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Original article

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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Abstract

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

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

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

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

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

Article summary

Strengths and limitations of this study

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

- 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.
- In this study, CPKP-positive blood and respiratory samples were more frequently associated with infections in different body sites.
- Only the results obtained for microbiological examination were considered.

Introduction

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

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

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

Methods

Study design.

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

Patient and Public Involvement

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

Definitions.

A patient was defined as carrier when only a CPKP-positive rectal swab was detected and as infected when a CPKP-positive sample other than rectal swab was found, in presence of signs and symptoms of infection, according to CDC criteria for specific types of infections [6]. CPKP-positive samples other than rectal swab were grouped in blood (including blood, vascular catheter, and cerebrospinal fluid), respiratory (including bronchial aspirate, bronchoalveolar lavage, sputum, pleural fluid, pharyngeal swab, nasopharyngeal aspirate and nasal swab), urine (including urine and urinary catheter), and other (including bile, peritoneal, ascitic and abdominal drainage fluids, pus, bioptic and prothesic materials, sperm, tongue, wound, cutaneous, vaginal and urethral swabs). Multiple CPKP-positive samples categorized in the same group and belonging to the same patient were considered only once, as a unique sample.

Study setting and population.

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

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

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 methodssucceeded 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].

Statistical analysis.

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

Results

Among the 22,939 "at-risk" patients (32,477 rectal swabs), carbapenem-resistant *K. pneumoniae* strains were detected in 1178 cases (5.1%) and the production of carbapenemase was revealed in 1150 cases (5%). Intensive care and long-term care units accounted for the highest number of patients with CPKP-positive rectal swabs (188 cases each), with a prevalence of 1.9% and 15.8%, respectively (Fig.1a). The frequency of patients with CPKP-positive rectal swabs ranged from 4.4% to 4.7% in the 2012-2014 period, reached the highest peak (6.3%) in 2015, and showed a fluctuating trend from 2016 to 2018 (Fig.1b). With regard to the results of the molecular genotyping assays, all targeted types of carbapenemase genes were detected among the analysed rectal swabs. The *bla*KPC was predominant (79%, 909/1150) followed by *bla*VIM (16.7%, 192/1150), while *bla*OXA-48 and *bla*NDM were more rarely observed, accounting for 0.3% (3/1150) and 0.2% (2/1150), respectively (Fig. 2). In 0.8% (9/1150, 8 class B *bla*NDM- and *bla*VIM-negative and 1 class A *bla*KPC-negative) of the CPKP strains, none of the targeted genes was revealed, if excluding those class B cases (35) for which only *bla*NDM was tested. With reference to temporal distribution, from 2012 to 2014 a decrease of *bla*KPC was observed in contrast to a correspondent increase of *bla*VIM, which started gradually decreasing from 2015. When the peak of positive rectal

swabs (204) was observed in 2015, *bla*KPC reached the maximum peak frequency (173), doubling that of 2014 and subsequently slightly decreased with a fluctuating trend (Fig. 2).

When the 22,939 patients submitted to CRE surveillance (group 1) were combined with the 1094 CPKP infected patients (group 2), a total of 1662 CPKP-positive patients was found: 568 CPKP carriers (accounting for 49.4% of the 1150 patients with CPKP-positive rectal swab), 582 CPKP infected patients with a CPKP-positive rectal swab (accounting for 50.6% of the 1150 patients with CPKP positive rectal swab), 137 CPKP infected patients with a CPKP-negative rectal swab, and 375 CPKP infected patients not included in the active CRE surveillance.

Among the 1094 CPKP infected patients (719 included in the active CRE surveillance and 375 not included in the active CRE surveillance), accounting for 1283 CPKP-positive samples, urine (675) was the mostly involved sample all over the period, although a significant decrease from 2015 to 2016 was observed. Blood (175) accounted for about 25 cases per year, with a peak in 2017 (34) (Fig. 3).

With regard to the CPKP infected patients included in the active CRE surveillance, no significant difference was observed in the frequency of CPKP-positive rectal swabs in the different material groups. On the contrary, CPKP-positive blood (49%) and respiratory (31%) samples were more frequently associated than urine (17.7%) with CPKP-positive samples from 2- or more-site of infection ($p< 0.0001$), as well as CPKP-positive blood samples were more frequently associated with other CPKP-positive samples from 2- or more-site infection ($p< 0.001$) than the respiratory ones (Fig. 4).

With reference to the 1094 CPKP infected patients, 1034 (94.5%) were positive for one of the targeted carbapenemase genes (841 *bla*KPC, 188 *bla*VIM, 3 *bla*OXA-48, and 2 *bla*NDM) and 5 (0.5%) contained two of the targeted carbapenemase genes (4 *bla*KPC+*bla*VIM and 1 *bla*NDM+*bla*OXA48), for a total of 1044 carbapenemase genes detected (Fig. 5a). If excluding the 50 class B strains for which only *bla*NDM was tested, the remaining 5 cases were negative for the targeted carbapenemase genes (2 class A *bla*KPC-negative and 3 class B *bla*VIM- and *bla*NDM-

negative). When the carbapenemase gene analysis was performed by material grouping, *bla*KPC was the most frequently detected carbapenemase gene in all material groups, followed by *bla*VIM; however, the ratio of *bla*KPC and *bla*VIM was found to range from 6:1 to 10:1 for all material groups, except for urine for which *bla*VIM was more significantly detected (ratio 3:1) ($p<0.01$) (Fig. 5b).

Discussion

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

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

When the association between patients with CPKP-positive rectal swabs and those with CPKP infection (CPKP-positive samples other than rectal swab) was considered, it was observed that the 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. Taking into account the overall infected patients and excluding those not submitted to active CRE surveillance, no difference was observed in the frequency of patients with CPKP-positive rectal swabs among the different material groups. On the contrary, CPKP-positive blood and respiratory samples were more frequently associated with infections in different body sites, demonstrating that it is difficult to contain invasive infections (blood and respiratory samples) in a unique site and suggesting that carriage represents one of the most important risk factor for CPKP infection, as previously described for bloodstream infection [14,15].

With regard to the temporal distribution of the carbapenemase genes among CPKP-positive rectal swabs, from 2012 to 2014 the *bla*KPC and *bla*VIM showed an inverse trend: in fact, when the *bla*KPC decreased from 74.9% to 55.8%, the *bla*VIM increased consequently from 17% to 39.7%. In correspondence of the highest CPKP-positive rectal swab rate in 2015, the trend of the frequency of the *bla*KPC and *bla*VIM genes has reversed: that of *bla*KPC has continuously raised again, reaching 92.8%, whereas that of *bla*VIM progressively decreased to 3.2%. *bla*NDM and *bla*OXA-48 were only occasionally detected starting from 2016 and 2018, respectively. A similar temporal distribution was also observed among infected patients, in which *bla*KPC was prevalent during all the study period with a peak in 2015 and *bla*VIM was the second most frequently detected carbapenemase gene independently of the material grouping. These data are in contrast with those recently reported to the Italian national surveillance from 2014 to 2017 in bloodstream infections, for which the emergence of *bla*OXA-48, especially in CPKP isolates, and its assessment as the first most common gene after *bla*KPC, overcoming *bla*VIM, were described [16].

The overall high prevalence of *bla*VIM, mainly due to the relative peaks observed in 2013 and 2014 in our study, could be explained by a possible outbreak of the same clone of *bla*VIM among patients attending long-term care wards. As also already described [17,18] and supported by our study, in Italy *bla*KPC remains endemic and *bla*VIM is the predominant metallo-beta-lactamase whereas the *bla*NDM is only sporadically detected.

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

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Figure legend

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

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

Figure 3. Year distribution of CPKP infected patients by material grouping (blood, respiratory, urine and other sites).

Figure 4. Comparison of the results of active CPE surveillance among CPKP infected patients with one-site infection and in those with multiple-site infection by material grouping (blood, respiratory, urine).

