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# BMJ Open

## Seven-year active surveillance for carbapenemase-producing *Klebsiella pneumoniae* and correlation with infection in subjects attending an Italian tertiary-care hospital.

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3 1 **Original article**  
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6 3 **Seven-year active surveillance for carbapenemase-producing *Klebsiella pneumoniae* and**  
7 **correlation with infection in subjects attending an Italian tertiary-care hospital.**  
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## 31 Abstract

32 **Objectives.** The distribution of carbapenemase-producing *K. pneumoniae* (CPKP) phenotypes and  
33 genotypes in samples collected during 2011-2018 was evaluated.

34 **Setting.** The study was performed in a tertiary-care hospital located in northern Italy.

35 **Participants.** Two groups were considered: 22,939 “at-risk” patients submitted to active  
36 surveillance for CPKP detection in rectal swabs/stools and 1094 CPKP infected patients in which  
37 CPKP was detected in samples other than rectal swabs; the association between the 2 groups was  
38 investigated.

39 **Results.** CPKP-positive rectal swabs were detected in 5% (1150/22,939). A CPKP infection was  
40 revealed in 3.1% (719/22,939): 582 with CPKP-positive rectal swabs (50.6% of the 1150 CPKP-  
41 positive rectal swabs) and 137 with CPKP-negative rectal swab. The 49.4% (568/1150) of the  
42 patients with CPKP-positive rectal swab were carriers. The overall frequency of CPKP-positive  
43 patients (carriers and infected) was almost constant from 2012 to 2016 (excluding the 2015 peak)  
44 and then increased in 2017-2018. *bla*KPC was predominant followed by *bla*VIM. No difference  
45 was observed in the frequency of CPKP-positive rectal swab patients among the different material  
46 groups; on the contrary, the CPKP invasive infections more frequently involved different body  
47 sites.

48 **Conclusions.** The high prevalence of carriers without evidence of infection, representing a potential  
49 reservoir of CPKP, suggests to maintain the guard about this problem, emphasizing the importance  
50 of active surveillance for timely detection and separation of carriers, activation of contact  
51 precautions and antibiotic treatment guidance upon suspicion of infection.

## 53 Article summary

### 54 Strengths and limitations of this study

- 55 • This study involved a relevant number of patients whose samples, arriving from different  
56 hospital units, were analysed in a long period (7 years).

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- The study demonstrated that 49.4% of patients submitted to active surveillance with CPKP-positive rectal swabs were carriers, representing a potential reservoir for spread of CPKP strains detectable only by surveillance.

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- In this study, CPKP-positive blood and respiratory samples were more frequently associated with infections in different body sites.

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- Only the results obtained for microbiological examination were considered.

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## 65 Introduction

66 Multidrug-resistant (MDR) bacteria represent an increasing public health threat in health-care  
67 settings. Among MDR bacteria, carbapenemase-producing *Enterobacteriaceae* (CPE), especially  
68 *Klebsiella pneumoniae*, are a cause for concern being able to spread rapidly [1] and responsible of  
69 invasive infections associated with high mortality [2], recently inducing the Centers for Disease  
70 Control and Prevention to raise them to highest threat level [3]. The application of CPE control  
71 programs has been successful in some areas; however, the problem continues to worsen worldwide,  
72 requiring more effective prevention strategies [1,4].

73 Carbapenemases are enzymes included in the Amber classification in the A, B, or D classes. The  
74 class A and D enzymes are serine hydrolases, and the class B enzymes are catalases requiring  
75 1 or 2 zinc ions on the active site[5]. Although certain carbapenemases are typically associated with  
76 specific regions or countries, nowadays, due to globalization especially in terms of widespread  
77 international travel and broad access to medical care, such an association may change, emphasizing  
78 the need for routine local and national surveillance [4]. In particular, in Italy, besides the detection  
79 of Verona integron-encoded metallo-beta-lactamase (VIM)-producing *Enterobacteriaceae*, firstly  
80 detected in the early 2000s, *K. pneumoniae* carbapenemase (KPC) producers are widely spread  
81 whereas New Delhi metallo-beta-lactamase (NDM) and carbapenem-hydrolyzing oxacillinase-48  
82 (OXA-48) producers are only occasionally revealed [3].

83 The aim of this study was the evaluation of the distribution of the phenotypes and genotypes of the  
84 carbapenemase-producing *K. pneumoniae* (CPKP) strains circulating in two selected groups of  
85 patients (those examined for CPKP detection on rectal swabs as part of the National/Regional active  
86 CRE surveillance, and those with a CPKP infection) in a tertiary-care hospital during a seven-year  
87 period (2011-2018). The association between patients with CPKP-positive rectal swab and those  
88 with CPKP infection, as well as the overall analysis of CPKP infected patients were performed.

## 91 **Methods**

### 92 Study design.

93 The study was designed as a retrospective data collection. The total observation time was 7 years.

### 95 Patient and Public Involvement

96 Data were sought retrospectively from the records produced by the diagnostic flow of the  
97 laboratory, as answer to a clinical suspicion or to active CRE surveillance.

### 99 Definitions.

100 A patient was defined as carrier when only a CPKP-positive rectal swab was detected and as  
101 infected when a CPKP-positive sample other than rectal swab was found, in presence of signs and  
102 symptoms of infection, according to CDC criteria for specific types of infections [6].

103 CPKP-positive samples other than rectal swab were grouped in blood (including blood, vascular  
104 catheter, and cerebrospinal fluid), respiratory (including bronchial aspirate, bronchoalveolar lavage,  
105 sputum, pleural fluid, pharyngeal swab, nasopharyngeal aspirate and nasal swab), urine (including  
106 urine and urinary catheter), and other (including bile, peritoneal, ascitic and abdominal drainage  
107 fluids, pus, bioptic and prothetic materials, sperm, tongue, wound, cutaneous, vaginal and urethral  
108 swabs). Multiple CPKP-positive samples categorized in the same group and belonging to the same  
109 patient were considered only once, as a unique sample.

### 111 Study setting and population.

112 Two well-defined groups of patients attending a tertiary-care hospital (University-Hospital of  
113 Parma, Italy) from November 2011 to October 2018 were selected. The first group included 22,939  
114 “at-risk” patients (e.g. contacts of CPKP-positive patients; patients admitted to transplant surgery,  
115 to intensive care units or to any other “at-risk” unit such as long-term care units, oncology and  
116 haematology; patients known to be infected/colonized, with the last CPKP positivity dating back to



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3 117 more than 90 days from the new admission; patients coming from endemic countries such as Israel,  
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5 118 Greece, Pakistan and India; patients transferred from acute care and neurological rehabilitation  
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8 119 facilities; patients coming from nursing homes for the elderly; patients hospitalized in an acute care  
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10 120 facility in the last 6 months), median age 70 years, range from 1 day to 108 years, examined on  
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12 121 admission as part of the National/Regional active CRE surveillance for the detection of CPKP  
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15 122 strains in rectal swabs/stools (hereafter referred as rectal swab), for a total of 32,477 rectal swabs  
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17 123 due to multiple sampling when required by CRE surveillance [7]. In case of multiple CPKP-positive  
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19 124 samples, only the first one of each patient was considered. The second group included 1094 CPKP  
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21 125 infected patients (median age 78 years, range from 20 days to 102 years), either involved or not  
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24 126 involved in active CRE surveillance.

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### Microbiological methods.

The rectal swabs were inoculated onto chromogenic agar (Brilliance CRE medium, Oxoid, Milan, Italy) and the blue colonies referring to presumptive CRE were subcultured on MacConkey agar with a carbapenem disk, as previously described [3]. CPKP strains from clinical samples other than rectal swabs were isolated as previously described [3]. All *K. pneumoniae* strains were identified by MALDI-TOF MS and submitted to antimicrobial susceptibility testing (Gram-negative NMIC/ID88 or NMIC/ID94 Combo Panels, Becton Dickinson, Sparks, MD, USA). When a carbapenem nonsusceptible *K. pneumoniae* strain was revealed, the carbapenemase production confirmation was performed by phenotypical analysis and genotypical characterization. The phenotypical analysis, according to Regional guidelines [7], included the modified Hodge test in combination with the disk diffusion inhibition test (KPC+MBL Confirm ID Kit/ KPC, MBL and OXA-48 Confirm Kit, Rosco Diagnostica, Taastrup, Denmark), performed according to the manufacturer's instructions. For genotypical characterization, 2 molecular methods succeeded during the study period: the first detecting *bla*KPC, *bla*NDM, and *bla*VIM was used until 2015, and the second one also detecting *bla*OXA-48 was used since 2015, as previously described [3].

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45 144 Statistical analysis.

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8 145 Chi-square test was used for comparison of the frequency of CPKP-positive rectal swabs among  
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10 146 CPKP infected patients by material grouping, the frequency of involvement of multiple materials  
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12 147 among CPKP infected patients, and the distribution of carbapenemase genes in the different  
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15 148 material groups. Statistical significance was set at  $p < 0.01$ .

