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Prevalence, molecular epidemiology and intra-hospital acquisition of Klebsiella pneumoniae strains producing carbapenemases in an Italian teaching hospital from January 2015 to September 2016

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ABSTRACT

Objectives: We described Klebsiella pneumoniae producing carbapenemase (CPKP) spread from 01/01/2015 to 13/09/16 in a tertiary level hospital.
Methods: The first positive surveillance rectal swab (SRS) or clinical sample (CS) collected in the medical department (MD), surgical department (SD) and intensive care department (ICD) were included in the study. A validated in-house Real-Time PCR method was used to detect carbapenemases; multilocus sequence typing (MLST) was used for further characterization of the strains.
Results: 21535 patients were included: 213 CPKP strains from surveillance rectal swab (SRS) and 98 from clinical samples (CS) were collected. The percentage of CPKP detected in SRS with respect to CS increased in the medical MD from 2015 to 2016 ($p = 0.01$) and in ICD from 2012 to 2015 ($p = 0.0001$), while it decreased in SD from 2014 to 2016 ($p = 0.003$); 68.5% of the positive SRS had a previous negative SRS; CPKP was more frequently identified in CS than in SRS in MD. Twelve strains harboured more than one carbapenemase gene. Many other species harbouring a carbapenemase gene were collected.
Conclusions: MDs need more inclusive surveillance criteria. The late detection of positive SRS underlined the risk of colonization during hospitalization.

Introduction

The endemic spread of *Klebsiella pneumoniae* strains producing carbapenemases (CPKP) has serious implications for both public health and infection control practices in Italy: a countrywide survey reported an 11.9% rate of carbapenem-resistant strains in consecutive non-replicate clinical isolates, with carbapenemase production detected in 85% of the strains (Giani et al., 2013). Bacteria that have become resistant to carbapenems cause infections in debilitated and immunocompromised patients that are associated with high morbidity and mortality (Falcone et al., 2016; Palacios-Baena et al., 2016). Therapeutic options are limited and there is still a lack of consensus on the optimal approach: patients treated with regimens including carbapenems in addition to tigecycline or colistin seemed to have better outcomes (Sekirov et al., 2016). Moreover, colistin-resistant CPKP may directly colonize or infect patients and a colistin resistant (CoR) strain can be identified in subjects with a previous detection of a colistin sensitive (CoS) strain (Parisi et al., 2015). The local epidemiology and the specific health setting (i.e. the availability of isolation rooms) may impact the efficacy of hospital infection control strategies even in a geographically limited area in a high-resource country (Gagliotti et al., 2014). One of the major keys to control the spread of these pathogens is the prompt screening and isolation or cohorting of colonized subjects: an active surveillance with rectal swab (RS) cultures increases the frequency of proportion of CPKP first isolated by RS relative to those identified by clinical samples (Parisi et al., 2015). Nosocomial spread of CPKP is a multifaceted

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and dynamic phenomena: a positive surveillance RS (SRS) can be detected after more than a previous negative result, patients may have a different environmental contamination capacity, the detection of CPKP in SRS allows starting control measures to avoid the transmission but the predictability of the susceptibility profile is 88.7% with respect to the clinical samples and errors are observed mainly for colistin susceptibility (Parisi et al., 2015; Lerner et al., 2015; Perez et al., 2016).

Here we report an updated epidemiological and molecular description of CPKP spread over the last 20 months in a tertiary level hospital in Padova (Italy), which has been under a surveillance program since 2012 (Parisi et al., 2015).

Patients and Methods

A retrospective analysis of SRS and clinical samples (CS) collected from 01/01/2015 to 13/09/2016 was performed. Adult patients admitted to the Intensive Care Department (ICD) were monitored with SRS upon admission and at least weekly thereafter, patients of the Surgery Department (SD) were tested only upon admission; patients admitted to the Medicine Department (MD) were screened if they were hospitalized in the last two months or if they arrived from long-term care facilities. Some isolates were obtained from patients with potential epidemiological links to persons from whom CPKP were isolated (e.g., patients in the same room or ward). Only the first positive CPKP strain isolated from each patient was included. All samples were collected as part of routine management/surveillance. Isolation rooms for colonized/infected patients were set up or the subjects were transferred to the infectious diseases ward. Isolation was not feasible in all cases because of insufficient bed capacity cases, but the colonized or infected patients were cohorted in the same room whenever it was possible. All contact precautions were improved. The study was approved by the Ethical Committee for Clinical Experimentation, Padua Province (Ethics Review 3418/AO/15). The Padua Teaching Hospital is a highly accessed tertiary care hospital with 1300 recovery beds.