Figure 5. Distribution of CPKP genes in infected patients by year (a) and by material grouping (blood, respiratory, urine and other sites) (b).

Contributorship: 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 he 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.

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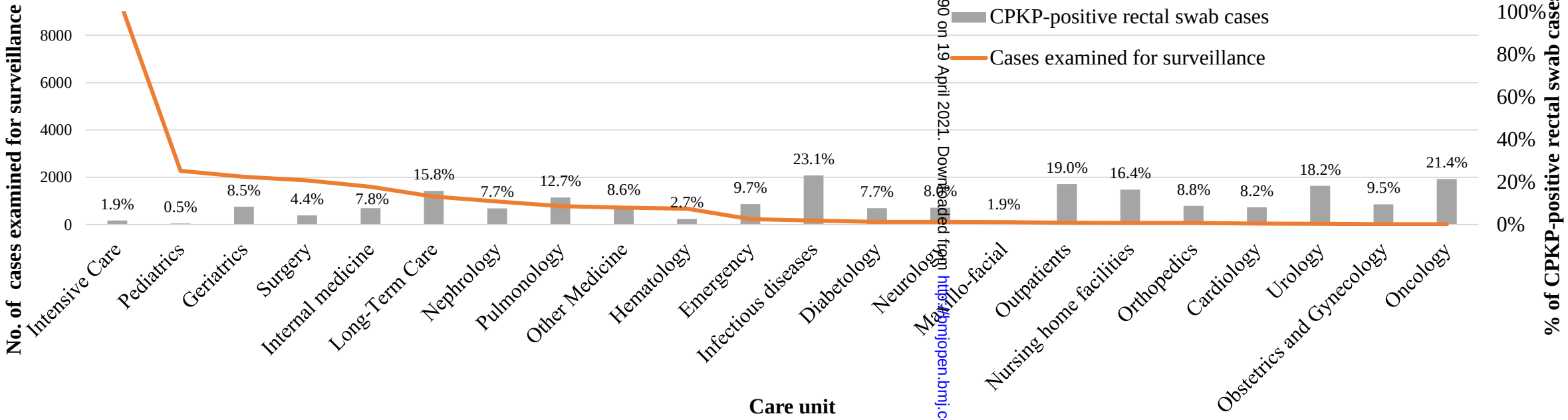
(a)

FIGURE 1



(b)

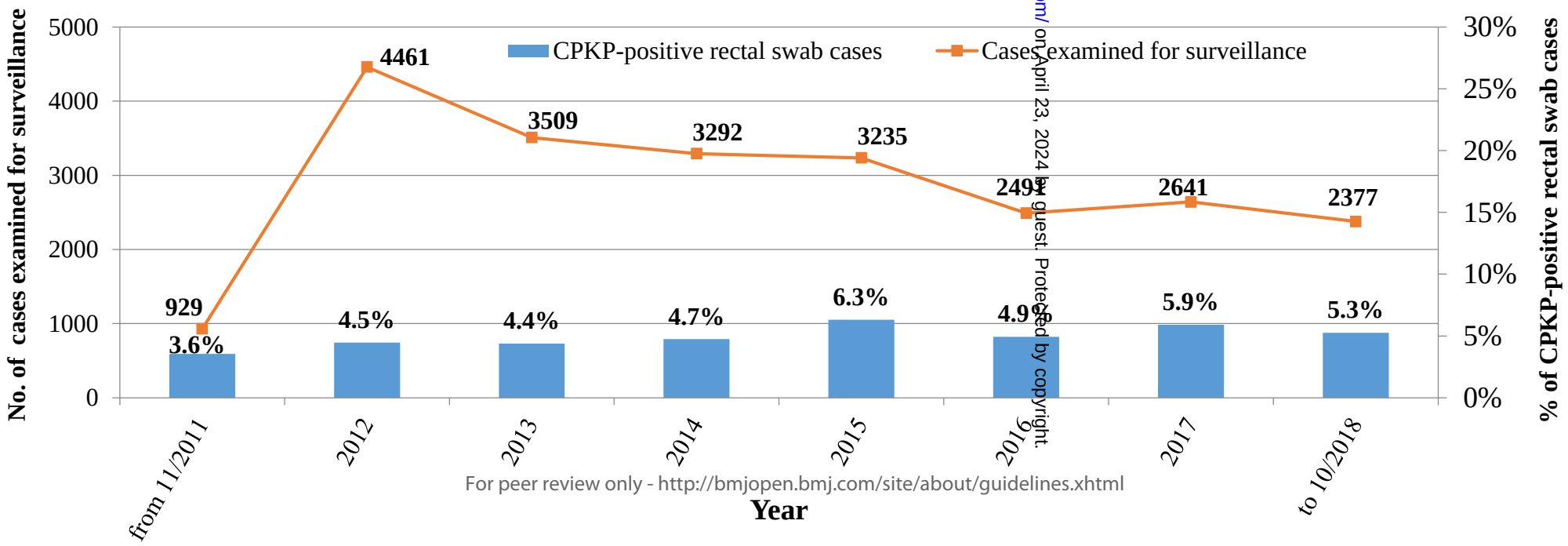
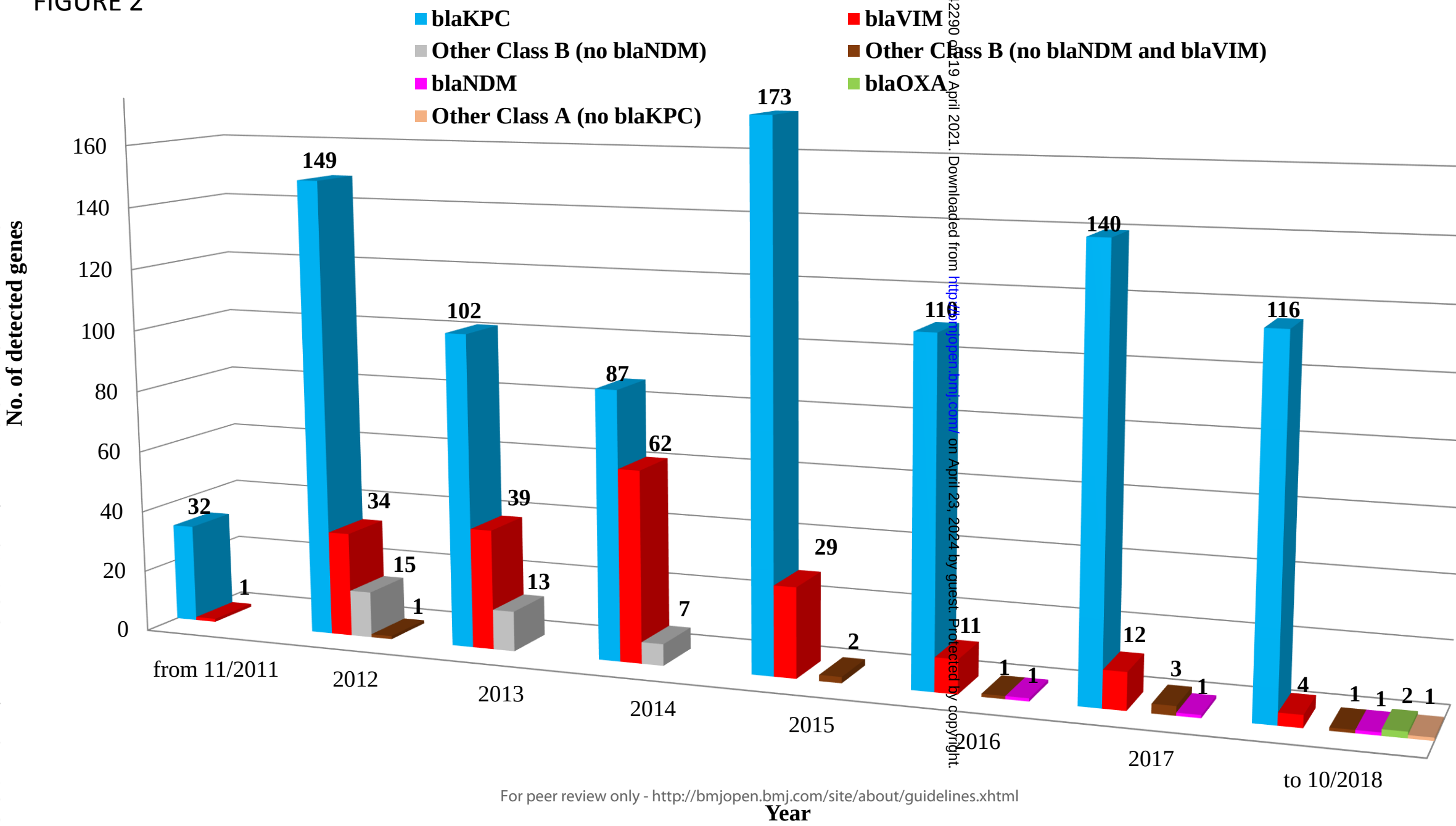


