonies were confirmed and tested for antibiotic resistance by an automated system. *K. pneumoniae* resistant to carbapenems were tested for carbapenemase production by modified Hodge test.

Results: A total of 2526 environmental samples were collected from November 2010 to July 2014. Of those, 111 resulted positive for *K. pneumoniae* while KPC were 95 (85.6% of all *K. pneumoniae*, 3.8% of total samples). KPC was recovered in all patient rooms with similar proportion (5.1-5.6%) with the exception of patient 6 bed room where it was lower (2.4%). The pathogen was not recovered in rooms dedicated to healthcare personnel and doctors. Among surfaces, the highest proportion of KPC resulted on bedrail (6.8%), more than double than other surfaces. Washbasins had nearly half of samples KPC positive (1.2%).

Conclusions: Despite previous studies suggested that environment plays a minor role in the transmission of carbapenem-resistant enterobacteriaceae, our data highlighted that surfaces represents a significant reservoir for KPC possibly supporting transiently contamination of hands of healthcare workers in our ICU. Our results confirm that KPC are more likely found on surfaces closer to the patient than on those situated further away.

Keywords: *Klebsiella pneumoniae*; carbapenemase-producing *Klebsiella pneumoniae*; Intensive Care Unit; environmental contamination.

Introduction

Healthcare-associated infections (HAI) remain a major cause of patient morbidity and mortality. Although the main source of nosocomial pathogens is likely the patient's endogenous flora, an estimated 20% to 40% of HAI have been attributed to cross infection via the hands of health care personnel, who have become contaminated from direct contact with the patient or indirectly by

touching contaminated environmental surfaces (Otter et al., 2013; Weber and Rutala, 2013).

The development of resistance is a normal evolutionary process for microorganisms, but it is accelerated by the selective pressure exerted by widespread use of antibacterial drugs. Resistant strains are able to propagate and spread where there is non-compliance with infection prevention and control measures (WHO, 2014). In Italy a recent study conducted on 49 hospitals reported that

14.8% of patients admitted to the Intensive Care Unit (ICU), 30.9% of intubated patients, 21.4% of patients with central venous catheter and 13.2% of patients with urinary catheter acquired an HAI. The same study reported that respiratory (24,1%), urinary (20,8%) and surgical site (16,2%) were the most frequent infections (Ricchizzi and Moro 2013).

Carbapenem-resistant Enterobacteriaceae are an increasing cause of HAI worldwide (WHO, 2014). Mechanisms of resistance to carbapenems include production of β -lactamases, efflux pumps, and mutations that alter the expression and/or function of porins and penicillin binding proteins (K.M. Papp-Wallace et al., 2011). Combinations of these mechanisms can cause high levels of resistance to carbapenems in certain bacterial species, such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. In particular *K. pneumoniae* carbapenemase (KPC) strain was first identified in USA from 1996 and has subsequently been reported worldwide (Tofield et al. 2013).

A recent review addressed the impact on health and economic burden due to infections caused by *K. pneumoniae* resistant to third-generation cephalosporins and carbapenems and found that patients with KPC had a significant increase in both all-cause mortality and 30-day mortality (WHO, 2014).

The risk of infection for patients increases with the level of microbial contamination of surfaces (Russotto et al. 2015). Therefore, interventions aiming at contrasting surface contamination may lead to reduction of *Klebsiella* spread in hospital care units. The aims of this study was to investigate the prevalence of KPC on environmental samples collected during nearly 4 years of monitoring in an Intensive Care Unit (ICU) of a teaching hospital.

Materials and Methods

This study was conducted in the ICU, Policlinico Umberto I teaching hospital in Rome (Italy) from November 2010 to July 2014. The ward is divided in one 6 bed patient room (d5) a single bed room (d4), two 2 contiguous bed patient rooms served by the same cart medication (d1) and three 2 bed patient room (d2).

The samples was collected in the patient zone (e.g. bedrails, ventilators, cart medications) and in healthcare area (washbasin, computer device, computer keyboard, touch screen emogas analysis and other equipment and environmental surfaces) by using swab method (ISO 18593:2004).

