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# Intra-hospital acquisition of colonization and infection by *Klebsiella pneumoniae* strains producing carbapenemases and carriage evolution: A longitudinal analysis in an Italian teaching hospital from January 2017 to August 2019

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ABSTRACT

*Objectives:* We present an updated picture (1/1/2017–31/08/2019) of the frequency of carbapenemase producing *Klebsiella pneumoniae* (CPKP) in surveillance rectal swabs (SRS) and in clinical samples (CS) of patients admitted to a tertiary level hospital, focusing on longitudinal evolution of CPKP detected in SRS and on colistin resistant strains.
*Methods:* Retrospective longitudinal analysis. Only the first positive CPKP strain isolated from each patient was included.
*Results:* 638 CPKP strains were identified (471 in SRS and 167 in CS). SRS frequency increased over time in the medical department, remained high in the surgical department (SD) and decreased in the intensive care department. Most SRS–71.3%–and 49.1% of CS had nosocomial origin; about half of the SRS were identified in the SD. Regarding SRS evolution, carriage was confirmed in 39.5% of patients, no more testing in 25.5%, clinical involvement in 24.8 %, and negative result in 10.2%. Rates of colistin resistance were 20.1% in 2017, 31.2% in 2018 and 26.9% in 2019.
*Conclusions:* CPKP diffusion is still an important issue despite the surveillance program. It is vital to enhance medical staff's awareness on this because most CPKP first detections in SRS occurred during hospital stay due to a nosocomial acquisition with a comparable picture over time. Colistin resistance is increasing.


## Introduction

Carbapenem-resistant *K. pneumoniae* detection has greatly increased in Italy, after the first isolation reported in 2008 (Giani et al., 2009). The European Survey demonstrated that 192 out of the 195 *K. pneumoniae* clinical isolates non-susceptible to carbapenems submitted from 22 Italian hospitals between November 1, 2013, and April 30, 2014 were PCR positive for one of the following: *K. pneumoniae* carbapenemase [KPC], New Delhi metallo-β-lactamase [NDM], oxacillinase-48 [OXA-48-like], or Verona integron encoded metallo-β-lactamase [VIM]) (Grundmann et al., 2017).

Infections caused by carbapenemase producing *Klebsiella pneumoniae* (CPKP) are serious and life-threatening because of

*Abbreviations:* CoR, colistin resistant; CPKP, carbapenemase producing *Klebsiella pneumoniae*; CS, clinical samples; EUCAST, European Committee on Antimicrobial Susceptibility Testing; ICD, intensive care department; KPC, *K. pneumoniae* carbapenemase; LRT, lower respiratory tract; MD, medical department; NDM, New Delhi metallo-β-lactamase; OXA-48-like, oxacillinase-48; SD, surgical department; SRS, surveillance rectal swabs; UT, urinary tract; VIM, Verona integron encoded metallo-β-lactamase.

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the limited availability of treatment options (Suay-García and Pérez-Gracia 2019; Gupta et al., 2019): mortality rate in patients with bloodstream infection ranges from 33% to 51% (Brescini et al., 2019; Gomez-Simmonds et al., 2016; Giacobbe et al., 2015; Papadimitriou-Olivgeris et al., 2014), all-cause mortality rate ranges from 13.9% to 39.1% (Rodriguez-Gómez et al., 2019; Chiu et al., 2017; Kuntaman et al., 2018) in urinary tract infections, and it can be more than 40% in cases of lower respiratory tract infection (Capone et al., 2013; Tumbarello et al., 2015).

Asymptomatic rectal carriers are currently considered the main reservoir of CPKP in the hospital setting and their prompt identification and correct management represent the first step to control person-to-person transmission of the infection (Micozzi et al., 2017).CPKP can be primarily isolated from a clinical site or be detected after a previous asymptomatic gastrointestinal carriage: Borer et al. (2012) reported that 9.1% of subjects with CPKP colonization developed a clinical infection in a hospital wide study while higher percentages were reported by Papadimitriou-Olivgeris et al. (2013) and by Debby et al. (2012) in intensive care unit settings (22.6% and 41.7% respectively).