FIGURE 2



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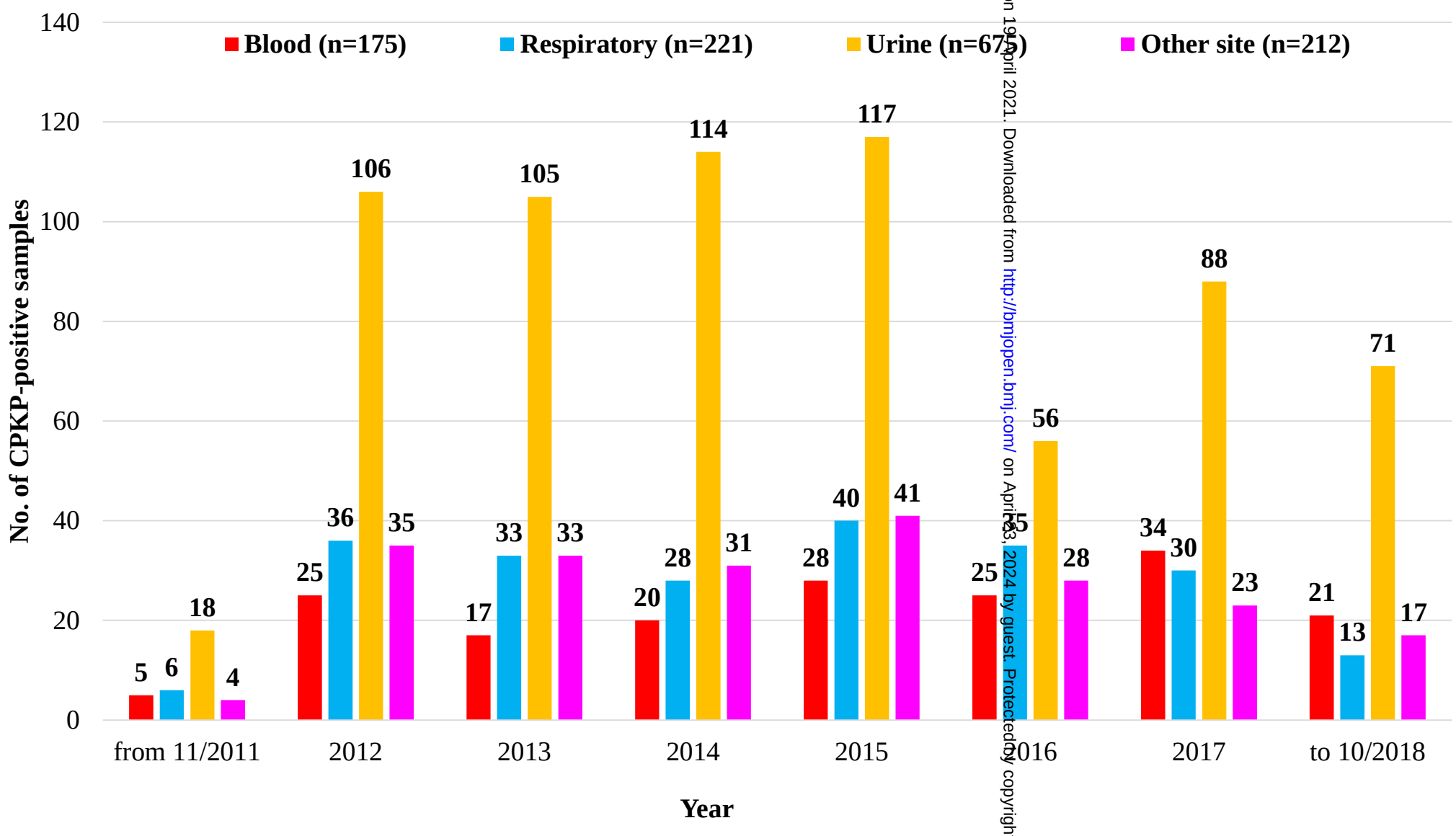