Microbial identification was performed in all strains by using bioMérieux Vitek® 2 and Vitek® MS. Antimicrobial susceptibly testing was performed with a Vitek® 2 automated system. Strains that exhibited reduced susceptibility to carbapenems (a MIC value $\geq$1 mg/L for ertapenem and/or imipenem and/or meropenem) were also tested using the dilution method (Thermo Scientific Sensititre™ system) for confirmation. Other phenotypic methods employed for the detection and confirmation of carbapenemase were the modified Hodge test and the Rosco Diagnostica KPC/MBL confirmation kit. Colistin susceptibility, initially evaluated using the Vitek® 2 automated system, was then confirmed with the dilution method on all strains because of possible over-estimation of resistance by automated methods (Sbrana et al., 2013). All MIC values were evaluated with European Committee on Antimicrobial Susceptibility Testing (EUCAST) Clinical Breakpoint Tables (European Committee, 2016). Multidrug-resistant (MDR) is defined as non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012).

Genotypic assays

A validated in-house Real-Time PCR method was used to detect KPC, KPC type, OXA-48, Verona integron-encoded metallo-β-lactamase (VIM) and New Delhi metallo-β-lactamase types (NDM) carbapenemases in cases of suspected carbapenemase-producing strains (Richter et al., 2011; Naas et al., 2013). A detailed description of primers and probes was reported in Table 1.

Multilocus sequence typing (MLST) was used according to the MLST website for further characterization of the strains to investigate possible cases of intra-hospital transmission and not for research purposes (MLST database, 2016).

Statistical methods

Data were expressed as absolute numbers and percentages. The number of CPKP strains was evaluated by material (SRS versus CS) and by the susceptibility or resistance to colistin. The results were compared with those of the previous survey performed in the same hospital from 2012 to 2014 in case of unchanged surveillance criteria: this analysis was made for ICD (years 2012-2014) and for the SD (data obtained in 2014). The Chi-squared test and Fisher's exact test were used to compare proportions (as appropriate), and the Chi-squared test for trends was used to evaluate the trends in proportions. Values of $p < 0.05$ were considered statistically significant. The statistical analyses were performed with MedCalc Statistical Software version 16.8 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2016).

Results

A total of 21,535 patients were included in the study (12,082 in 2015 and 9453 from 01/01/2016 to 13/09/2016): 311 consecutive non-replicated strains of CPKP were collected, as first isolate detection from each patient. Two hundred and seventy-eight (89.4%) were characterized using molecular methods: most were KPC strains (258 out of 278, 92.8%), 17 (6.1%) OXA-48 and 12 (4.3%) NDM positive isolates were identified, as single gene or associated

Table 1
List of primers and probes used to amplify $bla_{KPC1/2-12}$, $bla_{VIM-1-6,8-12,14-19,23-37}$, bla_{VIM-7}, bla_{VIM-13}, bla_{NDM}, bla_{OXA-48} by the in-house Real-Time PCR method.

Target	Name	Sequence (5′→3′)	Amplicon size (bp)
$bla_{KPC1/2-12}$	KPC1/2-12-F	GCCGTGCAATACAGTGATAACG	60
	KPC1/2-12-R	CGGGCCGCCCAACT	
$bla_{VIM-1-6,8-12,14-19,23-37}$	VIM-F-RT	TCGCACCCCACGCTGTA	58
	VIM-R-RT	GGTCTCATTGTCCGTGATGGT	
bla_{VIM-7}	VIM7-F-RT	TTCGCGTGACGCCTCAT	52
	VIM7-R-RT	GGCCGTGCGCGTACTG	
bla_{VIM-13}	VIM13-F-RT	TGTTTTTCGTACCCCAAGCTGTA	67
	VIM13-R-RT	AATGGTCTCATTGTCCGTGATG	
bla_{NDM}	NDM-F	GACCGCCCAGATCCTCAA	52
	NDM-R	CGCGACCGGCAGGTT	
bla_{OXA-48}	OXA-48-F	GTAGCAAAGGAATGGCAA	100
	OXA-48-R	CCTTGCTGCTTATTGTCA	