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2324 152 **Results**

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26 153 Among the 22,939 “at-risk” patients (32,477 rectal swabs), carbapenem-resistant *K. pneumoniae*  
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28 154 strains were detected in 1178 cases (5.1%) and the production of carbapenemase was revealed in  
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31 155 1150 cases (5%). Intensive care and long-term care units accounted for the highest number of  
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33 156 patients with CPKP-positive rectal swabs (188 cases each), with a prevalence of 1.9% and 15.8%,  
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35 157 respectively (Fig. 1a). The frequency of patients with CPKP-positive rectal swabs ranged from  
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38 158 4.4% to 4.7% in the 2012-2014 period, reached the highest peak (6.3%) in 2015, and showed a  
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40 159 fluctuating trend from 2016 to 2018 (Fig. 1b). With regard to the results of the molecular genotyping  
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42 160 assays, all targeted types of carbapenemase genes were detected among the analysed rectal swabs.  
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45 161 The *blaKPC* was predominant (79%, 909/1150) followed by *blaVIM* (16.7%, 192/1150), while  
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47 162 *blaOXA-48* and *blaNDM* were more rarely observed, accounting for 0.3% (3/1150) and 0.2%  
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49 163 (2/1150), respectively (Fig. 2). In 0.8% (9/1150, 8 class B *blaNDM*- and *blaVIM*-negative and 1  
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51 164 class A *blaKPC*-negative) of the CPKP strains, none of the targeted genes was revealed, if  
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54 165 excluding those class B cases (35) for which only *blaNDM* was tested. With reference to temporal  
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56 166 distribution, from 2012 to 2014 a decrease of *blaKPC* was observed in contrast to a correspondent  
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58 167 increase of *blaVIM*, which started gradually decreasing from 2015. When the peak of positive rectal  
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3 168 swabs (204) was observed in 2015, *blaKPC* reached the maximum peak frequency (173), doubling  
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5 169 that of 2014 and subsequently slightly decreased with a fluctuating trend (Fig. 2).  
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8 170 When the 22,939 patients submitted to CRE surveillance (group 1) were combined with the 1094  
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10 171 CPKP infected patients (group 2), a total of 1662 CPKP-positive patients was found: 568 CPKP  
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12 172 carriers (accounting for 49.4% of the 1150 patients with CPKP-positive rectal swab), 582 CPKP  
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14 173 infected patients with a CPKP-positive rectal swab (accounting for 50.6% of the 1150 patients with  
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17 174 CPKP positive rectal swab), 137 CPKP infected patients with a CPKP-negative rectal swab, and  
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19 175 375 CPKP infected patients not included in the active CRE surveillance.  
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21 176 Among the 1094 CPKP infected patients (719 included in the active CRE surveillance and 375 not  
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23 included in the active CRE surveillance), accounting for 1283 CPKP-positive samples, urine (675)  
24 177 was the mostly involved sample all over the period, although a significant decrease from 2015 to  
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26 178 2016 was observed. Blood (175) accounted for about 25 cases per year, with a peak in 2017 (34)  
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28 179 (Fig. 3).  
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33 181 With regard to the CPKP infected patients included in the active CRE surveillance, no significant  
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35 182 difference was observed in the frequency of CPKP-positive rectal swabs in the different material  
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37 183 groups. On the contrary, CPKP-positive blood (49%) and respiratory (31%) samples were more  
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39 frequently associated than urine (17.7%) with CPKP-positive samples from 2- or more-site of  
40 184 infection ( $p < 0.0001$ ), as well as CPKP-positive blood samples were more frequently associated  
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42 185 with other CPKP-positive samples from 2- or more-site infection ( $p < 0.001$ ) than the respiratory  
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44 186 ones (Fig. 4).  
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49 188 With reference to the 1094 CPKP infected patients, 1034 (94.5%) were positive for one of the  
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51 189 targeted carbapenemase genes (841 *blaKPC*, 188 *blaVIM*, 3 *blaOXA-48*, and 2 *blaNDM*) and 5  
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53 190 (0.5%) contained two of the targeted carbapenemase genes (4 *blaKPC+blaVIM* and 1  
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55 191 *blaNDM+blaOXA48*), for a total of 1044 carbapenemase genes detected (Fig. 5a). If excluding the  
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57 192 50 class B strains for which only *blaNDM* was tested, the remaining 5 cases were negative for the  
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59 193 targeted carbapenemase genes (2 class A *blaKPC*-negative and 3 class B *blaVIM*- and *blaNDM*-  
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3 194 negative). When the carbapenemase gene analysis was performed by material grouping, *blaKPC*  
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5 195 was the most frequently detected carbapenemase gene in all material groups, followed by *blaVIM*;  
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8 196 however, the ratio of *blaKPC* and *blaVIM* was found to range from 6:1 to 10:1 for all material  
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10 197 groups, except for urine for which *blaVIM* was more significantly detected (ratio 3:1) ( $p < 0.01$ )  
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12 198 (Fig. 5b).

## 17 200 Discussion

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20 201 Since 2013, the Centers for Disease Control and Prevention assigned the highest threat level to CRE  
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22 202 and declared that CRE require urgent public health attention [8,9]. Unlike previous Italian studies  
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24 203 reporting the colonization rate for selected patient categories [10–13], our data show the picture of  
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27 204 the spread of CPKP isolates in a tertiary-care hospital.

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29 205 In our experience, the results of the application of active CRE surveillance with the adoption of a  
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31 206 combination of phenotypic assays followed by genotypic characterization on “at-risk” patient  
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34 207 categories highlight the need not to lower the guard about this problem. In fact during the study  
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36 208 period, after an initial constant trend of the frequency of CPKP-positive rectal swab cases from  
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38 209 2012 to 2014 (ranging from 4.4% to 4.7%), in 2015 the highest peak (6.3%) was observed, in  
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41 210 agreement with the same pattern described for invasive infection at regional level [7], followed by a  
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43 211 decrease in 2016 (4.9%) and a subsequent increasing trend in the last 2 years. The highest  
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45 212 prevalence of CPKP-positive rectal swabs was observed in the long-term care units, if excluding  
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48 213 those units in which rectal swab screening was performed only on targeted patient, such as contacts  
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50 214 of carrier patients and/or transfer from “at-risk” care units, and obtained on a limited number of  
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52 215 rectal swabs.

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54 216 When the association between patients with CPKP-positive rectal swabs and those with CPKP  
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57 217 infection (CPKP-positive samples other than rectal swab) was considered, it was observed that the  
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59 218 49.4% of patients submitted to active surveillance with CPKP-positive rectal swabs were carriers,  
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3 219 representing a potential reservoir for spread of CPKP strains detectable only by surveillance.  
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5 220 Taking into account the overall infected patients and excluding those not submitted to active CRE  
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8 221 surveillance, no difference was observed in the frequency of patients with CPKP-positive rectal  
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10 222 swabs among the different material groups. On the contrary, CPKP-positive blood and respiratory  
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12 223 samples were more frequently associated with infections in different body sites, demonstrating that  
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14 224 it is difficult to contain invasive infections (blood and respiratory samples) in a unique site and  
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17 225 suggesting that carriage represents one of the most important risk factor for CPKP infection, as  
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19 226 previously described for bloodstream infection [14,15].  
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21 227 With regard to the temporal distribution of the carbapenemase genes among CPKP-positive rectal  
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24 228 swabs, from 2012 to 2014 the *blaKPC* and *blaVIM* showed an inverse trend: in fact, when the  
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26 229 *blaKPC* decreased from 74.9% to 55.8%, the *blaVIM* increased consequently from 17% to 39.7%.  
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28 230 In correspondence of the highest CPKP-positive rectal swab rate in 2015, the trend of the frequency  
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31 231 of the *blaKPC* and *blaVIM* genes has reversed: that of *blaKPC* has continuously raised again,  
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33 232 reaching 92.8%, whereas that of *blaVIM* progressively decreased to 3.2%. *blaNDM* and *blaOXA-*  
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35 233 48 were only occasionally detected starting from 2016 and 2018, respectively. A similar temporal  
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38 234 distribution was also observed among infected patients, in which *blaKPC* was prevalent during all  
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40 235 the study period with a peak in 2015 and *blaVIM* was the second most frequently detected  
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42 236 carbapenemase gene independently of the material grouping. These data are in contrast with those  
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45 237 recently reported to the Italian national surveillance from 2014 to 2017 in bloodstream infections,  
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47 238 for which the emergence of *blaOXA-48*, especially in CPKP isolates, and its assessment as the first  
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49 239 most common gene after *blaKPC*, overcoming *blaVIM*, were described [16].  
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51 240 The overall high prevalence of *blaVIM*, mainly due to the relative peaks observed in 2013 and 2014  
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54 241 in our study, could be explained by a possible outbreak of the same clone of *blaVIM* among  
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56 242 patients attending long-term care wards. As also already described [17,18] and supported by our  
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58 243 study, in Italy *blaKPC* remains endemic and *blaVIM* is the predominant metallo-beta-lactamase  
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60 244 whereas the *blaNDM* is only sporadically detected.

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245 Carbapenemases have a global distribution, but substantial over time variability can be observed not  
246 only at continental and national level, but also among different settings in the same region.  
247 Awareness on the distribution of the specific mechanisms of carbapenem resistance within  
248 *Enterobacteriaceae*, in particular *K. pneumoniae*, and their temporal trend is crucial in the  
249 prevention of their spread and selection of appropriate patient management. These data emphasize  
250 the importance of active surveillance for timely detection and separation of carriers, activation of  
251 contact precautions and, after risk evaluation, antibiotic treatment guidance upon suspicion of  
252 infection, besides the evaluation of the risk factors for invasive infections, avoiding unnecessary  
253 potential toxic antimicrobial therapy in low-risk patients and for starting adequate treatment  
254 promptly in those at high risk.

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3 314 **Figure legend**

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6 315 Figure 1. Distribution of CPKP-positive rectal swab cases by care unit (a) and by year (b).

7  
8 316 Figure 2. Year distribution of CPKP genes in 1150 patients with CPKP-positive rectal swab.

9  
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11 317 Figure 3. Year distribution of CPKP infected patients by material grouping (blood, respiratory,  
12  
13 318 urine and other sites).

14  
15 319 Figure 4. Comparison of the results of active CPE surveillance among CPKP infected patients with  
16  
17  
18 320 one-site infection and in those with multiple-site infection by material grouping (blood, respiratory,  
19  
20 321 urine).

21  
22 322 Figure 5. Distribution of CPKP genes in infected patients by year (a) and by material grouping  
23  
24  
25 323 (blood, respiratory, urine and other sites) (b).

26  
27 324  
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29 325 **Contributorship:** AC, MM, CC conceived and designed the study; AC, MB, MM, SM, SC, AR,  
30  
31 326 IR, ADM, MG, SL acquired the data; AC, MB, MM, SM, MCA, FDC analysed and interpreted the  
32  
33 327 data; AC, MB, MM, SM, CC drafted the article or revised it critically; AC, MB, MM, SM, SC, AR,  
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36 328 IR, ADM, MG, SL, MCA, CC, FDC finally approved the version to be submitted.

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41 330 **Competing interests:** the authors declare no competing interests.

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45 332 **Data sharing statement:** No additional data available.

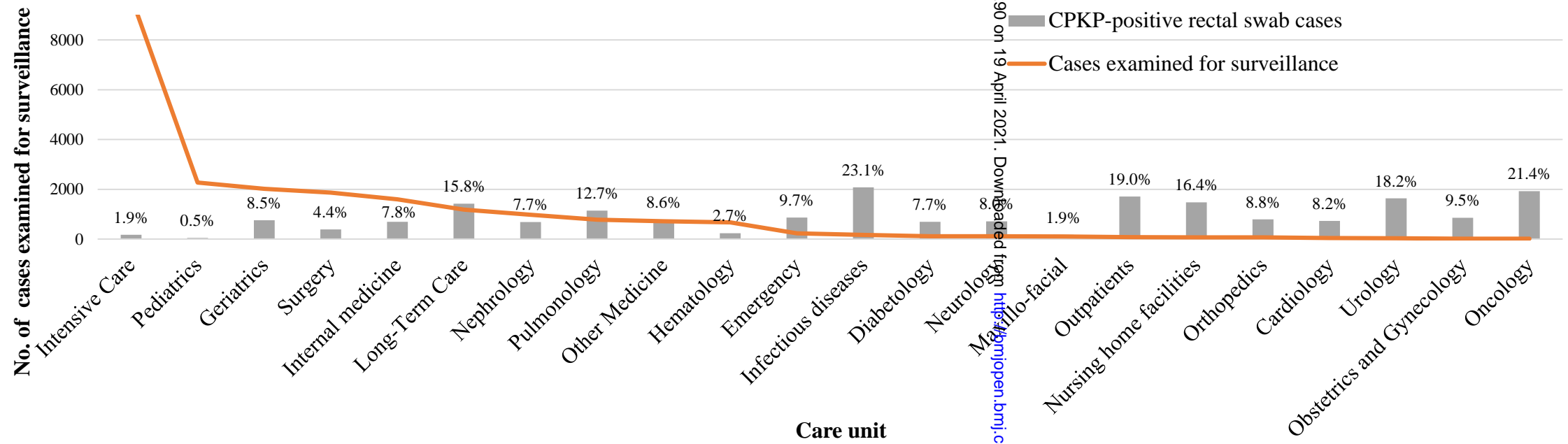
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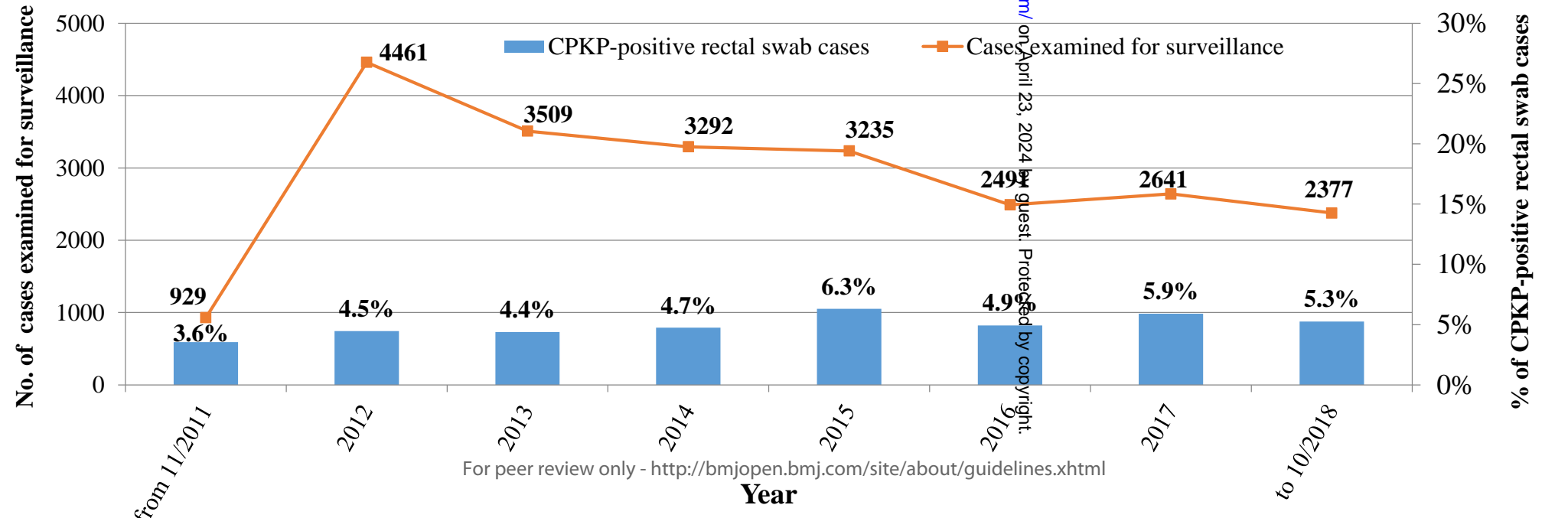
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FIGURE 1