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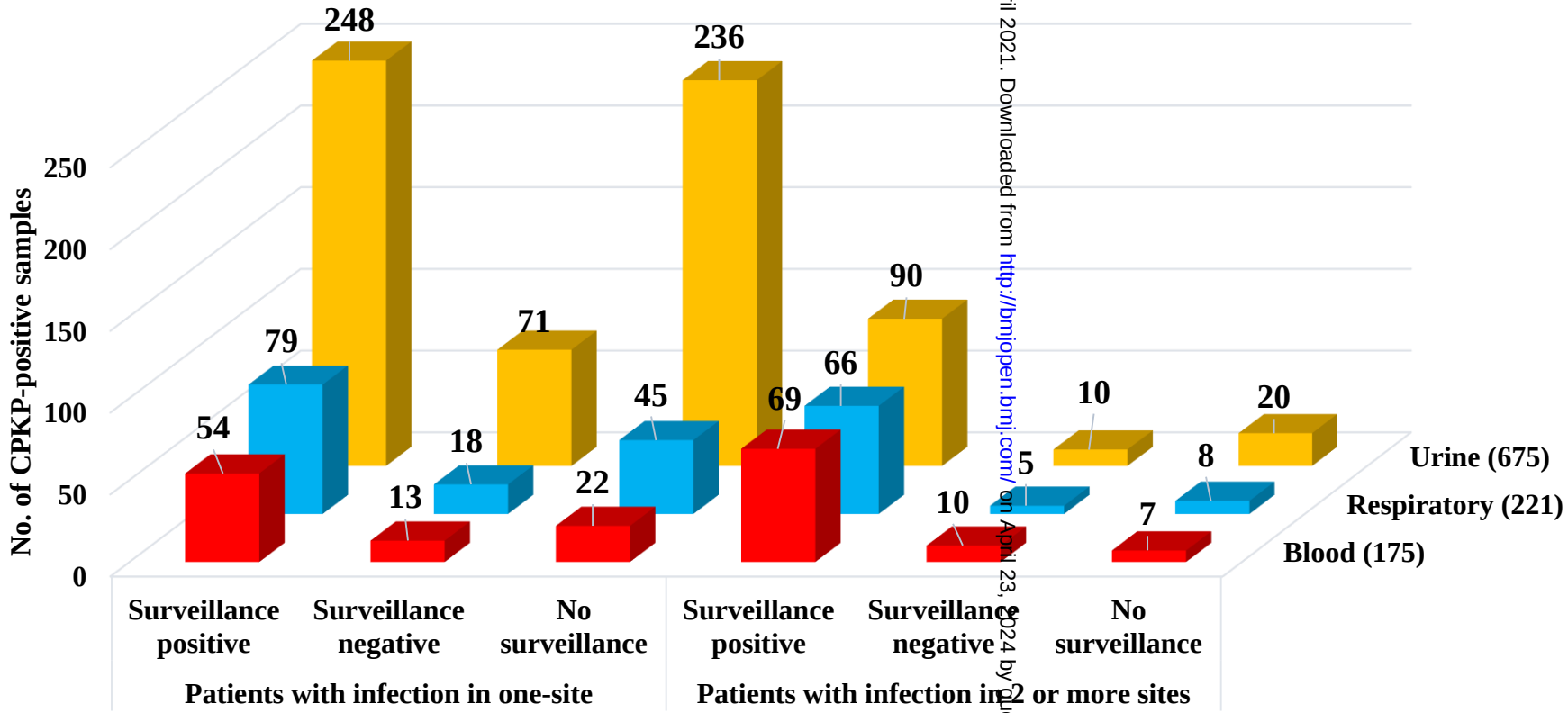
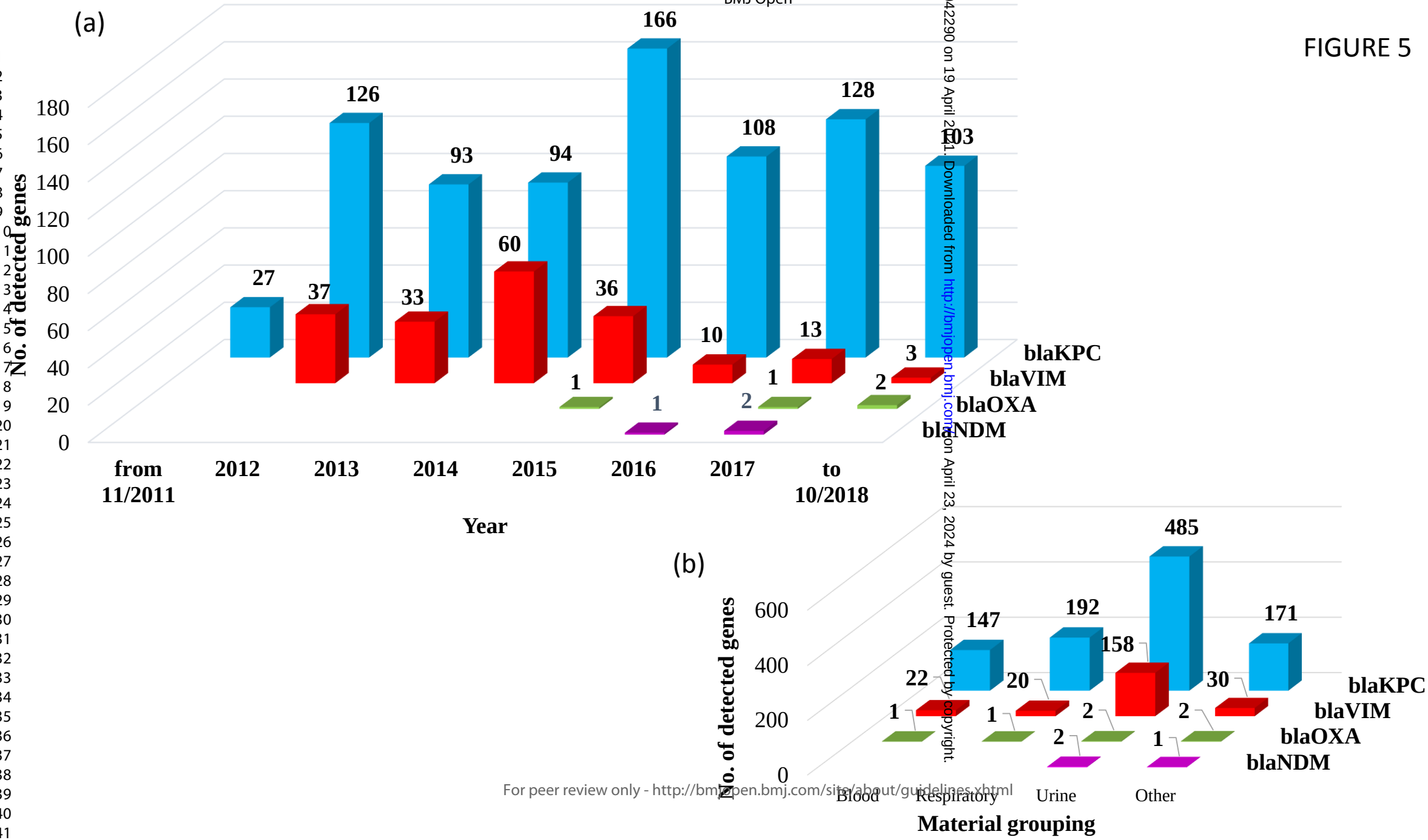


FIGURE 5



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Original article

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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Abstract

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

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

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

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

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

Article summary

Strengths and limitations of this study

- This study describes the distribution of the CPKP phenotypes and genotypes detected in a large number of samples over a long period (7 years), with particular reference to their temporal trend.
- The results reported in this study from an unselected patient population attending a single tertiary-care hospital may contribute to the global data production.
- The association between patients with CPKP-positive rectal swab and those with CPKP infection was performed taking into account also the different material groups.
- The molecular genotypic characterization based only on the four major carbapenemase genes could have missed the more rarely circulating genotypes.
- The lack of further genetic typing hampered to add consideration about any molecular epidemiological link among the isolates.

Introduction

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

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

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

Methods

Study design.

The study was designed as a retrospective data collection. The total observation time was 7 years. Data were sought retrospectively from the records produced by the diagnostic flow of the laboratory, as answer to a clinical suspicion or to active CRE surveillance [6].

Patient and Public Involvement.

Patients were not involved in the study.

Definitions.

A patient was defined as carrier when only a CPKP-positive rectal swab was detected and as infected when a CPKP-positive sample other than rectal swab was found, in presence of signs and symptoms of infection, according to CDC criteria for specific types of infections [7].
CPKP-positive samples other than rectal swab were grouped in blood (including blood, vascular catheter, and cerebrospinal fluid), respiratory (including bronchial aspirate, bronchoalveolar lavage, sputum, pleural fluid, pharyngeal swab, nasopharyngeal aspirate and nasal swab), urine (including urine and urinary catheter), and other (including bile, peritoneal, ascitic and abdominal drainage fluids, pus, bioptic and prothesic materials, sperm, tongue, wound, cutaneous, vaginal and urethral swabs).

Study setting and population.

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

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

Inclusion and exclusion criteria

Inclusion criteria: The first group included "at-risk" patients examined on admission as part of the National/Regional active CRE surveillance for the detection of CPKP strains in rectal swabs/stools (hereafter referred as rectal swab) [6], according to the following indications: 1) contacts of CPKP-positive patients; 2) patients admitted to transplant surgery, intensive care units or any other "at-risk" unit such as long-term care units, oncology and haematology; 3) patients known to be infected/colonized, with the last CPKP positivity dating back to more than 90 days from the new admission; 4) patients coming from endemic countries, such as Israel, Greece, Pakistan and India; 5) patients transferred from acute care and neurological rehabilitation facilities; 6) patients coming from nursing homes for the elderly; 7) patients hospitalized in an acute care facility in the last 6 months.

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

Exclusion criteria: no exclusion criteria were adopted.

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 [6], 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), able to differentiate KPC, MBL and OXA-48 like carbapenemases, performed according to the manufacturer's instructions. For the genotypical characterization, 2 molecular methods were used during the study period: the first, detecting *bla*KPC, *bla*NDM, and retrospectively, on the available *bla*NDM-negative MBL-producing isolates, *bla*VIM, was used until 2015, and the second one also detecting *bla*OXA-48 was used since 2015, as previously described [3].

Statistical analysis.