In our previous works, we described the spread of CPKP in an Italian teaching hospital with an ongoing active surveillance program (Parisi et al., 2015; Bartolini et al., 2017). The proportion of CPKP first isolated by surveillance rectal swabs (SRS) with relation to those identified by clinical samples (CS) increased from 2012 to 2014 (Parisi et al., 2015) and the percentage of CPKP detected in SRS with respect to CS increased in the medical department (MD) from 2015 to 2016 and in intensive care department (ICD) from 2012 to 2015 while it decreased in the surgical department (SD) from 2014 to 2016 (Bartolini et al., 2017).The percentage of colistin resistant (CoR) strains detected in SRS was higher than 20% in 2015 and 2016 (Bartolini et al., 2017), worrisome data since colistin-resistant *K. pneumoniae* infections have a high mortality rate (Capone et al., 2013; Balkhair et al., 2019; Menekşe et al., 2019).

The aim of this study was to give an updated picture (January, 1 2017–August, 31 2019) of the relative frequency of CPKP in SRS and in clinical samples of patients admitted to a tertiary level hospital, with a special focus on longitudinal evolution of CPKP detected in SRS and on CoR resistant strain diffusion.

## Patients and methods

### *Study design*

We conducted a retrospective longitudinal analysis on the evolution of SRS and of CS positive for CPKP isolated in a tertiary level teaching hospital from January 1, 2017 to August 31, 2019.

The hospital guidelines came from a surveillance program started in 2012; adult patients admitted to the ICD were monitored with SRS upon admission and at least weekly thereafter; patients of the SD were screened only upon admission; patients admitted to the MD were tested only if they were hospitalized in the last two months or if they came from long-term care facilities. Additionally, isolates obtained during surveys of subjects hospitalized in the same room or ward of positively diagnosed patients were included in the study. Carriers were recovered in isolation rooms when possible or cohorted in the same room, and all contact precautions were improved. No data about the adherence to hospital guidelines by different wards or departments were available.

The Ethical Committee for Clinical Experimentation, Padua Province (Ethics Review 3418/AO/15) approved the study. All samples were collected as part of routine management/surveillance, and were anonymised prior to research use.

Only the first positive CPKP strain isolated from each patient was included, regardless of whether it was a surveillance swab or a clinical sample.

Other collected data were gender, age, intervals (as days) between the first and subsequent identifications, SRS and CS performed after the first CPKP detection in SRS, and details on the identified isolates other than CPKP.

Four different analyses were performed on the patients who took part in the study.

The first was the description of the relative frequency of SRS and of CS by year in MD, SD, and ICD.

The second was classification of SRS and of CS acquisition. SRS acquisition was classified as follows: (1) nosocomial (at least one negative SRS performed at least 3 days before the positive one) (Horan et al., 2008; Nicolle et al., 2011); (2) possibly nosocomial (no previous negative SRS, but patient hospitalized for 7 days at least); (3) co-morbidities related (no previous negative SRS in patients undergoing dialysis, patients with cancer, transplantation, chronic heart disease, on immunosuppressive therapy, referred to a day hospital setting or transferred from other hospitals or from long term care facilities (Friedman et al., 2002); (4) unknown origin (not included in the first three categories).

The last definition was applied with no changes in CS acquisition classification, while the other categories had slightly different inclusion criteria. A positive CS was defined as of nosocomial origin when at least one negative SRS was performed at least 3 days before the positive CS and/or a previous negative CS of the same clinical site made 3–7 days before positive CS detection was known. It was possibly nosocomial if no negative CS of the same clinical site was known but hospital stay was 7 days at least and co-morbidities related if no previous CS of the same clinical site was available in the same groups of patients listed for SRS.

The third was the description of longitudinal evolution of SRS. Parameters evaluated were the rate of subjects not retested and the results of those retested. The detection of CPKP in a clinical site was accepted as true evolution only if a previous negative test for CPKP in the same clinical site was performed 3–15 days before. CS were classified in four groups according to the site of CPKP detection: lower respiratory tract, urinary tract, blood, and any other different site.

The fourth was the description of the diffusion rate of CoR in SRS and in CS by year in the three Departments.

### *Laboratory analysis*

Screening test for carbapenemase production on SRS was performed by inoculation of the strains on BD BBL™ CHROMagar™ CPE (C-CPE) with the automated WASP® system (Copan, Brescia, Italy). In addition, an ertapenem disk (10 μg, BD BBL Sensi-Disk™) was placed on several media to identify suspected carbapenemase-producing colonies (screening cut-off ≤25 mm) (EUCAST_detection_of_resistance_mechanisms_v1.0 and v2.0).