(a)



(b)



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FIGURE 2

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- blaKPC
- blaVIM
- Other Class B (no blaNDM)
- Other Class B (no blaNDM and blaVIM)
- blaNDM
- blaOXA
- Other Class A (no blaKPC)

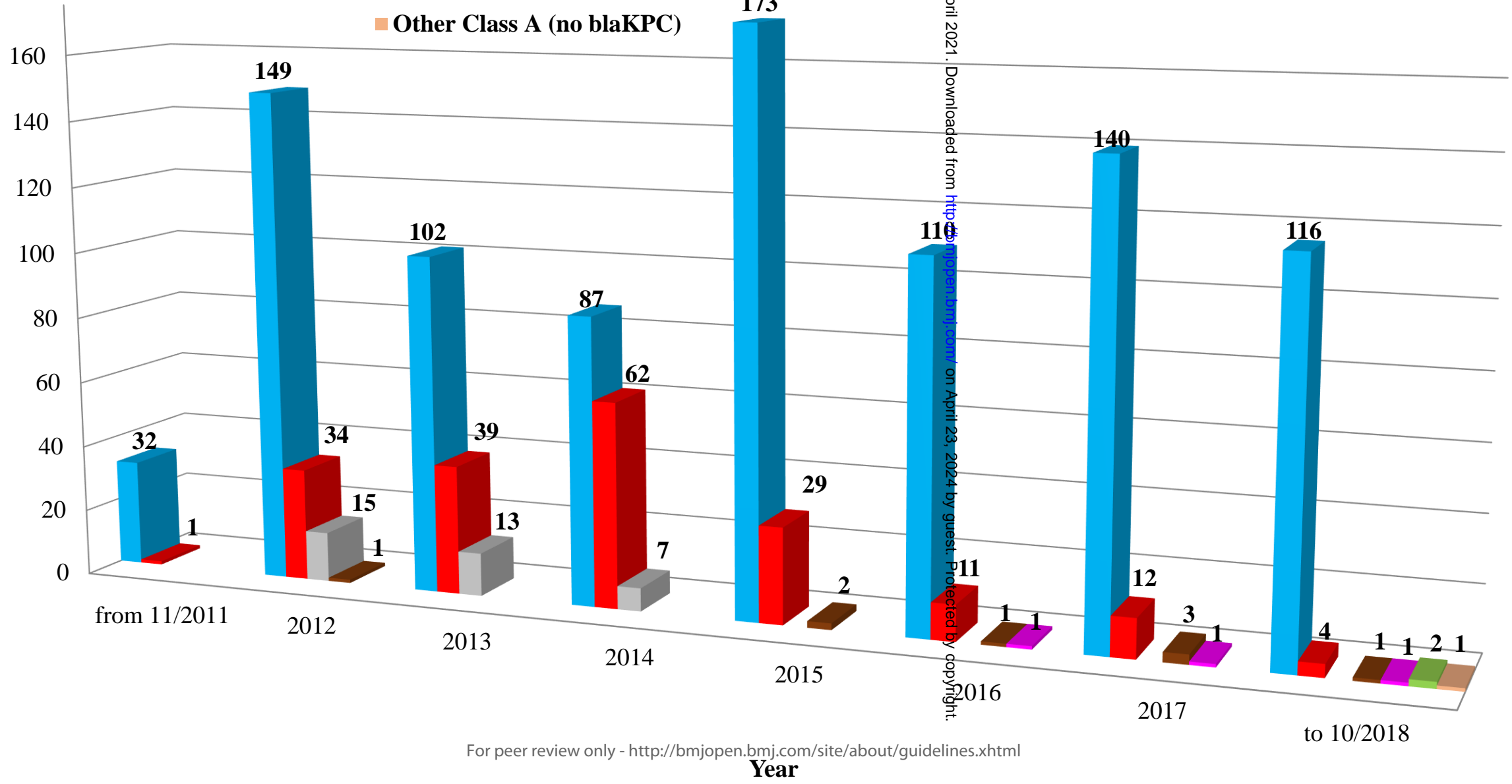
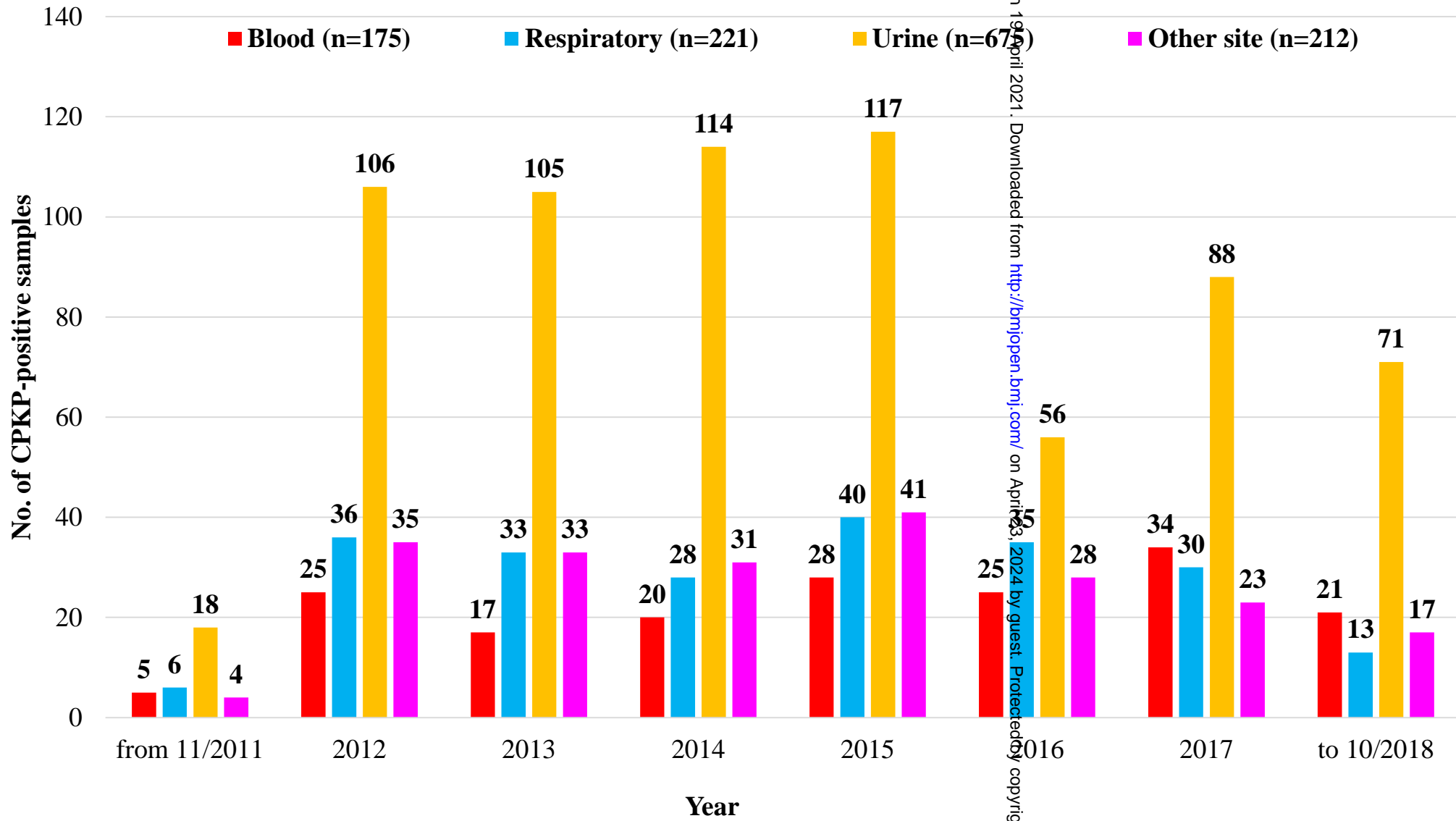
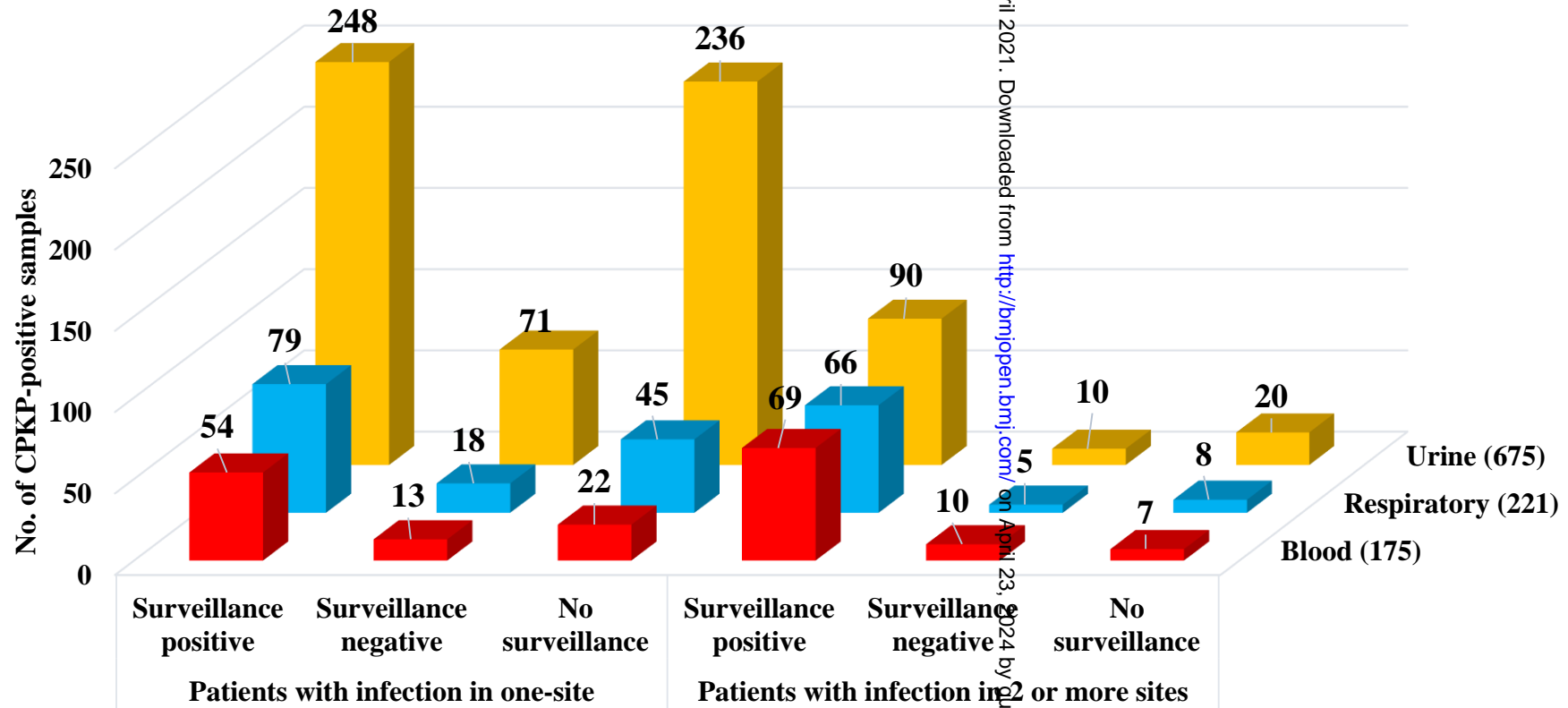