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

Results

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

to 4.7% in the 2012-2014 period, reached the highest peak (6.3%) in 2015, and showed a fluctuating trend from 2016 to 2018 (Fig.1b). With regard to the results of the molecular genotyping assays, all targeted types of carbapenemase genes were detected among the analysed rectal swabs. The *bla*KPC was predominant (79%, 909/1150) followed by *bla*VIM (16.7%, 192/1150), while *bla*OXA-48 and *bla*NDM were more rarely observed, accounting for 0.3% (3/1150) and 0.2% (2/1150), respectively (Fig. 2). In 0.8% (9/1150, 8 class B other than *bla*NDM and *bla*VIM and 1 class A other than *bla*KPC) of the CPKP strains, none of the targeted genes was revealed, if excluding the class B (35) *bla*NDM-negative strains for which *bla*VIM was not tested. With reference to temporal distribution, from 2012 to 2014 a decrease of *bla*KPC was observed in contrast to a correspondent increase of *bla*VIM, which started gradually decreasing from 2015. When the peak of positive rectal swabs (204) was observed in 2015, *bla*KPC reached the maximum peak frequency (173), doubling that of 2014 and subsequently slightly decreased with a fluctuating trend (Fig. 2).

When the 22,939 patients submitted to CRE surveillance (group 1) were combined with the 1094 CPKP infected patients (group 2), a total of 1662 CPKP-positive patients was found: 568 CPKP carriers (accounting for 49.4% of the 1150 patients with CPKP-positive rectal swab), 582 CPKP infected patients with a CPKP-positive rectal swab (accounting for 50.6% of the 1150 patients with CPKP positive rectal swab), 137 CPKP infected patients with a CPKP-negative rectal swab, and 375 CPKP infected patients not included in the active CRE surveillance (Fig. 3).

Among the 1094 CPKP infected patients (719 included in the active CRE surveillance and 375 not included in the active CRE surveillance), accounting for 1283 CPKP-positive samples, urine (675) was the mostly involved sample all over the period, although a significant decrease from 2015 to 2016 was observed. Blood (175) accounted for about 25 cases per year, with a peak in 2017 (34) (Fig. 4).

With regard to the CPKP infected patients included in the active CRE surveillance, no significant difference was observed in the frequency of CPKP-positive rectal swabs in the different material

groups. On the contrary, CPKP-positive blood (49%) and respiratory (31%) samples were more frequently associated than urine (17.7%) with CPKP-positive samples from 2- or more-site of infection ($p< 0.0001$), as well as CPKP-positive blood samples were more frequently associated with other CPKP-positive samples from 2- or more-site infection ($p< 0.001$) than the respiratory ones (Fig. 5).

With reference to the 1094 CPKP infected patients, 1034 (94.5%) were positive for one of the targeted carbapenemase genes (841 *bla*KPC, 188 *bla*VIM, 3 *bla*OXA-48, and 2 *bla*NDM) and 5 (0.5%) contained two of the targeted carbapenemase genes (4 *bla*KPC+*bla*VIM and 1 *bla*NDM+*bla*OXA48), for a total of 1044 carbapenemase genes detected (Fig. 6a). If excluding the 50 class B *bla*NDM-negative strains for which *bla*VIM was not tested, the remaining 5 cases were negative for the targeted carbapenemase genes (2 class A *bla*KPC-negative and 3 class B *bla*VIM- and *bla*NDM-negative). When the carbapenemase gene analysis was performed by material grouping, *bla*KPC was the most frequently detected carbapenemase gene in all material groups, followed by *bla*VIM; however, the ratio of *bla*KPC and *bla*VIM was found to range from 6:1 to 10:1 for all material groups, except for urine for which *bla*VIM was more significantly detected (ratio 3:1) ($p<0.01$) (Fig. 6b).

Discussion

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

In our experience, the results of the application of active CRE surveillance with the adoption of a combination of phenotypic assays followed by genotypic characterization on “at-risk” patient categories highlight the need not to lower the guard about this problem. In fact during the study period, after an initial constant trend of the frequency of CPKP-positive rectal swab cases from

2012 to 2014 (ranging from 4.4% to 4.7%), in 2015 the highest peak (6.3%) was observed, in agreement with the same pattern described for invasive infection at regional level [6], followed by a decrease in 2016 (4.9%) and a subsequent increasing trend in the last 2 years. The highest prevalence of CPKP-positive rectal swabs was observed in the long-term care units, if excluding those units in which rectal swab screening was performed only on targeted patients, such as contacts of carrier patients and/or transfer from "at-risk" care units, and obtained on a limited number of rectal swabs.

When the association between patients with CPKP-positive rectal swab and those with CPKP infection (CPKP-positive samples other than rectal swab) was considered, it was observed that the 49.4% of patients submitted to active surveillance with CPKP-positive rectal swab were carriers, representing a potential reservoir for spread of CPKP strains detectable only by surveillance. Taking into account the overall infected patients and excluding those not submitted to active CRE surveillance, no difference was observed in the frequency of patients with CPKP-positive rectal swab among the different material groups. On the contrary, CPKP-positive blood and respiratory samples were more frequently associated with infections in multiple body sites, as expected due to the difficulty in containing invasive infections (blood and respiratory samples) in a unique site and suggesting that carriage represents one of the most important risk factors for CPKP infection, as previously described for bloodstream infection [14,15].

With regard to the temporal distribution of the carbapenemase genes among CPKP-positive rectal swabs, from 2012 to 2014 the *bla*KPC and *bla*VIM showed an inverse trend: in fact, when the *bla*KPC decreased from 74.9% to 55.8%, the *bla*VIM increased consequently from 17% to 39.7%. In correspondence of the highest CPKP-positive rectal swab rate in 2015, the trend of the frequency of the *bla*KPC and *bla*VIM genes has reversed: that of *bla*KPC has continuously raised again, reaching 92.8%, whereas that of *bla*VIM progressively decreased to 3.2%. *bla*NDM and *bla*OXA-48 were only occasionally detected starting from 2016 and 2018, respectively. A similar temporal distribution was also observed among infected patients, in which *bla*KPC was prevalent during all

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

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

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 he 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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Figure legend

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

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

Figure 3. Study result flow diagram.

Figure 4. Year distribution of CPKP infected patients by material grouping (blood, respiratory, urine and other sites).

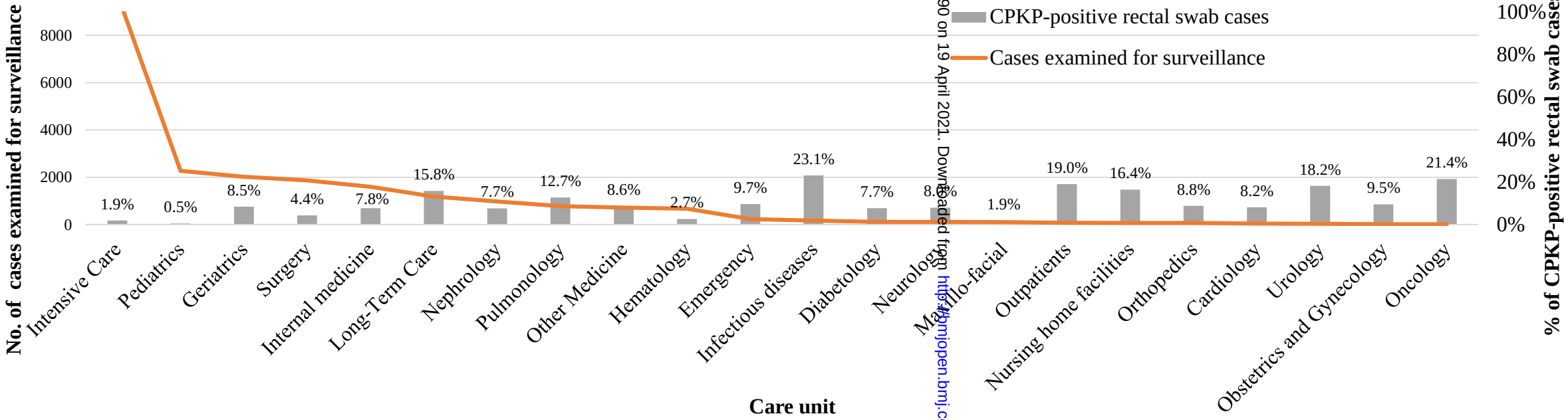
Figure 5. Comparison of the results of active CPE surveillance among CPKP infected patients with one-site infection and in those with multiple-site infection by material grouping (blood, respiratory, urine).