After the incubation on the medium and the microbial identification with MALDI-TOF, the antimicrobial susceptibility testing of the strains obtained through the surveillance rectal swabs was performed using the dilution method (Thermo Scientific Sensititre™ system) and the susceptibility testing for the pathogens isolated from clinical samples was performed through VITEK® 2 automated system.

Results were interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints. Colistin susceptibility was determined as prescribed by EUCAST guidelines and resistance was set at MIC breakpoint >2 mg/L (EUCASTv 7.1, v 8.0, v 8.1 and v 9.0).

Genotypic detection of carbapenemases was obtained with a validated in-house PCR method for KPC type, OXA-48, VIM, and NDM carbapenemases when a carbapenemase-producing strain was suspected (Richter et al., 2011).

**Table 1**
Carbapenemase single gene and double gene detection in CPKP. Data were described as absolute number and as percentage respect to the total number of strains tested in 2017, 2018 and 2019.

| Single gene detection | | | |
|---|---|---|---|
| | 2017 (110 strains) | 2018 (169 strains) | 2019 (129 strains) |
| KPC, n (%) | 109 (99.1) | 158 (93.5) | 121 (93.8) |
| OXA 48[2], n (%) | 0 | 2 (1.2) | 0 |
| OXA 23, n (%) | 0 | 0 | 4 (3.1) |
| VIM, n (%) | 0 | 8 (4.7) | 3 (2.2) |
| NDM, n (%) | 1 (0.9) | 1 (0.6) | 1 (0.8) |
| Double gene detection | | | |
| | 2017 (8 strains) | 2018 (5 strains) | 2019 (4 strains) |
| KPC + NDM, n (%)[6] | 1 (12.5) | 0 | 0 |
| KPC + OXA-48, n (%) | 1 (12.5) | 1 (20) | 0 |
| KPC + VIM, n (%) | 3 (37.5) | 3 (60) | 3 (75) |
| NDM + OXA-48, n (%) | 3 (37.5) | 0 | 1 (25) |
| NDM + VIM, n (%) | 0 | 1 (20) | 0 |

CPKP: carbapenemase producing *Klebsiella pneumoniae*.
KPC: class A carbapenemases.
OXA-48: oxacillinases (OXA-48-like enzymes).
OXA-23: carbapenem-hydrolyzing OXA-type carbapenemase.
VIM: Verona integron-encoded metallo-β-lactamase.
NDM: New Delhi metallo-β-lactamase types.

*Statistical analysis*

The number of CPKP strains was evaluated by material (SRS versus CS), by department (MD, SD and ICD), and by year. The Chi-squared test and Fisher's exact test were used as appropriate. The Mann–Whitney U test was applied to compare continuous variables between groups. Values of $p < 0.05$ were considered statistically significant.

The statistical analyses were performed with MedCalc Statistical Software version 19.1 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org; 2019).

## Results

Most patients were male (66.7% in 2017, 68.5% in 2018 and 69.9% in 2019). Patients with CS were older than colonized subjects in 2017 (71 years, IQR 59–83 years vs 66 years, IQR 56–76 years, $p = 0.0219$), no significant age difference in 2018 (71 years, IQR 52–81 years vs 67 years, IQR 54–75 years) and 2019 (63 years, IQR 51–77 years vs 69 years, IQR 60–78 years).

A total of 638 first strains of CPKP were identified in SRS (471, 73.8%) and in CS (167, 26.2%) during the study period (January 1, 2017–August 31, 2019) — 243 strains in 2017, 222 strains in 2018, and 173 strains in 2019.

Overall, SRS were represented more and the frequency was comparable in 2017 and 2018 (68.3% and 74.3% respectively), and it was 80.9% in 2019 (chi squared for trend $p = 0.0153$).

Four hundred and twenty-five strains (66.6% of all strains) were characterized by molecular analysis. Overall, KPC resulted the most frequent type of carbapenemases detected during the study period, both as a single gene and associated to others, with a comparable yearly frequency of 96.6% in 2017, 93.1% in 2018, and 93.2% till August 31, 2019. A single carbapenemase was detected in 408 strains and two carbapenemases were identified at the same time in 17 strains. OXA-23 was identified only as a single gene and for the first time in 2019; it was more represented than OXA-48. NDM was identified only in 3 subjects as a single gene but it was found in 6 out of 17 (35.3%) combined genes, a frequency only second to VIM in the case of gene combination (identified in 10 strains, 58.8%). A complete description of carbapenemase detected by year is reported in Table 1.