FIGURE 3



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FIGURE 4

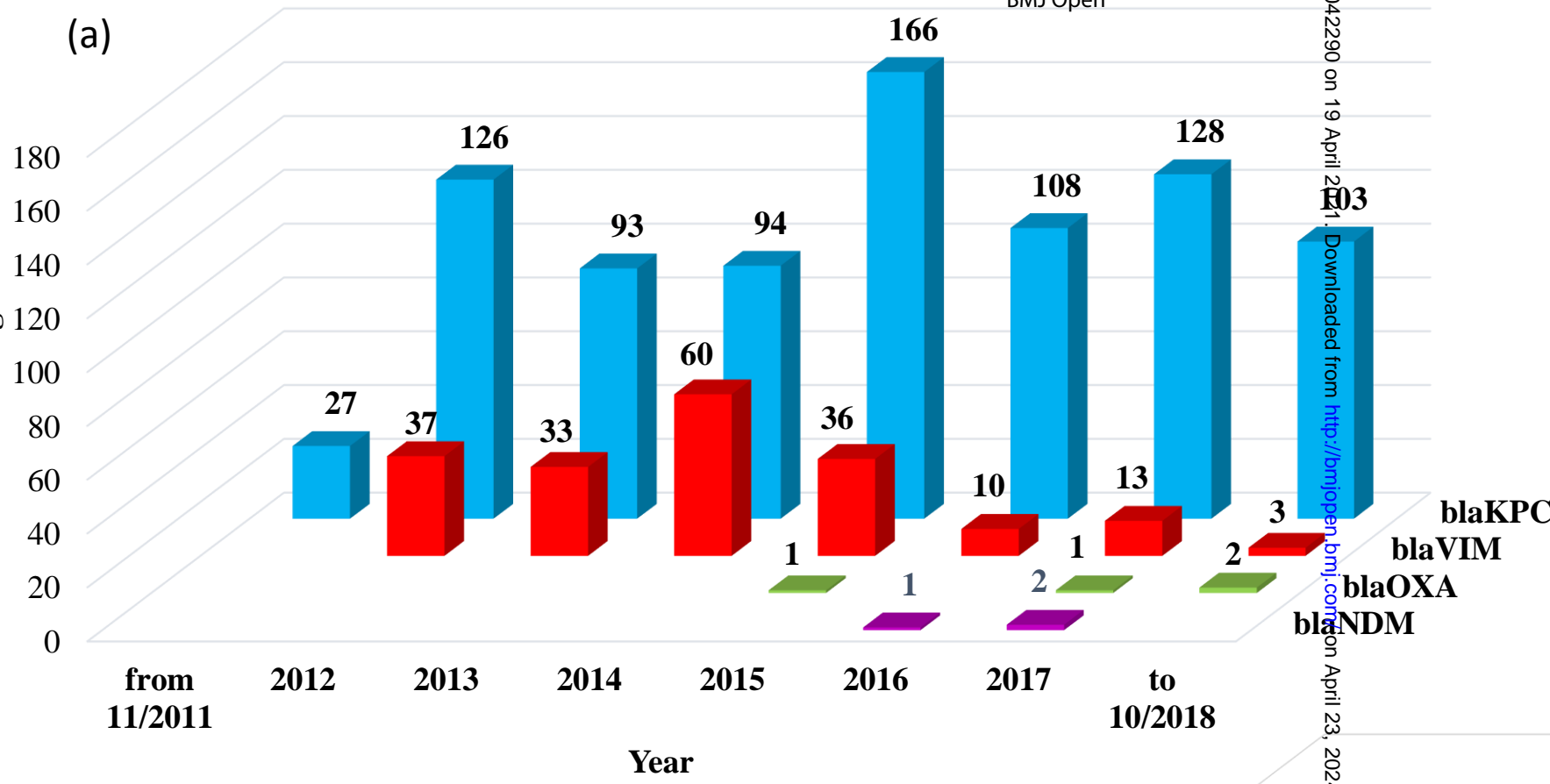


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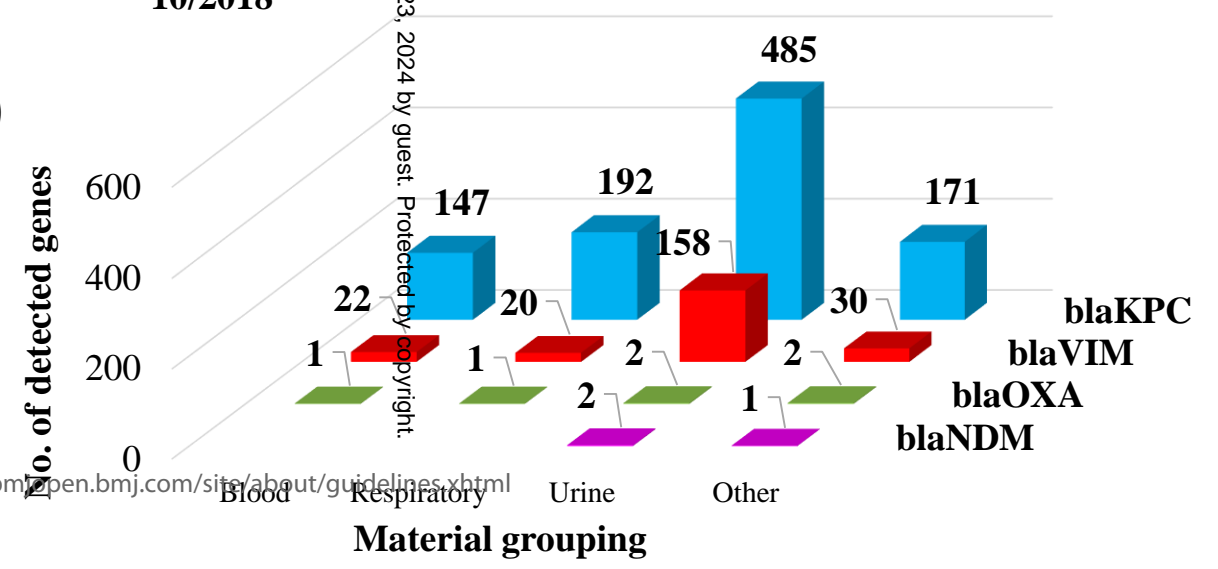
FIGURE 5

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(b)



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# BMJ Open

## Active surveillance for carbapenemase-producing *Klebsiella pneumoniae* and correlation with infection in subjects attending an Italian tertiary-care hospital: a seven-year retrospective study.

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3 1 **Original article**  
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6 3 **Active surveillance for carbapenemase-producing *Klebsiella pneumoniae* and correlation with**  
7 **infection in subjects attending an Italian tertiary-care hospital: a seven-year retrospective**  
8 **study.**  
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## 31 Abstract

32 **Objectives.** The distribution of carbapenemase-producing *K. pneumoniae* (CPKP) phenotypes and  
33 genotypes in samples collected during 2011-2018 was evaluated. The association between patients  
34 with CPKP-positive rectal swab and those with CPKP infection, as well as the overall analysis of  
35 CPKP infected patients, was performed.

36 **Setting.** The study was performed in a tertiary-care hospital located in Northern Italy.

37 **Participants.** Two groups were considered: 22,939 “at-risk” patients submitted to active  
38 surveillance for CPKP detection in rectal swabs/stools and 1094 CPKP infected patients in which  
39 CPKP was detected in samples other than rectal swabs.

40 **Results.** CPKP-positive rectal swabs were detected in 5% (1150/22,939). A CPKP infection was  
41 revealed in 3.1% (719/22,939) of patients: 582 with CPKP-positive rectal swab (50.6% of the 1150  
42 CPKP-positive rectal swabs) and 137 with CPKP-negative rectal swab. The 49.4% (568/1150) of  
43 the patients with CPKP-positive rectal swab were carriers. The overall frequency of CPKP-positive  
44 patients (carriers and infected) was almost constant from 2012 to 2016 (excluding the 2015 peak)  
45 and then increased in 2017-2018. *blaKPC* was predominant followed by *blaVIM*. No difference  
46 was observed in the frequency of CPKP-positive rectal swab patients among the different material  
47 groups. Among the targeted carbapenemase genes, *blaVIM* was more significantly detected from  
48 urine than from other samples.

49 **Conclusions.** The high prevalence of carriers without evidence of infection, representing a potential  
50 reservoir of CPKP, suggests to maintain the guard about this problem, emphasizing the importance  
51 of active surveillance for timely detection and separation of carriers, activation of contact  
52 precautions and antibiotic treatment guidance upon suspicion of infection.