KPC: class A carbapenemases.
OXA-48: oxacillinases (OXA-48-like enzymes).
VIM: Verona integron-encoded metallo-β-lactamase.
NDM: New Delhi metallo-β-lactamase types.
bp: base pair.

Table 2
Description of the carbapenemase genes detected in *Klebsiella Pneumoniae* strains. Data were described as absolute number and percentage with respect to the total number of strains isolated in the specific year.

Year	KPC (n, %)	OXA-48 (n, %)	NDM (n, %)	KPC + VIM (n, %)	NDM + OXA-48 (n, %)	More than one gene detected (n, %)
2015[1]	195 (94.3)	6 (2.9)	1 (0.5)	1 (0.5)	2 (0.9)	1 KPC/NDM (0.5) 1 KPC/NDM + OXA48 (0.5)
2016[2]	63 (88.7)	1 (1.4)	0	0	7 (9.9)	0

KPC: class A carbapenemases.
OXA-48: oxacillinases (OXA-48-like enzymes).
NDM: New Delhi metallo-β-lactamase types.
VIM: Verona integron-encoded metallo-β-lactamase.
[1] Data available for 207/216 strains.
[2] Period 01/01/2016-13/09/2016: data available for 71/95 strains.

with each other or with KPC. Of note, 12 strains harboured more than one carbapenemase gene (Table 2). All the available isolates were tested: 20 isolates were not frozen and 13 were found contaminated when they were thawed.

Specifically, we collected 213 CPKP strains from SRS and 98 from clinical samples. The percentage of CPKP detected in SRS with respect to those detected in CS increased in the MD from 2015 to 2016 ($p = 0.01$) and in ICD from 2012 to 2015 (chi square for trend $p = 0.0001$). Conversely, a decreasing trend in the percentage of positive SRS was found in patients admitted to SD (from 2014 to 2016, $p = 0.003$ and from 2014 to 2015 $p = 0.0573$). Absolute data are detailed in Figure 1.

All SRS and CS data obtained in the previous survey (Parisi et al., 2015) are reported in supplementary Figure S1 in the online version at DOI: 10.1016/j.ijid.2017.04.007(doi:10.1016/j.ijid.2017.04.007). The percentage of positive SRS with respect to the number of the patients involved in the surveillance was lower in SD with respect to MD and to ICD both in 2015 ($p = 0.0065$ and $p < 0.0001$) and in 2016 ($p < 0.0001$ and $p = 0.0256$) (Table 3). The results obtained in 2012-2014 are described in supplementary Figure S2 in the online version at DOI: 10.1016/j.ijid.2017.04.007 (doi:10.1016/j.ijid.2017.04.007).

In 2015, 68.5% of the CPK detected in SRS had at least a previous negative SRS during the same hospital admission. The frequency of the late detection during active surveillance was significantly higher in the SD [44 patients, (67.7%)] and in ICD [51 patients, (85%)] with respect to that reported in MD (3 patients, 16.7%) ($p = 0.0001$ and $p < 0.0001$ respectively). Overall, from January to September 2016 the percentage of positive SRS identified after a negative test was 62.9%, similar to that of 2015: in SD 23 patients (71.8%), in ICD 15 patients (62.5%) and in MD 6 patients, (42.9%). In 2015, patients had an interval from the first negative SRS to positive SRS of 13 days (median, range 1- > 40 days). In 2016, the results are comparable with a time interval of 12 days (median, range 1- > 40 days). The isolate obtained from patients with a possible epidemiological link to known positive subjects were included in this analysis. The percentage of CPKP strains identified in SRS versus that found in CS was significantly lower in MD than in SD and in ICD both in 2015 ($p < 0.0001$) and in 2016 ($p = 0.03$ and $p = 0.01$ respectively).