*Relative frequency of SRS and CS by year in MD, SD and ICD*

In 2017 the relative frequency of SRS in MD was lower than 50% but it increased to 60% in 2018 ($p = 0.0322$ respect to 2017) and it reached 76.9% in 2019 (chi squared for trend $p = 0.0001$); SRS percentage detection was always about 90% in SD and it showed a decreasing trend in ICD, starting from a value of 78.2% in 2017. In 2017, SRS frequency in MD was lower than in SD and ICD ($p < 0.0001$) in 2018, and in 2019 the SRS detection in SD was higher than in MD ($p < 0.0001$ and $p = 0.006$) and in ICD ($p = 0.0131$ and $p = 0.0001$). A detailed description is shown in Figure 1.[2]

*Analysis of SRS and CS acquisition*

In 2017–2019, CPKP carriage in SRS had a nosocomial origin (336 out of 471, 71.3%); the overall relative frequency of patients with nosocomial and possibly nosocomial origin was 83.7% in 2017, 81.2% in 2018, and 72.1% till August 2019 ($p = 0.0142$ with respect to 2017). The decreasing trend in 2019 preliminary data was confirmed including only subjects with nosocomial acquisition (62.9%) and the difference was significant with respect to 2017 (75.9%, $p = 0.0133$) and to 2018 (73.9%, $p = 0.0376$). Furthermore, we analyzed the relative frequency by year and department of patients with a positive SRS of nosocomial origin: about half SRS were identified in the SD (54% in 2017, 50.8% in 2018 and 51.1% in 2019), and no difference in relative percentage throughout the study period was found in the three departments.

During the study period (2017–2019), nosocomial acquisition of CPKP in CS was found in 49.1% of subjects, a frequency significantly lower with respect to rectal colonization ($p < 0.0001$); conversely both possibly nosocomial and co-morbidities related acquisition were more frequent in CS with respect to SRS (22.8% vs 8.1%, $p < 0.0001$ and 20.4% vs 10.8%, $p = 0.019$ respectively). Relative frequency of nosocomial CS in MD was significantly higher in 2017 than in 2019 (48.7% vs 5.6%, $p = 0.0021$), and it was significantly higher in 2019 with respect to 2017 (83.3% vs 28.2%, $p = 0.0001$) and 2018 (83.3% vs 48%, $p = 0.0194$) in ICD.

One hundred and sixty-seven patients had CPKP first detected in a CS. Most patients had a single CS detection (143 patients, 85.6%); 13 patients (7.8%) reported a contemporary positivity of SRS and 11 subjects (6.6%) had more than a positive clinical sample at the same time. Overall, the site which more frequently had a single detection was the urinary tract (UT) (61 patients), while the

[2] From January, 1 to August 31, SRS: surveillance rectal swabs, CS: clinical samples, CPKP: carbapenemase producing *Klebsiella pneumoniae*.