## 55 Article summary

### 56 Strengths and limitations of this study

- 1  
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3 57 • This study describes the distribution of the CPKP phenotypes and genotypes detected in a  
4 58 large number of samples over a long period (7 years), with particular reference to their  
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6  
7 59 temporal trend.  
8  
9 60 • The results reported in this study from an unselected patient population attending a single  
10 61 tertiary-care hospital may contribute to the global data production.  
11  
12 62 • The association between patients with CPKP-positive rectal swab and those with CPKP  
13 63 infection was performed taking into account also the different material groups.  
14  
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16 64 • The molecular genotypic characterization based only on the four major carbapenemase  
17 65 genes could have missed the more rarely circulating genotypes.  
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20 66 • The lack of further genetic typing hampered to add consideration about any molecular  
21 67 epidemiological link among the isolates.  
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23 68

## 69 Introduction

70 Multidrug-resistant (MDR) bacteria represent an increasing public health threat in health-care  
71 settings. Among MDR bacteria, carbapenemase-producing *Enterobacteriaceae* (CPE), especially  
72 *Klebsiella pneumoniae*, are a cause for concern being able to spread rapidly [1] and responsible for  
73 invasive infections associated with high mortality [2], recently inducing the Centers for Disease  
74 Control and Prevention (CDC) to raise them to the highest threat level [3]. The application of CPE  
75 control programs has been successful in some areas; however, the problem continues to worsen  
76 worldwide, requiring more effective prevention strategies [1,4].

77 Carbapenemases are enzymes included in the Amber classification in the A, B, or D classes. The  
78 class A and D enzymes are serine hydrolases, and the class B or metallo-beta-lactamase (MBL)  
79 enzymes are catalases requiring 1 or 2 zinc ions on the active site [5]. Although certain  
80 carbapenemases are typically associated with specific regions or countries, nowadays, due to  
81 globalization especially in terms of widespread international travel and broad access to medical  
82 care, such an association may change, emphasizing the need for routine local and national  
83 surveillance [4]. In particular, in Italy, besides the detection of Verona integron-encoded metallo-  
84 beta-lactamase (VIM)-producing *Enterobacteriaceae*, firstly detected in the early 2000s, *K.*  
85 *pneumoniae* carbapenamase (KPC) producers are widely spread whereas New Delhi metallo-beta-  
86 lactamase (NDM) and carbapenem-hydrolyzing oxacillinase-48 (OXA-48) producers are only  
87 occasionally revealed [3].

88 The aim of this study was the evaluation of the distribution of the phenotypes and genotypes of the  
89 carbapenemase-producing *K. pneumoniae* (CPKP) strains circulating in two selected groups of  
90 patients (those examined for CPKP detection on rectal swab as part of the National/Regional active  
91 CRE surveillance, and those with a CPKP infection) in a tertiary-care hospital during a seven-year  
92 period (2011-2018). The association between patients with CPKP-positive rectal swab and those  
93 with CPKP infection, as well as the overall analysis of CPKP infected patients, was performed.

94

## 95 **Methods**

### 96 Study design.

97 The study was designed as a retrospective data collection. The total observation time was 7 years.

98 Data were sought retrospectively from the records produced by the diagnostic flow of the  
99 laboratory, as answer to a clinical suspicion or to active CRE surveillance [6].

### 101 Patient and Public Involvement.

102 Patients were not involved in the study.

### 104 Definitions.

105 A patient was defined as carrier when only a CPKP-positive rectal swab was detected and as  
106 infected when a CPKP-positive sample other than rectal swab was found, in presence of signs and  
107 symptoms of infection, according to CDC criteria for specific types of infections [7].

108 CPKP-positive samples other than rectal swab were grouped in blood (including blood, vascular  
109 catheter, and cerebrospinal fluid), respiratory (including bronchial aspirate, bronchoalveolar lavage,  
110 sputum, pleural fluid, pharyngeal swab, nasopharyngeal aspirate and nasal swab), urine (including  
111 urine and urinary catheter), and other (including bile, peritoneal, ascitic and abdominal drainage  
112 fluids, pus, bioptic and prothetic materials, sperm, tongue, wound, cutaneous, vaginal and urethral  
113 swabs).

### 115 Study setting and population.

116 Two well-defined groups of patients attending a tertiary-care hospital (University-Hospital of  
117 Parma, Italy) from November 2011 to October 2018 were selected. The first group included 22,939  
118 “at-risk” patients (median age 70 years, range from 1 day to 108 years), for a total of 32,477 rectal  
119 swabs due to multiple sampling when required by CRE surveillance [6]. The second group included  
120 1094 CPKP infected patients (median age 78 years, range from 20 days to 102 years), either

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3 121 involved or not involved in active CRE surveillance. In case of multiple CPKP-positive samples,  
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5 122 only the first one of each patient was considered. Multiple CPKP-positive samples categorized in  
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8 123 the same material group (blood, respiratory, urine, and other) and belonging to the same infected  
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10 124 patient were considered only once, as a unique sample.

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12 125

### 13 14 15 126 Inclusion and exclusion criteria

16  
17 127 Inclusion criteria: The first group included “at-risk” patients examined on admission as part of the  
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19 128 National/Regional active CRE surveillance for the detection of CPKP strains in rectal swabs/stools  
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21 129 (hereafter referred as rectal swab) [6], according to the following indications: 1) contacts of CPKP-  
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24 130 positive patients; 2) patients admitted to transplant surgery, intensive care units or any other “at-  
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26 131 risk” unit such as long-term care units, oncology and haematology; 3) patients known to be  
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28 132 infected/colonized, with the last CPKP positivity dating back to more than 90 days from the new  
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31 133 admission; 4) patients coming from endemic countries, such as Israel, Greece, Pakistan and India;  
32  
33 134 5) patients transferred from acute care and neurological rehabilitation facilities; 6) patients coming  
34  
35 135 from nursing homes for the elderly; 7) patients hospitalized in an acute care facility in the last 6  
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37  
38 136 months.

39  
40 137 The second group included patients with a CPKP infection in at least a sample other than rectal  
41  
42 138 swab, either involved or not involved in active CRE surveillance.

43  
44 139 Exclusion criteria: no exclusion criteria were adopted.

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### 48 49 141 Microbiological methods.

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51 142 The rectal swabs were inoculated onto chromogenic agar (Brilliance CRE medium, Oxoid, Milan,  
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54 143 Italy) and the blue colonies referring to presumptive CRE were subcultured on MacConkey agar  
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56 144 with a carbapenem disk, as previously described [3]. CPKP strains from clinical samples other than  
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58 145 rectal swabs were isolated, as previously described [3]. All *K. pneumoniae* strains were identified  
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146 by MALDI-TOF MS and submitted to antimicrobial susceptibility testing (Gram-negative

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3 147 NMIC/ID88 or NMIC/ID94 Combo Panels, Becton Dickinson, Sparks, MD, USA). When a  
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5 148 carbapenem nonsusceptible *K. pneumoniae* strain was revealed, the carbapenemase production  
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8 149 confirmation was performed by phenotypical analysis and genotypical characterization. The  
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10 150 phenotypical analysis, according to Regional guidelines [6], included the modified Hodge test in  
11  
12 151 combination with the disk diffusion inhibition test (KPC+MBL Confirm ID Kit/ KPC, MBL and  
13  
14 152 OXA-48 Confirm Kit, Rosco Diagnostica, Taastrup, Denmark), able to differentiate KPC, MBL and  
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16  
17 153 OXA-48 like carbapenemases, performed according to the manufacturer's instructions. For the  
18  
19 154 genotypical characterization, 2 molecular methods were used during the study period: the first,  
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21 155 detecting *blaKPC*, *blaNDM*, and retrospectively, on the available *blaNDM*-negative MBL-  
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24 156 producing isolates, *blaVIM*, was used until 2015, and the second one also detecting *blaOXA-48*  
25  
26 157 was used since 2015, as previously described [3].  
27  
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29 158

### 30 159 Statistical analysis.

31 160 Chi-square test was used for comparison of the frequency of CPKP-positive rectal swabs among  
32  
33 161 CPKP infected patients by material grouping, the frequency of involvement of multiple materials  
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35 162 among CPKP infected patients, and the distribution of carbapenemase genes in the different  
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38 163 material groups. Statistical significance was set at  $p < 0.01$ .  
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## 49 167 **Results**

50  
51 168 Among the 22,939 "at-risk" patients (32,477 rectal swabs), carbapenem-resistant *K. pneumoniae*  
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53 169 strains were detected in 1178 cases (5.1%) and the production of carbapenemase was revealed in  
54  
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56 170 1150 cases (5%). Intensive care and long-term care units accounted for the highest number of  
57  
58 171 patients with CPKP-positive rectal swabs (188 cases each), with a prevalence of 1.9% and 15.8%,  
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60 172 respectively (Fig.1a). The frequency of patients with CPKP-positive rectal swab ranged from 4.4%

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3 173 to 4.7% in the 2012-2014 period, reached the highest peak (6.3%) in 2015, and showed a fluctuating  
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5 174 trend from 2016 to 2018 (Fig. 1b). With regard to the results of the molecular genotyping assays, all  
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8 175 targeted types of carbapenemase genes were detected among the analysed rectal swabs. The  
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10 176 *blaKPC* was predominant (79%, 909/1150) followed by *blaVIM* (16.7%, 192/1150), while  
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12 177 *blaOXA-48* and *blaNDM* were more rarely observed, accounting for 0.3% (3/1150) and 0.2%  
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15 178 (2/1150), respectively (Fig. 2). In 0.8% (9/1150, 8 class B other than *blaNDM* and *blaVIM* and 1  
16  
17 179 class A other than *blaKPC*) of the CPKP strains, none of the targeted genes was revealed, if  
18  
19 180 excluding the class B (35) *blaNDM*-negative strains for which *blaVIM* was not tested. With  
20  
21 181 reference to temporal distribution, from 2012 to 2014 a decrease of *blaKPC* was observed in  
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23  
24 182 contrast to a correspondent increase of *blaVIM*, which started gradually decreasing from 2015.  
25  
26 183 When the peak of positive rectal swabs (204) was observed in 2015, *blaKPC* reached the maximum  
27  
28 184 peak frequency (173), doubling that of 2014 and subsequently slightly decreased with a fluctuating  
29  
30  
31 185 trend (Fig. 2).  
32  
33 186 When the 22,939 patients submitted to CRE surveillance (group 1) were combined with the 1094  
34  
35 187 CPKP infected patients (group 2), a total of 1662 CPKP-positive patients was found: 568 CPKP  
36  
37 188 carriers (accounting for 49.4% of the 1150 patients with CPKP-positive rectal swab), 582 CPKP  
38  
39 189 infected patients with a CPKP-positive rectal swab (accounting for 50.6% of the 1150 patients with  
40  
41  
42 190 CPKP positive rectal swab), 137 CPKP infected patients with a CPKP-negative rectal swab, and  
43  
44  
45 191 375 CPKP infected patients not included in the active CRE surveillance (Fig. 3).  
46  
47 192 Among the 1094 CPKP infected patients (719 included in the active CRE surveillance and 375 not  
48  
49 193 included in the active CRE surveillance), accounting for 1283 CPKP-positive samples, urine (675)  
50  
51 194 was the mostly involved sample all over the period, although a significant decrease from 2015 to  
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53  
54 195 2016 was observed. Blood (175) accounted for about 25 cases per year, with a peak in 2017 (34)  
55  
56 196 (Fig. 4).  
57  
58 197 With regard to the CPKP infected patients included in the active CRE surveillance, no significant  
59  
60 198 difference was observed in the frequency of CPKP-positive rectal swabs in the different material



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3 199 groups. On the contrary, CPKP-positive blood (49%) and respiratory (31%) samples were more  
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5 200 frequently associated than urine (17.7%) with CPKP-positive samples from 2- or more-site of  
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7  
8 201 infection ( $p < 0.0001$ ), as well as CPKP-positive blood samples were more frequently associated  
9  
10 202 with other CPKP-positive samples from 2- or more-site infection ( $p < 0.001$ ) than the respiratory  
11  
12 203 ones (Fig. 5).