The MLST analysis revealed the circulation of 16 different CPKP strains without the predominance of a specific ST: in two patients two different strains were identified at the same time in a sample. Interestingly, ST-745 (a new single locus variant of ST-512 identified in our hospital in 2011) circulation persisted over time (Table 4). A complete description of MLST analysis performed in 2012-2014 (5) was reported in supplementary Figure S3 in the online version at DOI: 10.1016/j.ijid.2017.04.007(doi:10.1016/j.ijid.2017.04.007).

Some CPKP clusters were identified, possibly due to episodic depth of contact subjects after the discovery of a first positive isolate. At least five clusters were observed in different wards.

In 2015, in an SD ward, six subjects with ST-512 strain were diagnosed as SRS positive in a 72 day period: all subjects had one to three previous negative SRS. In the same ward, in a 22 day period (included in the previous one), another five patients were found to be SRS positive for ST-745 (in all cases after a one to six negative SRS).

In 2016, in the same ward, another six subjects were diagnosed as SRS positive in a 64 day period. All had an ST-512 strain and all but one patient had from one to five previous negative SRS. In five of these patients a concordant positive clinical sample was later detected (one as responsible for bacteremia). Other clusters were observed in another two SD wards and the patients had always a previous negative SRS. In the first ward four ST-745 strains were identified in a 16 days interval; in the second ward, five ST-307 strains were detected in 31 days. Finally, in an ICD ward nine ST-554 strains were isolated in an 88 day period, all after one to 38 negative SRS (range 4-133 days); in these cases, five out of nine patients had a subsequent positive clinical sample (identified in blood in three cases).

Many other species harbouring a carbapenemase gene were collected, mainly from clinical samples: *Enterobacter cloacae*, *Klebsiella oxytoca*, *Escherichia coli*, *Serratia marcescens* and *Citrobacter freundii* (Table 5).

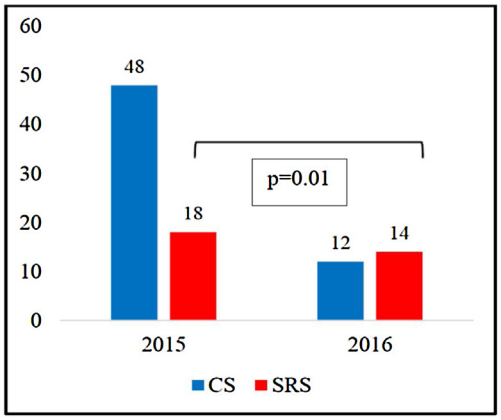
Overall, the percentage of CoR strains was 15.7% (34 samples) in 2015 and 18.9% in 2016 (18 samples). Colistin resistant strains were more frequently detected in SRS than in CS (5 out of 73 versus 29 out of 143) in 2015 and in 2016 (15 out of 70 versus 3 out of 25). All but 2 CoR strains had an MDR profile. A detailed description of colistin MIC values is reported in Figure 2.

Table 3
Percentage of positive SRS with respect to the number of the patients involved in the surveillance in the Medical Department, Surgical Department and Intensive Care Department in 2015 and 2016[1]. The percentage of positive SRS was calculated with respect to the number of patients evaluated in each Department.

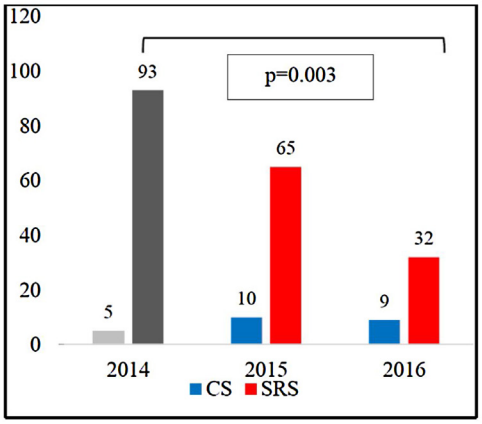
Year	Medical Department[2]			Surgical Department[2]			Intensive Care Department		
	Total SRS (n)	Patients (n)	Percentage of positive SRS	Total SRS (n)	Patients (n)	Percentage of positive SRS	Total SRS (n)	Patients (n)	Percentage of positive SRS
2015	2.711	1.077	1.7	14174	7.886	0.8	9.793	3.119	1.9
2016[1]	1567	721	1.9	10276	6172	0.5	6620	2560	0.9

SRS: Surveillance rectal swab.
[1] Period 01/01/2016-13/09/2016.
[2] some isolates were obtained from patients with potential epidemiological links to persons from whom CPKP were isolated.