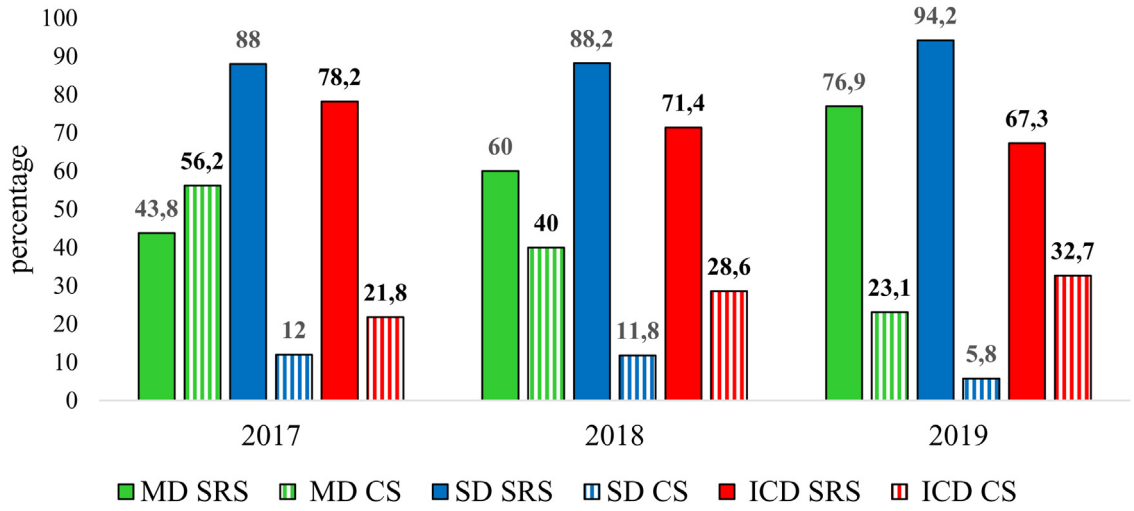


**Figure 1.** Percentage of CPKP detected in SRS and in CS in the Medical Department, Surgical Department and Intensive Care Department in 2017, 2018 and 2019[1]. Data are reported as relative percentages for each department.

lower respiratory tract (LRT) was the specific site with the higher percentage of combined CS-SRS detection (Table 2).

Blood was found in 7 of the 11 patients with multiple positive CS (Supplementary Figure 1).

*Analysis of longitudinal evolution of SRS*

Evolution of the 471 patients colonized by CPKP was as follows: no further testing available (120 subjects, 25.5%), CPKP clearance (48 subjects, 10.2%), persistent carriage (186 patients, 39.5%) or detection in CS (117 patients, 24.8%). The intervals between positive SRS and the three possible evolutions were comparable: 7 days (median, IQR 4–18 days) in subjects who turned negative, 7 days (median, IQR 3–8 days) in patients who confirmed colonization, and 8 days (median, IQR 3–18 days) when a clinical site was involved. Amongst the 117 subjects with CS, 13 (11.1%) had CPKP detected in more than one site (Supplementary Figure 2) and 66 had a previous negative culture or another infectious agent detected. In all clinical sites other than the urinary tract, more than a third of subjects had a previous positivity: 8 of the 31 isolates other than CPKP (25.8%) were *E. coli* (Table 3).

*Colistin resistance*

Overall, 415 SRS (88.1% of all SRS) and 152 CS (91% of all CS) had colistin sensitivity tested: more than a quarter (148 strains, 26.1%) were CoR and the frequency was comparable in SRS (106, 25.5%) and in CS (42, 27.6%).

Analysis by year showed an overall frequency of 20.1% in 2017, 31.2% in 2018 (significantly higher than in 2017, p = 0.0118), and 26.9% in 2019: no statistical significance was found between CoR SRS and CoR CS percentage in MD, SD and ICD throughout the study period, with the only exception of the higher frequency of CoR in CS (75% vs 22.9%, p = 0.0006) in ICD in 2019 (Figure 2).

Lower respiratory tract and urinary tract are the sites with the higher relative percentage of detection, blood was detected less among the CoR samples, but the absolute frequency was 33.3% (Supplementary Table 1). Two patients had a CoR detection in two different sites: blood and lower respiratory tract in one case, and urinary tract and pharyngeal mucosa in the other case.

Considering the total number of CoR strains identified throughout the study period, about half of them were isolated in SD (76 samples, 51.4%), about a third in ICD (49 samples, 33.1%), and a lower percentage in MD (23 strains, 15.5%).

## Discussion

To the best of our knowledge, this study reports the most updated report of CPKP detection and acquisition in SRS and CS in three different clinical settings of an Italian tertiary level hospital with an ongoing surveillance program since 2012.

**Table 2**
Description of single CS detection and of the combined CS and SRS positivity (156 patients). Data are expressed as absolute number, absolute and relative percentage.

| | n (%)[a] | Relative percentage[b] | Relative percentage[c] | Relative percentage[d] |
|---|---|---|---|---|
| Respiratory tract | 34 (21.8) | 23.8 | – | – |
| Respiratory tract + SRS | 4 (2.6) | – | 30.8 | 10.5 |
| Urinary tract | 61 (39.1) | 42.7 | – | – |
| Urinary tract + SRS | 1 (0.6) | – | 7.7 | 1.6 |
| Blood | 13 (8.3) | 9.1 | – | – |
| Blood + SRS | 1 (0.6) | – | 7.7 | 7.1 |
| Other sites[5] | 35 (22.4) | 24.5 | – | – |
| Other sites + SRS | 7 (4.5) | – | 53.9 | 16.7 |

CS: clinical sample; SRS: surveillance rectal swab.
[a] Percentage respect to the 156 patients.
[b] Percentage respect to the 143 patients with single CS.
[c] Percentage respect to the 13 patients with combined detection.
[d] Percentage respect to the total number of CS of the specific site.