13  
14 204 With reference to the 1094 CPKP infected patients, 1034 (94.5%) were positive for one of the  
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16  
17 205 targeted carbapenemase genes (841 *blaKPC*, 188 *blaVIM*, 3 *blaOXA-48*, and 2 *blaNDM*) and 5  
18  
19 206 (0.5%) contained two of the targeted carbapenemase genes (4 *blaKPC+blaVIM* and 1  
20  
21 207 *blaNDM+blaOXA48*), for a total of 1044 carbapenemase genes detected (Fig. 6a). If excluding the  
22  
23  
24 208 50 class B *blaNDM*-negative strains for which *blaVIM* was not tested, the remaining 5 cases were  
25  
26 209 negative for the targeted carbapenemase genes (2 class A *blaKPC*-negative and 3 class B *blaVIM*-  
27  
28 210 and *blaNDM*-negative). When the carbapenemase gene analysis was performed by material  
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31 211 grouping, *blaKPC* was the most frequently detected carbapenemase gene in all material groups,  
32  
33 212 followed by *blaVIM*; however, the ratio of *blaKPC* and *blaVIM* was found to range from 6:1 to  
34  
35 213 10:1 for all material groups, except for urine for which *blaVIM* was more significantly detected  
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38 214 (ratio 3:1) ( $p < 0.01$ ) (Fig. 6b).

## 39 40 215 **Discussion**

41  
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43 216 Since 2013, the CDC assigned the highest threat level to CRE and declared that CRE require urgent  
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45 217 public health attention [8,9]. Unlike previous Italian studies reporting the colonization rate for  
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47 218 selected patient categories [10–13], our data show the picture of the circulation of CPKP isolates in  
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50 219 a single tertiary-care hospital on a big sample size over a long period.

51  
52 220 In our experience, the results of the application of active CRE surveillance with the adoption of a  
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54 221 combination of phenotypic assays followed by genotypic characterization on “at-risk” patient  
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57 222 categories highlight the need not to lower the guard about this problem. In fact during the study  
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59 223 period, after an initial constant trend of the frequency of CPKP-positive rectal swab cases from  
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3 224 2012 to 2014 (ranging from 4.4% to 4.7%), in 2015 the highest peak (6.3%) was observed, in  
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5 225 agreement with the same pattern described for invasive infection at regional level [6], followed by a  
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7  
8 226 decrease in 2016 (4.9%) and a subsequent increasing trend in the last 2 years. The highest  
9  
10 227 prevalence of CPKP-positive rectal swabs was observed in the long-term care units, if excluding  
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12 228 those units in which rectal swab screening was performed only on targeted patients, such as contacts  
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14  
15 229 of carrier patients and/or transfer from “at-risk” care units, and obtained on a limited number of  
16  
17 230 rectal swabs.  
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19 231 When the association between patients with CPKP-positive rectal swab and those with CPKP  
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21 232 infection (CPKP-positive samples other than rectal swab) was considered, it was observed that the  
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24 233 49.4% of patients submitted to active surveillance with CPKP-positive rectal swab were carriers,  
25  
26 234 representing a potential reservoir for spread of CPKP strains detectable only by surveillance.  
27  
28 235 Taking into account the overall infected patients and excluding those not submitted to active CRE  
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30 236 surveillance, no difference was observed in the frequency of patients with CPKP-positive rectal  
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33 237 swab among the different material groups. On the contrary, CPKP-positive blood and respiratory  
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35 238 samples were more frequently associated with infections in multiple body sites, as expected due to  
36  
37  
38 239 the difficulty in containing invasive infections (blood and respiratory samples) in a unique site and  
39  
40 240 suggesting that carriage represents one of the most important risk factors for CPKP infection, as  
41  
42 241 previously described for bloodstream infection [14,15].  
43  
44 242 With regard to the temporal distribution of the carbapenemase genes among CPKP-positive rectal  
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46  
47 243 swabs, from 2012 to 2014 the *blaKPC* and *blaVIM* showed an inverse trend: in fact, when the  
48  
49 244 *blaKPC* decreased from 74.9% to 55.8%, the *blaVIM* increased consequently from 17% to 39.7%.  
50  
51 245 In correspondence of the highest CPKP-positive rectal swab rate in 2015, the trend of the frequency  
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53  
54 246 of the *blaKPC* and *blaVIM* genes has reversed: that of *blaKPC* has continuously raised again,  
55  
56 247 reaching 92.8%, whereas that of *blaVIM* progressively decreased to 3.2%. *blaNDM* and *blaOXA-*  
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58 248 48 were only occasionally detected starting from 2016 and 2018, respectively. A similar temporal  
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60 249 distribution was also observed among infected patients, in which *blaKPC* was prevalent during all

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250 the study period with a peak in 2015 and *blaVIM* was the second most frequently detected  
251 carbapenemase gene independently of the material grouping. However, *blaVIM* was more  
252 significantly detected from urine than from other samples. As already described [16,17], in Italy  
253 *blaKPC* remains endemic and *blaVIM* is the predominant MBL whereas the *blaNDM* is only  
254 sporadically detected, in agreement with the findings in our single-centre study. However, our data  
255 are in contrast with those recently reported to the Italian national surveillance from 2014 to 2017 in  
256 bloodstream infections, for which the emergence of *blaOXA-48*, especially in CPKP isolates, and  
257 its assessment as the first most common gene after *blaKPC*, overcoming *blaVIM*, were described  
258 [18]. The overall high prevalence of *blaVIM*, mainly due to the relative peaks observed in 2013 and  
259 2014 in our study, could be explained by a possible outbreak of the same clone of *blaVIM* among  
260 patients attending long-term care wards.

261 There are a few limitations in this study. First, this is a single-centre study and the findings may not  
262 generalize well to other settings due to multiple local factors. However, the carbapenemases have a  
263 global distribution, but substantial over time variability can be observed not only at continental and  
264 national level, but also among different settings in the same region: global data derive from single-  
265 centre studies. Awareness on the distribution of the specific mechanisms of carbapenem resistance  
266 within *Enterobacteriaceae*, in particular *K. pneumoniae*, and their temporal trend is crucial in the  
267 prevention of their spread and selection of appropriate patient management. Second, the genotypic  
268 characterization was limited to the detection of the four major carbapenemase genes that could have  
269 missed the more rarely circulating genotypes. Third, the lack of further genetic typing hampered to  
270 add consideration about any molecular epidemiological link among the isolates. Nonetheless, these  
271 data emphasize the importance of active surveillance for timely detection and separation of carriers,  
272 activation of contact precautions and, after risk evaluation, antibiotic treatment guidance upon  
273 suspicion of infection, besides the evaluation of the risk factors for invasive infections, avoiding  
274 unnecessary potential toxic antimicrobial therapy in low-risk patients and for starting adequate  
275 treatment promptly in those at high risk.

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**Contributorship statement:** AC, MM, CC conceived and designed the study; AC, MB, MM, SM, SC, AR, IR, ADM, MG, SL acquired the data; AC, MB, MM, SM, MCA, FDC analysed and interpreted the data; AC, MB, MM, SM, CC drafted the article or revised it critically; AC, MB, MM, SM, SC, AR, IR, ADM, MG, SL, MCA, CC, FDC finally approved the version to be submitted.

**Competing interests:** the authors declare no competing interests.

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**Data sharing statement:** All data relevant to the study are included in the article or uploaded as supplementary information

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3 350 **Figure legend**

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6 351 Figure 1. Distribution of CPKP-positive rectal swab cases by care unit (a) and by year (b).

7  
8 352 Figure 2. Year distribution of CPKP genes in 1150 patients with CPKP-positive rectal swab.

9  
10  
11 353 Figure 3. Study result flow diagram.

12  
13 354 Figure 4. Year distribution of CPKP infected patients by material grouping (blood, respiratory,  
14  
15 355 urine and other sites).

16  
17 356 Figure 5. Comparison of the results of active CPE surveillance among CPKP infected patients with  
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20 357 one-site infection and in those with multiple-site infection by material grouping (blood, respiratory,  
21  
22 358 urine).

23  
24 359 Figure 6. Distribution of CPKP genes in infected patients by year (a) and by material grouping  
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26  
27 360 (blood, respiratory, urine and other sites) (b).

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33 363 **Ethical approval:** data used for this study were reported in the medical records of the patients as

34  
35 364 answer to a clinical suspicion or to active CRE surveillance. Ethical approval at the University

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38 365 Hospital of Parma is required only in cases in which the clinical samples are to be used for

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41 366 applications other than diagnosis.