All samples were cultured and *K. pneumoniae* isolates were identified by standard microbiological techniques including enrichment phase on Brain Heart Infusion Broth (Oxoid, Germany) and incubation at 36 ± 1 °C for

24 hour, followed by isolation on Brilliance ESBL Agar Plates (Oxoid, Germany). Plates were incubated at 36 ± 1 °C for 24 hour to obtain presumptive identification of ESBL-producing *Klebsiella*. The presumptive colonies were confirmed and tested for antibiotic resistance by VITEK 2 Compact System (bioMerieux) and the results were interpreted according to the *European Committee on Antimicrobial Susceptibility Testing* (EUCAST).

Klebsiella pneumoniae resistant to carbapenems were tested for carbapenemase production by Modified Hodge test (MHT) (Amjad et al. 2011).

Quality control of the following organisms MHT Positive *K. pneumoniae* ATCC1705 and MHT Negative *K. pneumoniae* ATCC1706 were run with each monitoring.

Frequency tables and other descriptive statistics were calculated using R 2.14.1.

Results

Between 2010 and 2014 we conducted a total of 132 environmental monitoring campaigns from different critical surface of ICU ward in a Teaching Hospital Policlinico Umberto I. A total of 2526 environmental samples were collected and analysed for the recovery of *K. pneumoniae* (Tables 1-2).

Table 1. Proportion of sensible and resistant *K. pneumoniae* in different hospital rooms of intensive care unit (ICU) between 2010-2014. d1-d5: patient's rooms; s1: bathroom of health personnel, s2: dressing room, s3: medical office, s4: bronchoscopy, s5: medical device (duty room), s6: after surgery room. N=2526.

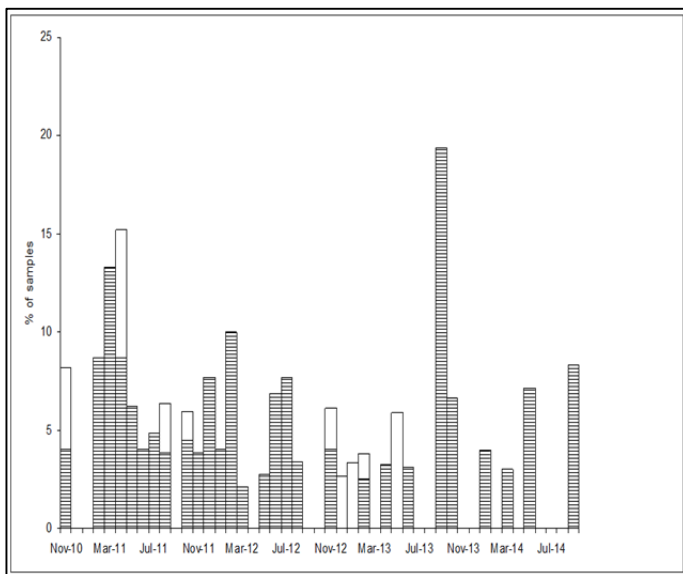
Room code	Room type	N.of beds	N. of samples collected	KPC %
d1	patient's room	4	175	5.1
d2	patient's room	2	303	5.3
d3	patient's room	4	595	5.5
d4	patient's room	1	195	4.6
d5	patient's room	6	1145	2.4
s1	bathroom of health personnel	-	41	0.0
s2	dressing room	-	25	0.0
s3	doctor's office	-	3	0.0
s4	bronchoscopy	-	5	0.0
s5	medical device washing room	-	37	0.0
s6	after surgery room	-	2	0.0

Table 2. Proportion of KPC on different surfaces of intensive care unit between 2010-2014. N=2526.

Surface type	Number of samples	KPC %
bed rail	775	6.8
cart medications	481	2.9
medical device	554	2.5
washbasin	331	1.2
computer device, computer keyboard	266	2.6
Other inanimate objects and environmental surfaces	119	2.5

Of those, 111 resulted positive for *K. pneumoniae* and KPC were 95 (85.6% of all *K. pneumoniae*, 3.8% of total samples). Temporal pattern of KPC on surfaces is shown in **Figure 1**.

Figure 1. Temporal pattern of %KPC on surface samples in the study period. White bars are *K. pneumoniae*; striped bars are KPC.



On surfaces, *K. pneumoniae* was sporadically present in various months following the first outbreak of October and November 2010 in patients (Figure 1). On average, KPC was 0,04% (min 0%, max 19,4), with peaks >10% in March 2011 and September 2013, following other patient outbreaks.