**Table 3**
Description of the 104 single detections of CPKP in clinical samples and of the available cultural results performed in the same clinical site.

| Site | n (%) | Days to evolution (median and IQR) | Previous negative n (%) | Previous positive other than CPKP n (%) | No previous data n (%) |
|---|---|---|---|---|---|
| Respiratory tract | 28 (26.9) | 6 (3–15) | 10 (35.7) | 10 (35.7)[a] | 8 (28.6) |
| Urinary tract | 28 (26.9) | 13 (5–28) | 12 (42.9) | 3 (10.7)[b] | 13 (46.4) |
| Blood | 15 (14.4) | 7 (3–29) | 6 (40) | 5 (33.3)[c] | 4 (26.7) |
| Other sites[e] | 33 (31.7) | 7 (3-20) | 7 (21.2) | 13 (39.4)[d] | 13 (39.4) |

CPKP: carbapenemase producing *Klebsiella pneumoniae*; CS: clinical samples.
[a] *E. coli* (2 patients), *Corynecterium spp* + *C. albicans*, *C. albicans*, *P. aeruginosa, methicillin resistant S. aureus*, *S. marcescens*, multidrug resistant *A. baumanii*, extended spectrum beta-lactamase *K. pneumoniae*, *P. aeruginosa* + *C. albicans*.
[b] Multidrug resistant *P. aeruginosa*, extended spectrum beta-lactamase *K. pneumoniae*, *P. aeruginosa*.
[c] *E. coli* (2 patients), *S. capitis*, *S. epidermidis*, multidrug resistant *A. baumanii*.
[d] *S. maltophilia*, methicillin resistant *S. aureus* (2 patients), *E. coli* (3 patients), extended spectrum beta-lactamase *E. coli*, *M. morganii* (2 patients), *S. gallinarum*, *P. aeruginosa* (2 patients), *A. baumanii*, *E. faecium* (2 patients), *C. albicans* (2 patients).
[e] Bile (3 patients), surgical drain (4 patients), wound swab and/or skin swab (13 patients), nasal and/or pharyngeal swab (13 patients).

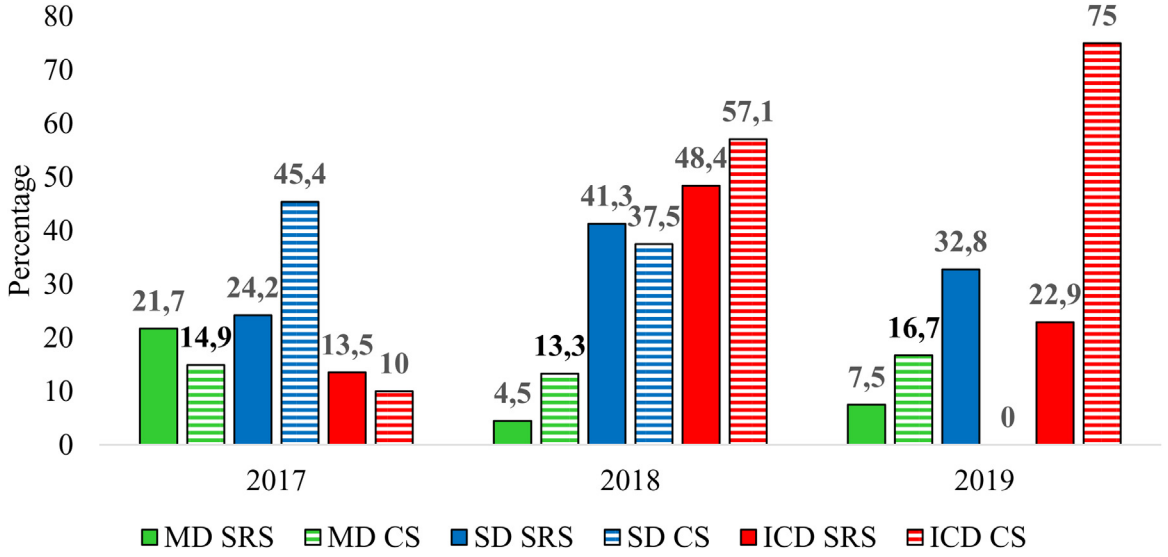


**Figure 2.** Percentage of colistin resistant strains (CoR) identified in 2017, 2018 and 2019 in the medical department (MD), surgical department (SD) and intensive care department (ICD).