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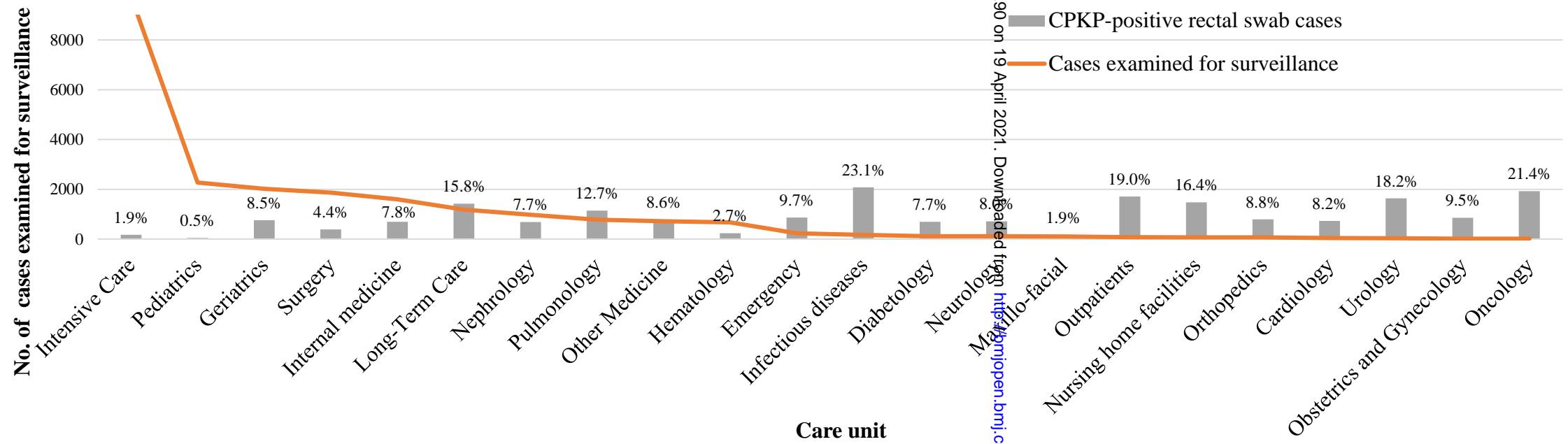
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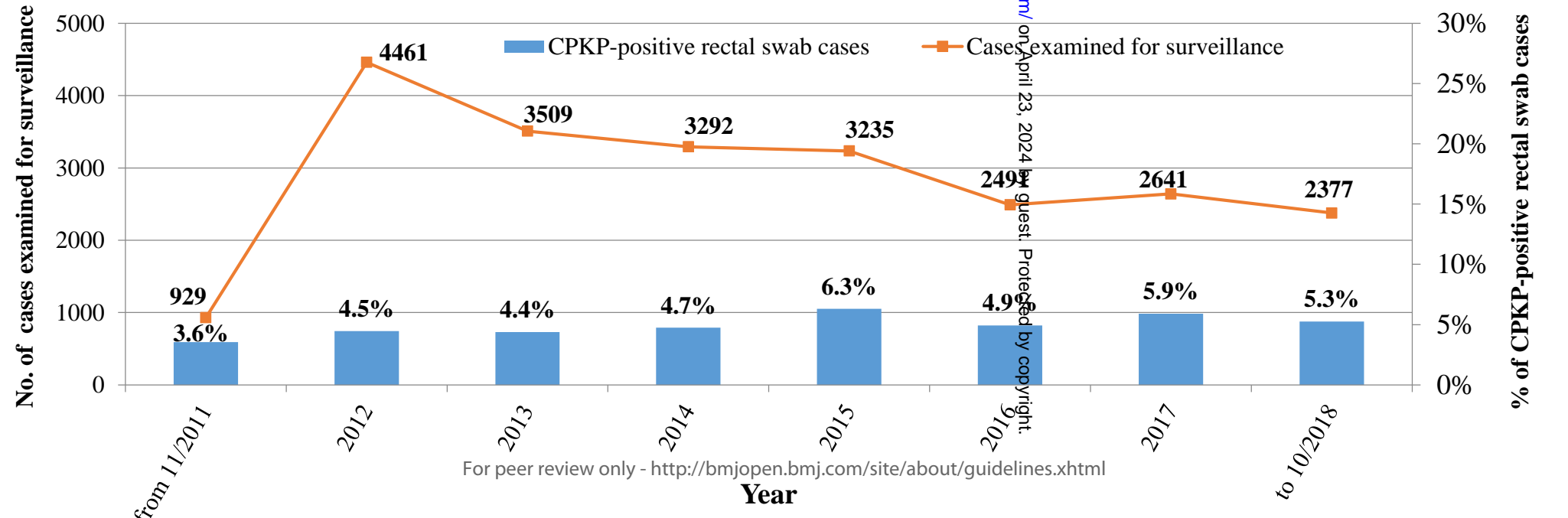


FIGURE 1

(a)



(b)



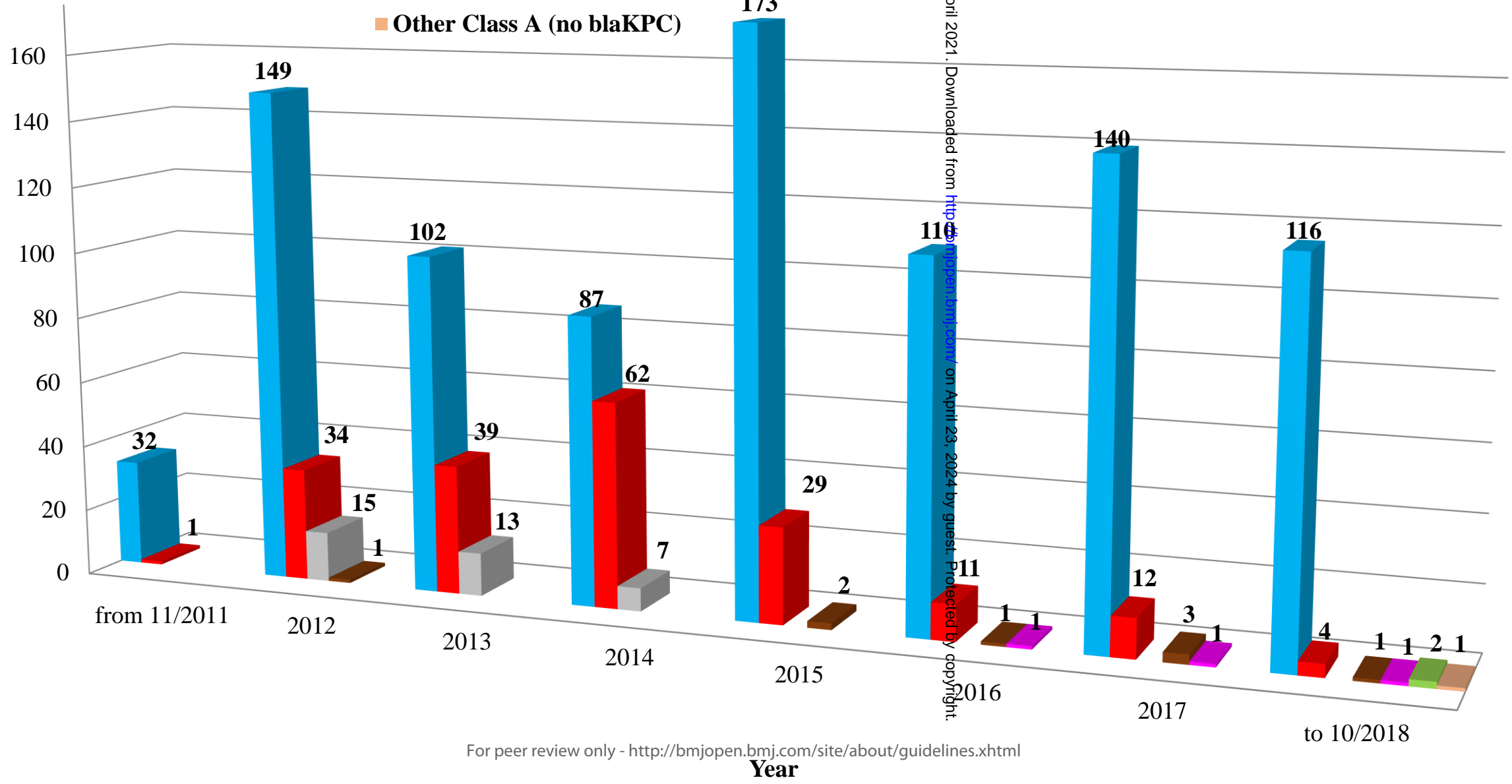
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FIGURE 2

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- blaKPC
- blaVIM
- Other Class B (no blaNDM)
- Other Class B (no blaNDM and blaVIM)
- blaNDM
- blaOXA
- Other Class A (no blaKPC)



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STUDY PERIOD:  
NOVEMBER 2011-OCTOBER 2018

22,939 «at-risk» patients submitted to active CRE surveillance

1094 CPKP infected patients

221,652 CPKP-negative patients

568 CPKP carriers

582 CPKP infected patients with positive rectal swab

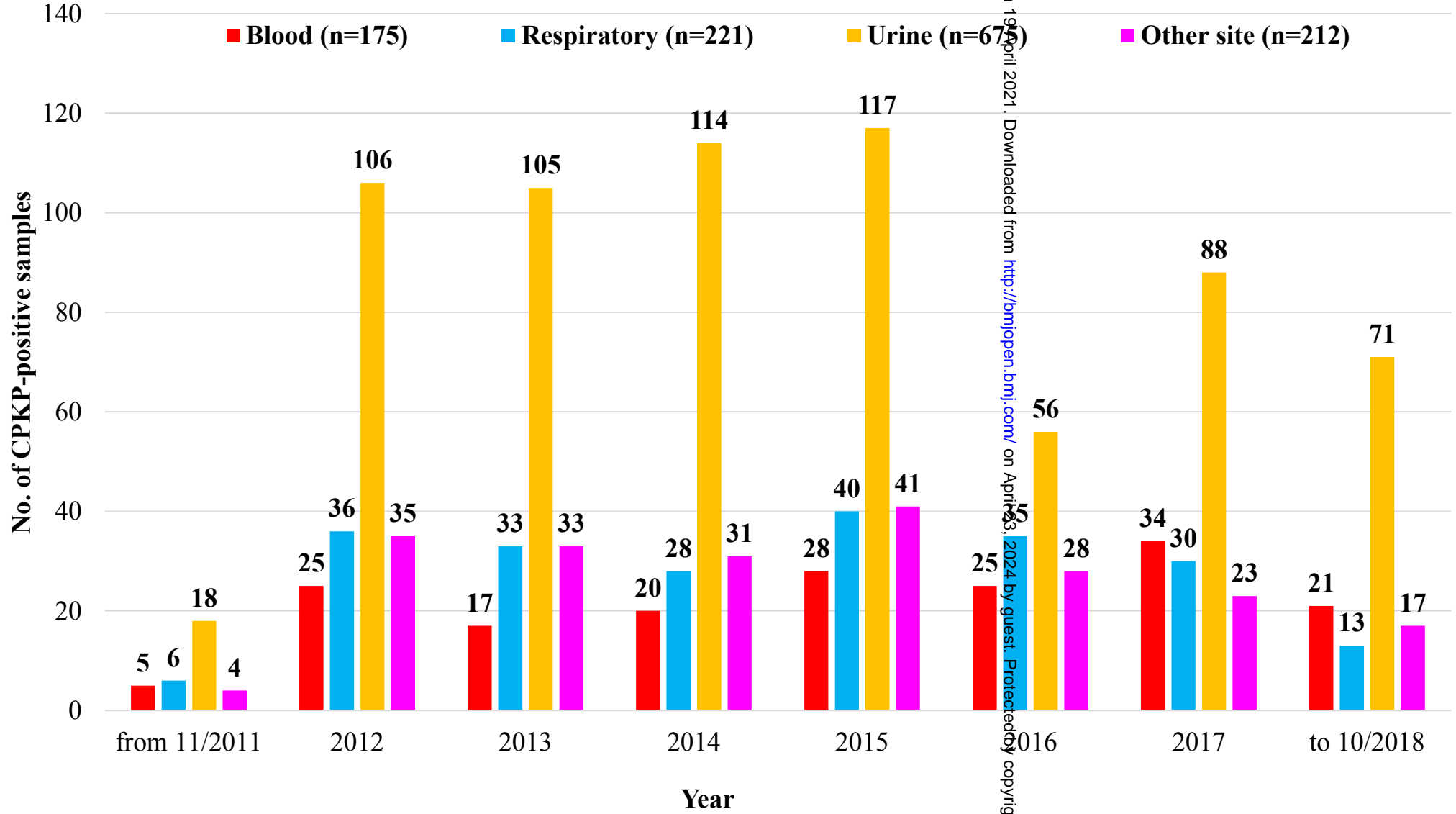
137 CPKP infected patients with negative rectal swab