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

Ethical approval: data used for this study were reported in the medical records of the patients as answer to a clinical suspicion or to active CRE surveillance. Ethical approval at the University Hospital of Parma is required only in cases in which the clinical samples are to be used for applications other than diagnosis.

FIGURE 1

(a)



(b)

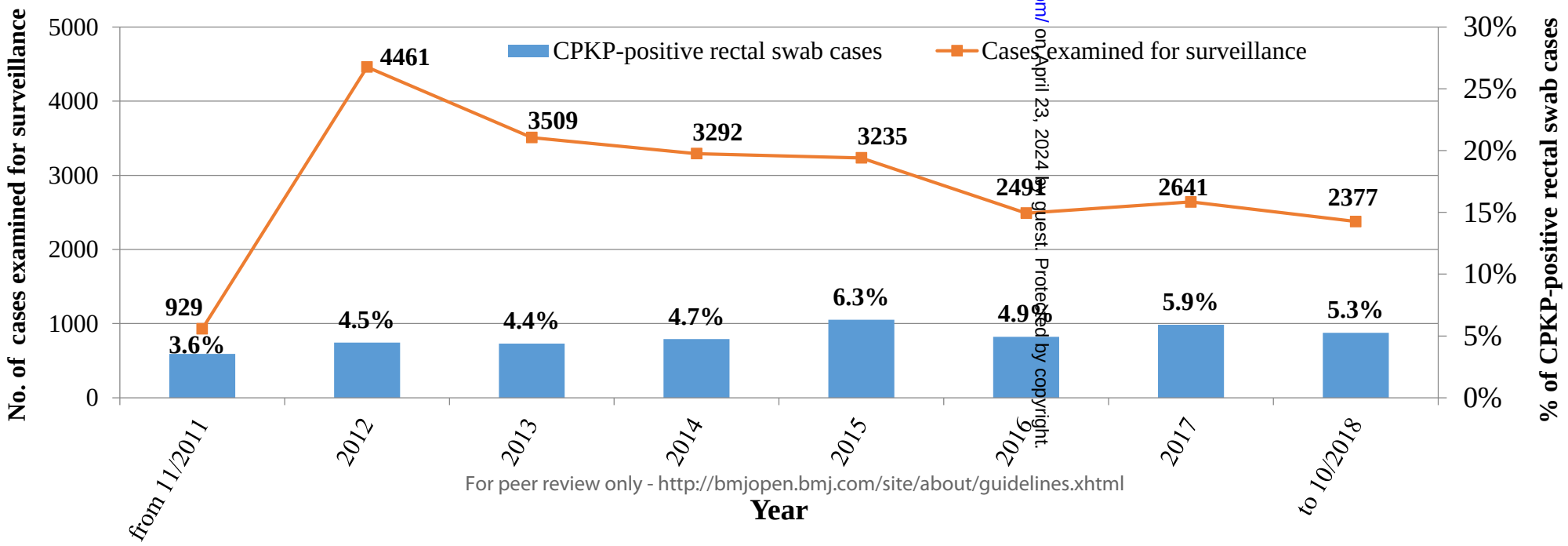
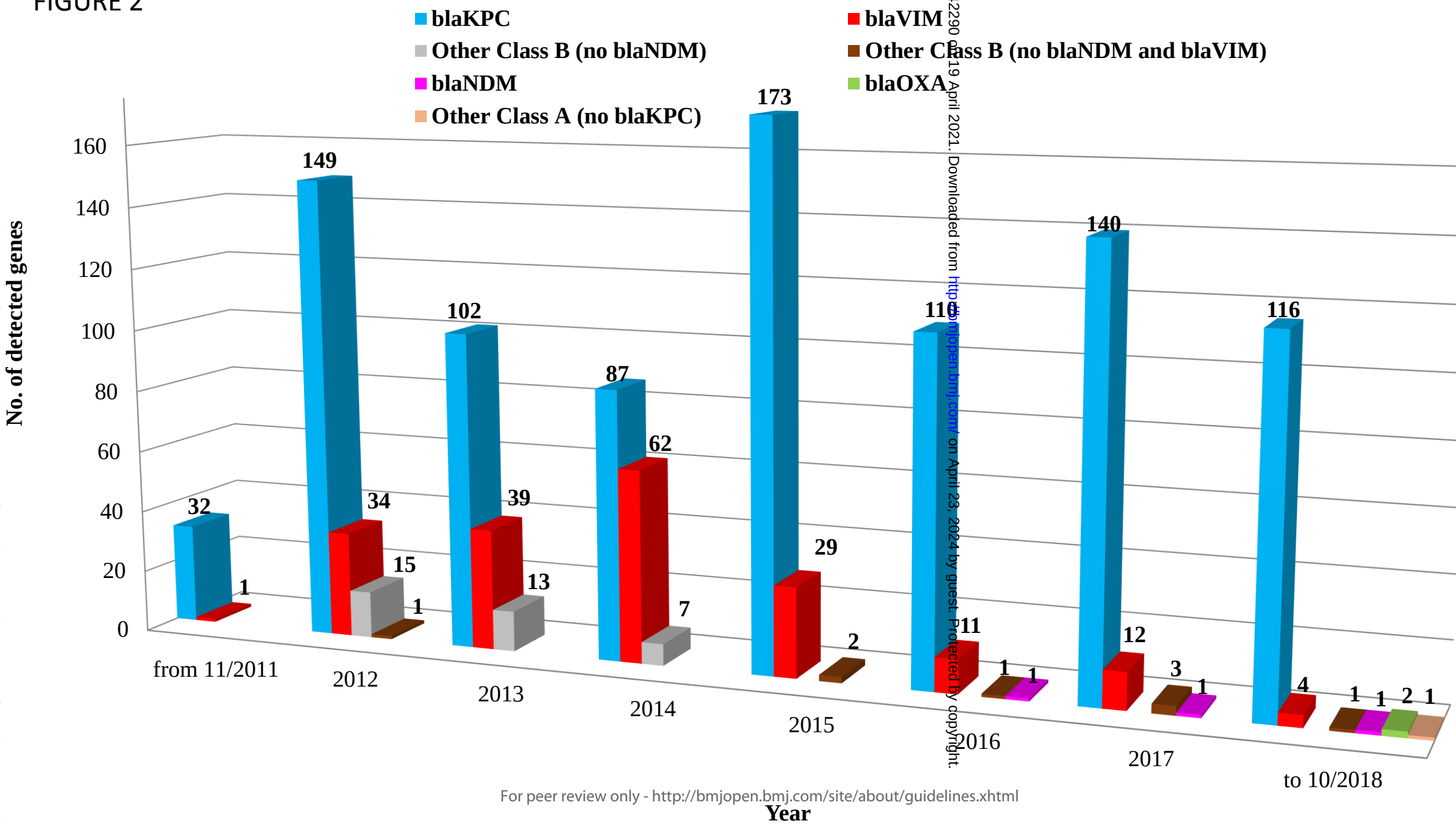