The availability of a wide setting of admitted patients gave us the opportunity to evaluate the overall "colonization risk" of the structure and to tailor the program in subjects who were transferred to different sections of the hospital. Our descriptive approach included both carriage and clinical site detection at the same time in a wide hospital analysis, different from other published studies in Italy (Brescini et al., 2019; Sotgiu et al., 2018; Rimoldi et al., 2017). Vilches et al. (2019) elaborated a multi-patch model to analyze the role of intra-hospital patient transfer on CPKP healthcare associate infections diffusion. ICD is the main site to focus on for infection diffusion control in this model. We observed a higher relative frequency of first CPKP detection in SRS than in CS in all settings, but MD in 2017 and three different figures in SRS/CS relative frequency were found in MD, SD, and ICD throughout the study period: there was a progressive increase of SRS frequency in MD, a persistently and high percentage in SD, and a decrease over time (even if not significant) in ICD. This last result suggested that our data are in accord with Vilches model and that surveillance strategy should be implemented in order to identify the carriers in ICD, whose relative frequency is lower than that found from 2014 to 2016 (Parisi et al., 2015; Bartolini et al., 2017). This concept was further strengthened by the data on overall nosocomial CPKP acquisition rates on SRS (71.3%) and on CS (49.1%). Only a few studies with a design including a global approach were conducted in other Italian hospitals. Ridolfo et al. (2016) reported 77.8% of CPKP detected in SRS and a 68.5% in CS in a cohort of 46 subjects with hospital-associated isolation in medical and surgical wards of a university hospital in 2012–2013. The lower value we reported in CS may be explained by our more restrictive inclusion criteria with respect to Ridolfo's work, but the SRS relative frequency was comparable and high. Our analysis by department showed a different picture for hospital-acquired SRS and CS: the former had a stable distribution over time while the latter was multifaceted. The highest numerosity of nosocomial SRS in SD throughout the study period may be justified by the presence of known predictors of CPKP detection (i.e., presence of surgical drain) (Tumbarello et al., 2014). However, the persistent frequency suggests the opportunity to check infection control measures and to address a specific antimicrobial stewardship program (Giacobbe et al., 2019). Colonization reduction rate is a clinical need because of the risk of bacterial translocation and subsequent endogenous localization in a clinical site. In our study, the relative frequency of colonized patients who had CPKP isolated from blood was 14.4%, quite similar to the reports by Giacobbe et al. (11%) (2017) on patients tested from January 2012 to March 2014. These authors demonstrated a previous bloodstream infection from other pathogens in 32.4% of

the subjects and reported that it was an independent risk factor for bloodstream infection in colonized subjects. A risk factor analysis was beyond the aims of our work, focused on a dynamic description of CPKP diffusion in different departments, but the frequency we reported was comparable (33.3%).

Our overall rates of colistin resistance (20.1% in 2017, 31.2% in 2018 and 26.9% in 2019) are higher than that reported by Cojutti et al. (2018) (8.2% in 169 isolates between 2013–2016) and by Sotgiu et al. (2018) (13% in 46 strains only from clinical samples from November 2015 to May 2017). Our data on a higher number of samples underlined the importance of an updated report, because the percentage of CPKP isolates with colistin resistance can increase heavily in a few years, as in the study by Tumbarello et al. (2015) (from 11% in 2010 to 27% in 2013). The variability may be due to the interplay of many factors; colistin administration has a known role on resistance development (Giacobbe et al., 2015), but Tansarli et al. (2018) elaborated a dynamic model in which only 69% of colistin resistance cases could be explained by colistin use and prior levels of colistin resistance, which was 22% in 2014 (Parisi et al., 2015) and 15.7% in 2015 (Bartolini et al., 2017). Other cases may have one or more explanations, such as hospital surface contamination (Caselli et al., 2018), cross-transmission of colistin-resistant isolates in the absence of colistin treatment, the presence of recovered subjects with known CoR CPKP carriage (Bogdanovich et al., 2011; Ah et al., 2014; Sypsa et al., 2012), superinfection with a CoR strain in a subject infected with a CoS CPKP (Parisi et al., 2015).