(a) Medical Department: years 2015 and 2016



(b) Surgical Department: years 2014-2016



c) Intensive Care Department: years 2012-2016.

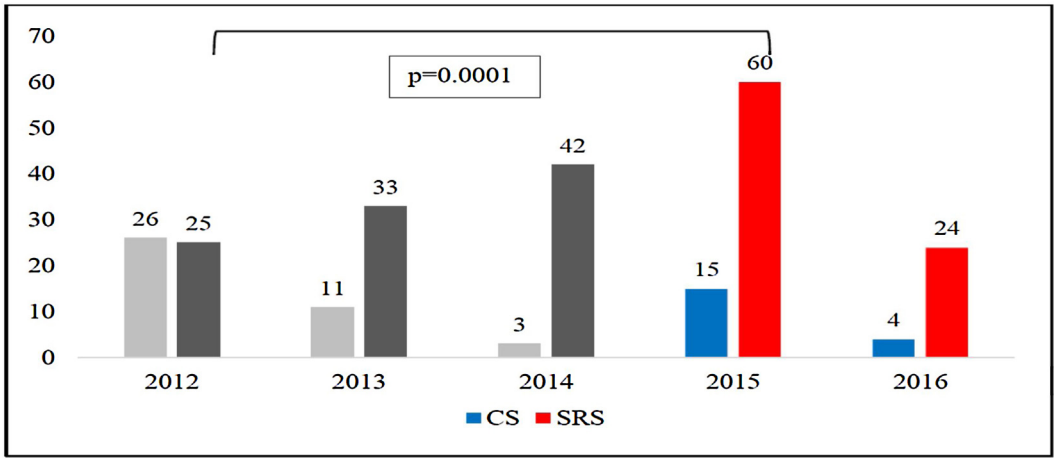


Figure 1. Carbapenemase producing Klebsiella pneumoniae strains isolated from surveillance rectal swabs (SRS) and from clinical samples (CS). The results are expressed as absolute numbers. Data obtained in 2015 and 2016 (from 01/01 to 13/09) were compared with those of the previous survey performed in the same hospital in case of unchanged surveillance criteria. In grey, previously published data (5)(CS light grey, SRS dark grey).
CS: clinical samples.
SRS: surveillance rectal swabs.

Discussion

This study showed the presence of epidemic clones of CPKP and of other CP Enterobacteriaceae despite an ongoing active surveillance cultures program started in 2012 and a peculiar epidemiological trend in SD: the number of CPKP detected in SRS from 2014 to 2016 is decreasing as an absolute number but increasing as a percentage with respect to CS (94.9% in 2014, 86.7% in 2015 and 78% in 2016), mainly represented as positive SRS after a negative survey.

Table 4
Description of the ST- identified in carbapenemase producing Klebsiella pneumoniae divided by Department and by material (SRS and CS) in 2015 and 2016[1].

Year 2015: clinical samples

	ST-258 (n)	ST-512 (n)	ST-745[2] (n)	ST-307 (n)	ST-554 (n)	ST-15 (n)	ST-16 (n)	ST-101 (n)	ST-11 (n)	Other ST and samples with 2 isolates at time
MD	8	8	2	18	4	1	2	1	1	–
SD	–	1	–	6	–	2	1	–	–	211 (1 pt) 398 (1 pt) 258/11 (1 pt)
ICD	2	2	4	2	–	–	–	–	–	–
Total[4]	10	11	6	26	4	3	3	1	1	3

Year 2016: clinical samples

	ST-258 (n)	ST-512 (n)	ST-745[2] (n)	ST-307 (n)	ST-554 (n)	ST-16 (n)	ST-101 (n)	ST-37 (n)	ST-45 (n)
MD	1	3	–	2	1	–	–	–	–
SD	1	2	–	1	–	–	–	–	1
ICD	1	–	–	1	–	–	–	–	–
Total[5]	3	5	–	4	1	–	–	–	1