FIGURE 2



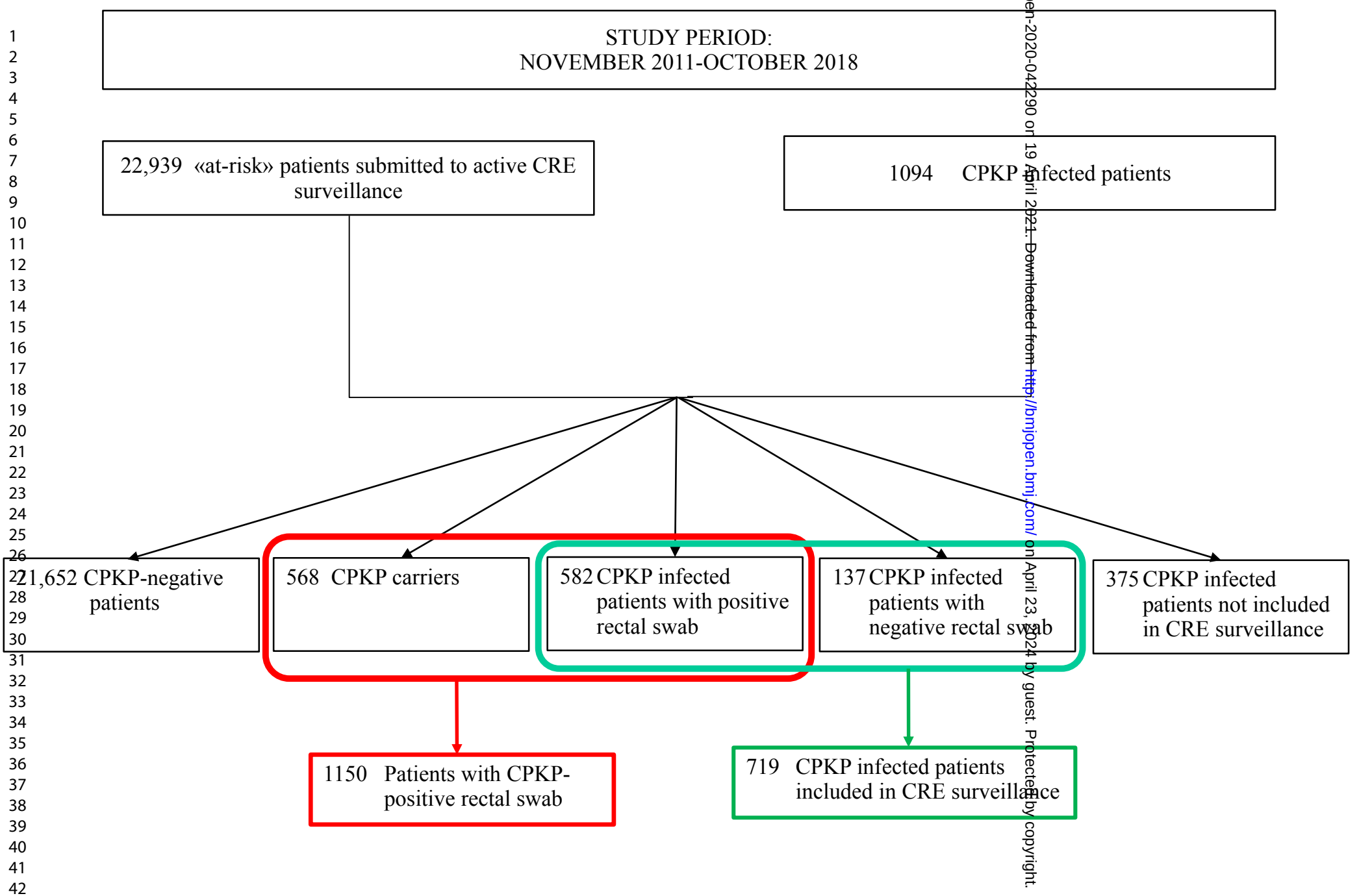


FIGURE 4

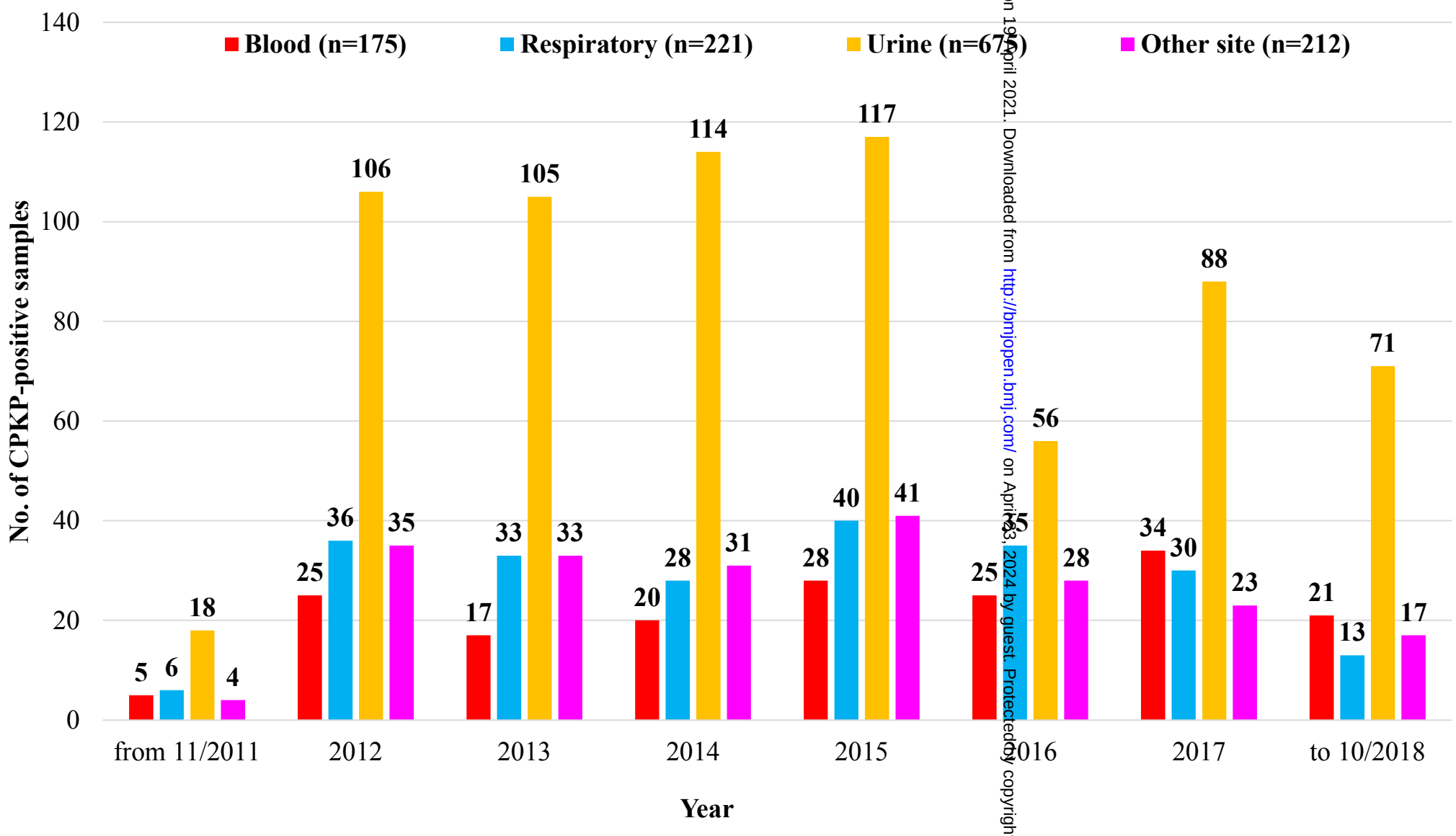


FIGURE 5

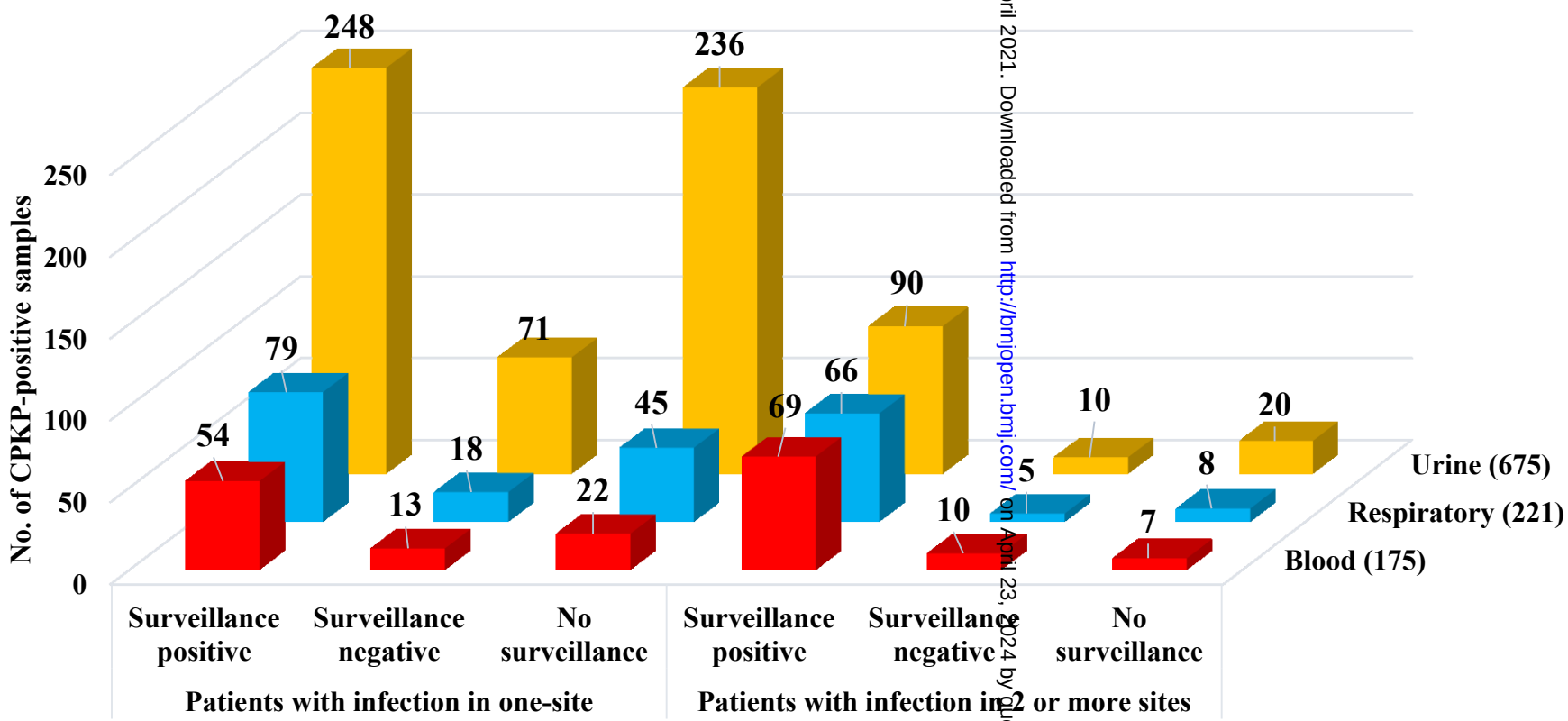
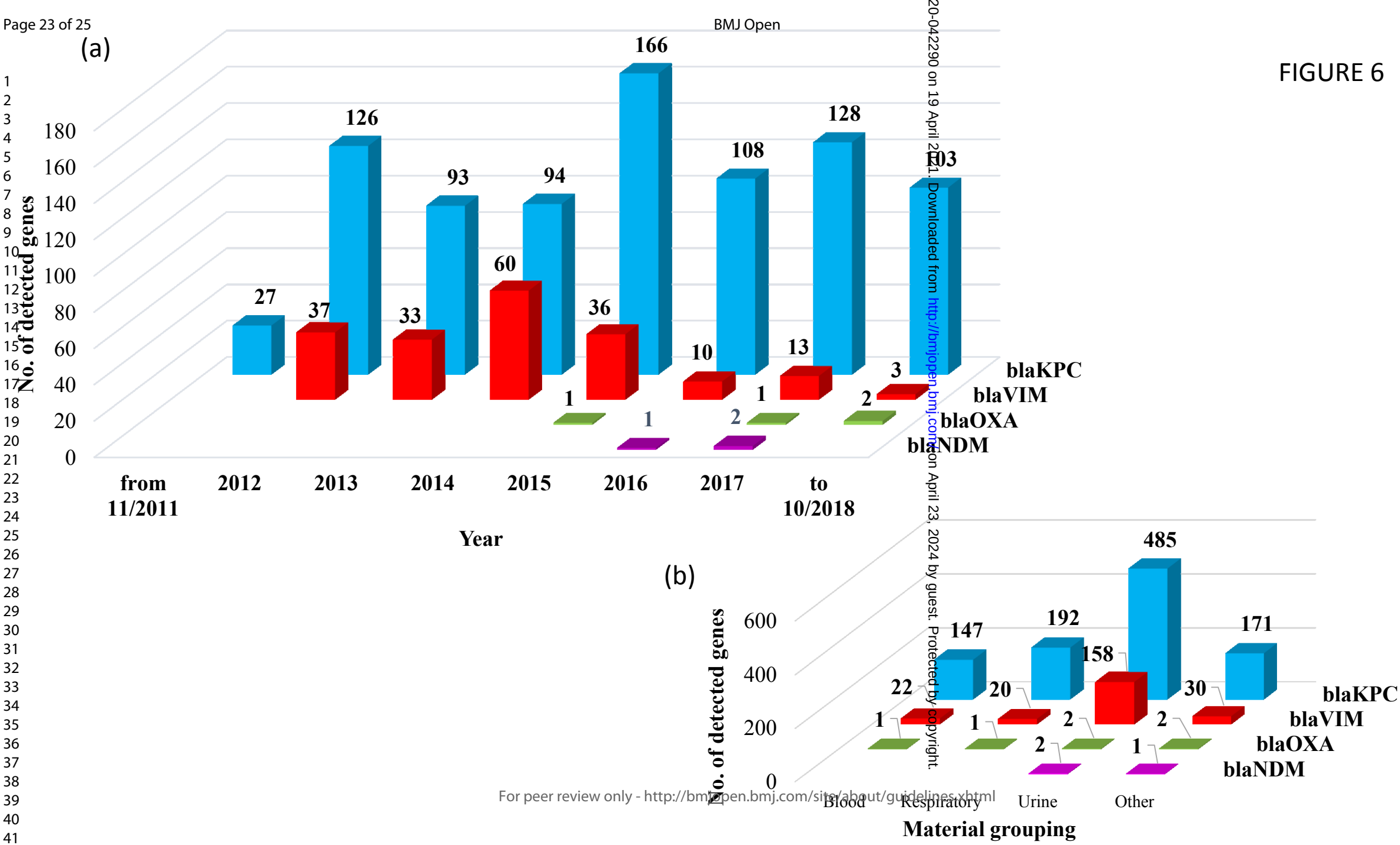


FIGURE 6



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

	Item No	Recommendation	Reported on page n., line n.
Title and abstract	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
Introduction			
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
Methods			
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.

Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 7, lines 156-159
		(*b*) Describe any methods used to examine subgroups and interactions	As mentioned above
		(*c*) Explain how missing data were addressed	Not applicable
		(*d*) If applicable, explain how loss to follow-up was addressed	Not applicable.
		(*e*) Describe any sensitivity analyses	Not applicable
Results			
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
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Figure 3
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
		(b) Indicate number of participants with missing data for each variable of interest	Page 8, lines 172-173 Page 9, lines 199-202
		(c) Summarise follow-up time (eg, average and total amount)	Not applicable
Outcome data	15*	Report numbers of outcome events or summary measures over time	Not applicable
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
		(*b*) Report category boundaries when continuous variables were categorized	Not applicable
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page 9, lines 189-195, 202-206
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 10, lines 215-219, 224-227 Page 11, lines 235-245

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
Other information			
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.