Furthermore, we observed a comparable frequency of CoR strain in SRS and in CS detected in the three departments in 2017, 2018 and 2019 (the higher frequency of CoR in CS in ICD in 2019 needs to be confirmed in the twelve-month analysis), but the CoR value ranged from 4.5% to 48.4% in SRS and from 10% to 57.1% in CS in the 2017–2018 period; parcelization of analysis, which is a characteristic of the study design, may have influenced these results, but they reflect the complexity of the antibiotic resistance picture and the need of local epidemiology knowledge.

In our study, most carbapenemases detected belonged to class A KPC-type, confirming the epidemiological data previously reported in other Italian studies (Sotgiu et al., 2018; Calia et al., 2017). Nine patients had NDM producing strains (six were double carbapenemase producers), a worrisome report because of the very limited therapeutic options (susceptible to aztreonam but not to ceftazidime/avibactam) (Lee et al., 2016; Petrosillo et al., 2019), but a very low burden with respect to the 350 cases notified in seven Tuscany hospitals and with no definite epidemiological source (ECDC, 2019).

Our study has some limits. First, no clinical or therapeutic data were available. However, the concordance between our results and those reported in other descriptions of Italian hospital epidemiology demonstrated the validity of the figures described. Second, molecular testing of carbapenemase was available only for two thirds of patients because they were performed according to laboratory guidelines and clinical needs and not for research aims: we are aware of the scarce molecular data and of their importance for infection control and public health purposes but unfortunately the characterization was not always possible even if it has improved over time throughout the study period.

In conclusion, CPKP diffusion inside the hospital is still an important issue despite the surveillance program, and this evidence has a great relevance not only for in-hospital subjects but also for outpatients because the current epidemic is greatly based on nosocomial spread (David et al., 2019). About half of patients included in our study had persistent CPKP carriage and this status implied a double healthcare risk for the community and for the subject. First, the patient can be responsible for CPKP transmission via non-invasive procedures (i.e. colonoscope and gastroscope-associated infection) (England et al., 2016; Bac and Bac, 2017) that are frequently performed in out-patients. The medical personnel should be informed and they should apply cleaning and disinfection protocols to avoid infections due to the instruments in other subjects. Second, colonized patients are at a risk of invasive infections with significant mortality (Borer et al., 2012; Giannella et al., 2014) and there is a dangerous loop because re-admission and duration of hospitalization are independent risk factors for persistent carriage (Kim et al., 2018).

The high percentage of intra hospital CPKP acquisition can be used as a starting point to discuss a different surveillance protocol and/or infection control measures implementation in the three different departments, which have now an epidemiological history lasting almost 7 years (Parisi et al., 2015; Bartolini et al., 2017). Furthermore, the unchanged relative frequency in 2017, 2018 and 2019 in the 3 departments suggests to check the application of prescribed protocols adopted and implemented since 2011 in daily clinical practice and to implement the education of the healthcare workers. Additional surveillance studies aimed to identify in each department the role of the known risk factors for CPKP acquisition and the influence of the ward organization, bed capacity for isolation and dedicated personnel number, allowing discussion of these items with political decision makers to better allocate resources. Nevertheless, the occurrence of transmission clusters and the endemic circulation of CPKP underline the inadequacy of controls of the application of the prescribed measures, making this work useful to reinforce attention to the need for more stringent measures.

## Conflicts of interest

The authors declare that they have no competing interests.

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## Ethical approval

The Ethical Committee for Clinical Experimentation, Padua Province (Ethics Review 3418/AO/15) approved the study. All samples were collected as part of routine management/surveillance, and were anonymised prior to research use.

## Authors' contributions

MB helped to interpret the findings, performed the statistical analysis, wrote the paper.

DZ: collected the data, helped to interpret the findings, wrote the paper.

IP: helped to interpret the findings, wrote the paper.

EDC: performed the laboratory tests, helped to interpret the findings.

RS: helped to interpret the findings, wrote the paper.

MAB: collected the samples, performed the laboratory tests.

MP: collected the samples, performed the laboratory tests.

FO: collected the samples, performed the laboratory tests.

EB: collected the samples, performed the laboratory tests.

GP: helped to interpret the findings and wrote the paper.

SGP: designed and coordinated the study, supervised the laboratory tests, collected the data, interpreted the findings, and wrote the paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2019.12.035.

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