Year 2015: surveillance rectal swabs

	ST-258 (n)	ST-512 (n)	ST-745[2] (n)	ST-307 (n)	ST-554 (n)	ST-15 (n)	ST-16 (n)	ST-101 (n)	ST-11 (n)	Other ST and samples with 2 isolates at time
MD	1	4	3	3	1	1	1	–	2	–
SD	7	23	14	8	6	1	2	1	–	–
ICD	8	17	7	11	9	–	1	–	–	147 (1 pt), 1458 (1 pt), 512/16 (1 pt)
Total[6]	16	44	24	22	16	2	4	1	2	3

Year 2016: surveillance rectal swabs

	ST-258 (n)	ST-512 (n)	ST-745[2] (n)	ST-307 (n)	ST-554 (n)	ST-16 (n)	ST-101 (n)	ST-37 (n)	ST-45
MD	1	3	–	–	1	1	1	–	–
SD	1	13	–	–	–	–	–	1	–
ICD	4	9	2	2	–	3	–	–	–
Total[7]	6	25	2	2	1	4	1	1	–

MD: medical department.
SD: surgical department.
ICD: intensive care department.
[1] Period 01/01/2016-13/09/2016.
[2] ST-512 single locus variant.
[4] MLST available for 68/73 strains.
[5] MLST available for 14/25 strains.
[6] MLST available for 134/143 strains.
[7] MLST available for 42/70 strains.

Table 5
Carbapenemase-producing species isolated in 2015-2016[1] as part of routine management/surveillance of the patients.

Species	Total number of strains	Dept	Sample	Gene
E. cloacae	19	ICD	16 CS	VIM
		ICD	2 SRS	1 VIM, 1 KPC + VIM
		MD	1 CS	VIM
K. oxytoca	4	ICD	1 SRS	KPC + VIM
			1 CS	VIM
		MD	1 CS	KPC
		SD	1 SRS	VIM
E. coli	3	SD	2 CS	1 KPC, 1 VIM
		MD	1 CS	KPC
S. marcescens	1	SD	1 CS	KPC
C. freundii	1	SD	1 CS	VIM

Dept: department.
CS: clinical sample.
SRS: Surveillance rectal swab.
KPC: class A carbapenemases.
VIM: Verona integron-encoded metallo-β-lactamase.
[1] Period 01/01/2016-13/09/2016.

Our data confirmed that KPC is still largely confined to KP but the potential to spread antibiotic resistance to other bacterial species, such as *Escherichia coli*, should be taken into account (Richter et al., 2011; Tzouvelekis et al., 2012).

KPC represented the most frequently identified carbapenemase genotype in Italy but the detection of several isolates with OXA-48 and NDM suggests a change in the Italian epidemiological scenario. NDM producing KP were rarely described in previous epidemiological studies: they were not detected in a survey involving 25 Italian laboratories in 2011 (Giani et al., 2013). KP harbouring NDM were previously isolated in five patients hospitalized in three different Italian healthcare facilities in the Bologna area: no definite explanation for this outbreak was reported (Gaibani et al., 2011).

Longitudinal analysis revealed a decrease in the relative percentage of positive SRS in the SD while an increase was reported in MD (from 2015 to 2016) and in ICD (from 2012 to 2015): moreover, most positive SRS in SD and ICD were identified after a previous negative SRS. These results suggest an increased risk of colonization during hospitalization in many hospital areas. Recent surgery is a predicting factor associated with CPKP positivity (da Silva et al., 2016) but local epidemiology plays an important role in timing of CPKP detection. The percentage of positive patients

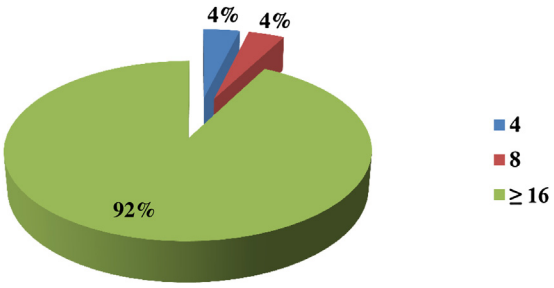


Figure 2. Colistin MIC values (mg/L) of CoR strains performed with Vitek® 2 system in 2015 and 2016[1]. Data are expressed as relative percentage.
[1]Period 01/01/2016-13/09/2016.
MIC: minimum inhibitory concentration.
CoR: colistin resistant.

colonized by CPKP at admission can be extremely different, ranging from 50% to 6.1% (Di Carlo et al., 2013; Salsano et al., 2016).