The majority of samples were collected in 6 bed patient room d5 (45.3% of total collected samples), followed by 4 bed patient rooms d3 (23.55% of total collected samples) and 2 bed patient room d2 (11.99% of total

collected samples; Table 1). Among the different surfaces, the majority of samples were collected on bed rail (30.7% of total collected samples), medical devices (21.9%) and cart medications (19.0%; Table 2).

Klebsiella pneumoniae carbapenemase was recovered in all patient rooms with similar proportion (5.1-5.6%; Table 1) with the exception of 6 bed room d5 where it was lower (2.4%; Table 1). The pathogen was not recovered in duty healthcare personnel/office/doctor rooms (0 % s1 to s6; Table 1). Among surfaces, the highest proportion of KPC (6.8%) resulted on bedrail, more than double than other surfaces (Table 2). Washbasins had nearly half of samples KPC positive (1.2%; Table 2).

Discussion

Although pathogen transfer from a colonized or infected patient to a susceptible patient most commonly occurs through the hands of healthcare personnel, contaminated inanimate surfaces and equipment can be directly or indirectly involved in the transmission pathways and have been often described as the source for outbreaks of nosocomial infections (Weber et al., 2013).

Evidence that contaminated surfaces contribute to the transmission of hospital pathogens comes from studies modeling transmission routes, microbiologic studies, observational epidemiologic studies, intervention studies, and outbreak reports (Otter et al., 2013).

Many Gram-negative species, such as *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp., *P. aeruginosa*, *Serratia marcescens*, or *Shigella* spp can survive on inanimate surfaces even for months and these species are found among the most frequent isolates from patients with nosocomial infections (Rüden, et al., 2006). The longer a nosocomial pathogen persists on a surface, the longer it may be a source of transmission and thus endanger a susceptible patient or healthcare worker (Kramer et al., 2006). *Klebsiella*, for examples, can survive on fomites till 30 months at ambient temperatures and constitute a risk for patients and health care personnel (Kramer et al., 2006).

Despite studies suggested that environment play a less important role in the transmission of carbapenem-resistant Enterobacteriaceae (CRE) which infrequently was isolated from environmental surfaces, (only 0,8% of 1160 environmental samples) (Weber et al., 2015), our data reported 3.8% of 2526 samples only for KPC, the most important CRE involved in HAI, suggesting that the surfaces represents an important reservoir for KPC supporting transiently contamination of hands of healthcare workers in our ICU.

Excluding the bed rail, which obviously represents the most contaminated surface (6,8%), in our studies cart medication followed by medical devices represent the main reservoir of KPC (2,9% and 2.5% respectively of

the positive samples), also described by *Tajeddin et al.* (2016). Our results confirm that the sites closer to the patient are more likely to give an infection risk of KPC than those situated further away as literature reported (*Dancer, 2009*).

Cleaning and disinfecting environmental surfaces with appropriate disinfectants is fundamental in reducing their potential contribution to the incidence of healthcare-associated infections (CDC Guidelines for Environmental Infection Control in Health-Care Facilities, 2003) and full description of current cleaning technologies and environmental contamination-monitoring systems has been reported by recently CDC guidelines (*Guh and Carling, 2015*).

The persistence of environmental microorganisms is now supported by the evidence of tolerance to biocides and oxidative stress, especially those micro-organisms endowed with antibiotic resistance (*Cordeiro Dias et al, 2017*).

Contamination of inanimate hand-touch sites near the patient may contribute to ICU-acquired colonization or infection, but further studies are needed to evaluate this correlation. However clinicians and researchers should be aware of the risk of cross-transmission of pathogens from surfaces in order to adopt appropriate infection control measures (*Russotto et al., 2015*).

It is therefore evident that environmental matrices, and in particular hand-touch sites near the patient, such as bed rail that shows the highest % of KPC, must be subjected to routine screening in order to monitor the level of contamination. This aspect is important to increase evidence in support of behavioral verification and sanitization procedures in terms of frequency and type of disinfectants.

As *Dancer (2009)* argues, “it is false economy to wait until an outbreak occurs before the clinical environment receives the attention it deserves”.

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