375 CPKP infected patients not included in CRE surveillance

1150 Patients with CPKP-positive rectal swab

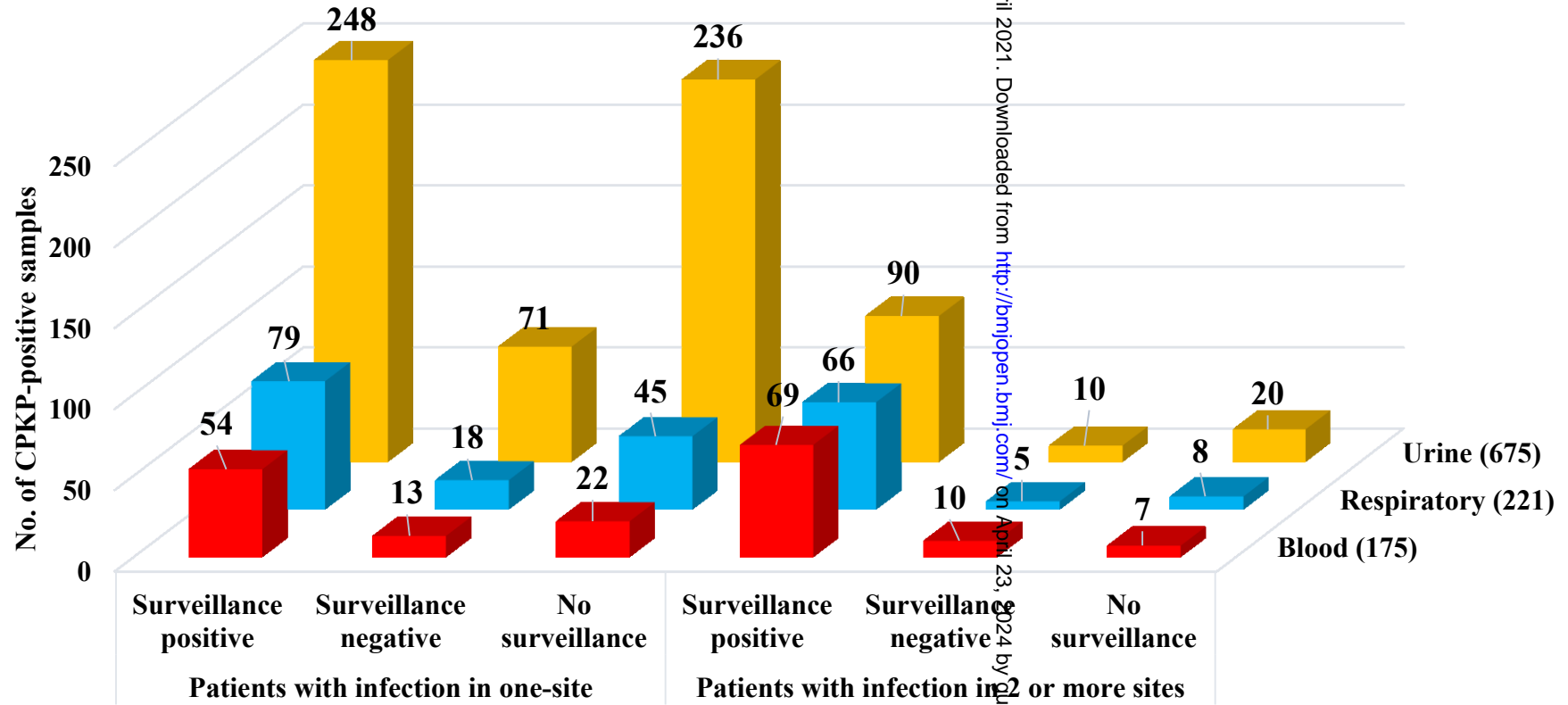
719 CPKP infected patients included in CRE surveillance

FIGURE 4



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FIGURE 5

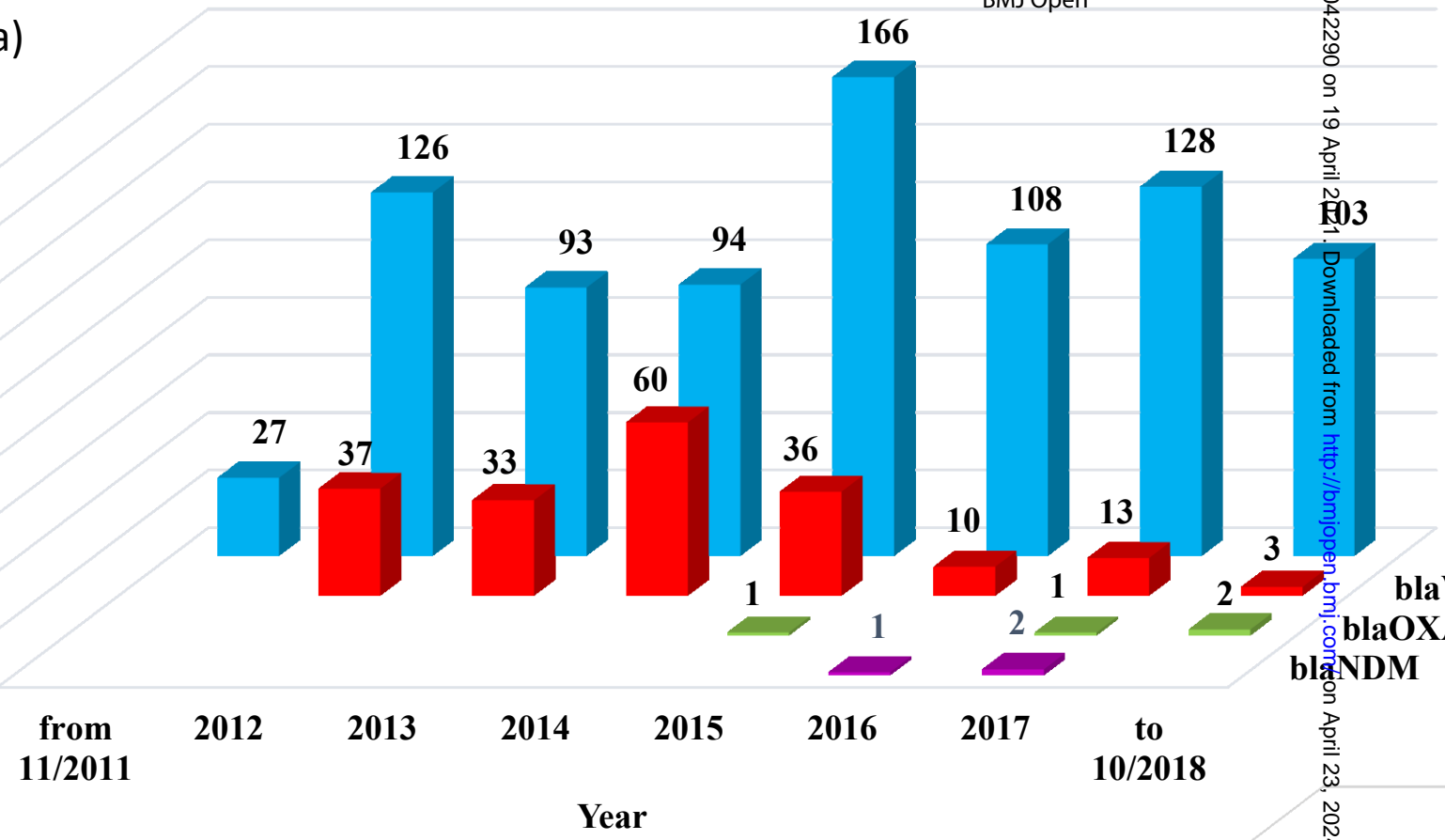


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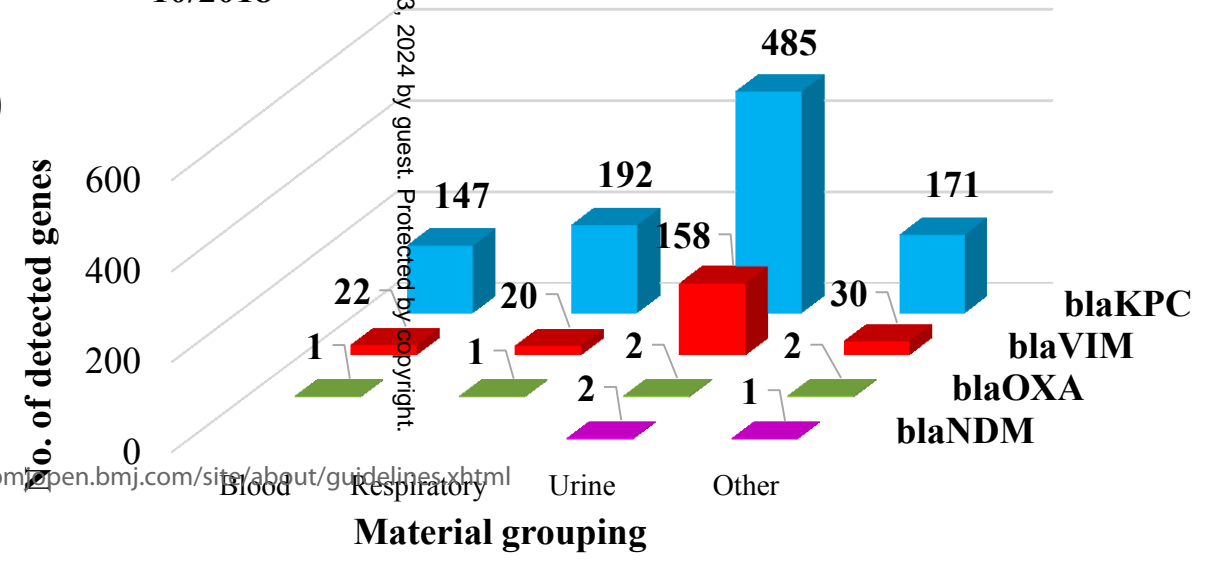
FIGURE 6

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(b)



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STROBE Statement—Checklist of items that should be included in reports of *cohort studies*

	Item No	Recommendation	Reported on page n., line n.
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Page 1, lines 3-5
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 2, lines 32-52
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page 4, lines 67-84
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 4, lines 85-90
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	Page 5, line 92-95
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 5, lines 111-116 Page 6, lines 117-120
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page 6, lines 122-135
		(b) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Not applicable.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 5, lines 94-95
Bias	9	Describe any efforts to address potential sources of bias	Not applicable
Study size	10	Explain how the study size was arrived at	Page 5, lines 113-116 Page 6, lines 117-120
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Not applicable.

1	Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Page 7, lines 156-159
2			(b) Describe any methods used to examine subgroups and interactions	As mentioned above
3			(c) Explain how missing data were addressed	Not applicable
4			(d) If applicable, explain how loss to follow-up was addressed	Not applicable.
5			(e) Describe any sensitivity analyses	Not applicable
6	<b>Results</b>			
7	Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Only partially applicable Page 8, lines 161-163, 178-185 Page 9, lines 186-188
8			(b) Give reasons for non-participation at each stage	Not applicable
9			(c) Consider use of a flow diagram	Figure 3
10	Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Only partially applicable Page 8, lines 163-165
11			(b) Indicate number of participants with missing data for each variable of interest	Page 8, lines 172-173 Page 9, lines 199-202
12			(c) Summarise follow-up time (eg, average and total amount)	Not applicable
13	Outcome data	15*	Report numbers of outcome events or summary measures over time	Not applicable
14	Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Not applicable
15			(b) Report category boundaries when continuous variables were categorized	Not applicable
16			(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
17	Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 9, lines 189-195, 202-206
18	<b>Discussion</b>			
19	Key results	18	Summarise key results with reference to study objectives	Page 10, lines 215-219, 224-227 Page 11, lines 235-245



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Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 11, lines 254-255, Page 12, lines 260-263
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 11, lines 245-253, 255-257 Page 12, lines 258-260, 263-268
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 11, lines 254-255
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 17, lines 352-353

\*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.