Furthermore, probably surgical patients underwent invasive procedures (including urinary catheterization and endoscopy procedures) more frequently with respect to patients admitted to MD and in a healthcare environment different from that of ICD, with a higher risk of person to person transmission.

MD had the highest number of CPKP identified in CS and the lowest in SRS: these data may be related to the different surveillance strategy, including only selected subjects at admission, different from SD and ICD. The ongoing active surveillance protocol is probably cost-effective: a matched case control analysis in 5 teaching hospitals in Italy showed that 2 or more hospitalizations occurring in the previous 12 months are the strongest predictor of CPKP detection (Tumbarello et al., 2014). On the other hand, the implementation of screening activities could identify all asymptomatic carriers. The clinical impact was not limited to the diffusion of CPKP and to the evolution to disease but can impact on comorbidities (Tascini et al., 2015).

The detection of 16 different STs with a predominance of three specific STs suggests the absence of a single reservoir for nosocomial pathogens in our study but, on the other hand, this finding could be related to the long-term survival of CPKP on the inanimate environment and equipment. Environmental cleaning and disinfection of equipment in proximity to a CPKP carrier are important infection control measures (Pantel et al., 2016). This pattern of polyclonal spread with a significant proportion of ST307 (non-ST258 ST) confirmed in a higher number of strains the report of Bonura et al in the six-month period March-August 2014 (Bonura et al., 2015).

We observed several outbreaks during the study period and the source of transmission was not always identified: contact with an index patient for ≥3 days is an independent risk factor for CP Enterobacteriaceae acquisition among contacts (Schwartz-Neiderman et al., 2016). In all cases a late acquisition of CPKP during hospitalization was demonstrated.

A reduction in number of positive CS cases, more evident in MD and in ICD from 2015 to 2016 was reported. This decreasing trend may be related to the ongoing surveillance strategy and is in accord with that reported by Gagliotti et al in a study period prior to ours (from November 2012 to March 2013) and with a different study design (questionnaire-based survey) (Gagliotti et al., 2014). However, it has to be underlined that CPKP diffusion also affects secondary care hospitals and that the local infection trends can be significantly different in a national or even regional setting because of structural characteristics (availability of single rooms) and/or adherence to control measures (Gagliotti et al., 2014; Corcione et al., 2015). Individual hospitals have to decide a local strategy based on the epidemiology in their own patient population: surveillance programs including molecular follow-up should be improved to monitor emerging resistance trends, as well as following the impact of intervention on hospital patients, on outpatients and on "cycling" of patients between institutions in the same region. In our opinion, the low number of CoR strains detected in CS can be considered an important result and a useful parameter to monitor surveillance efficacy.

This study has strengths and limitations. We described the detection of CPKP in SRS and in CS in three different clinical settings with three different surveillance strategies applied at one time in the same hospital. A daily and well-concerted effort is needed to identify and to interrupt transmission of CPKP: the analysis of updated results could help in improving outcomes.

All patients tested were included in the study, although we have no evidence on compliance with the recommendation from the staff. Finally, outbreaks of both CPKP and of other CP-Enterobacteriaceae were described, giving an updated and complete picture of CP diffusion in a tertiary level hospital.

We acknowledge the fact that our study might have suffered from several limitations. The first was the lack of clinical data: however, we focused on evolution of CPKP diffusion trend and on molecular epidemiology. Second, the level of adherence to the culture surveillance screening and the compliance of health personnel with established control measures was not monitored (i.e. medical records of patients admitted to MD were not checked to confirm inclusion or exclusion from survey). Third: we have no information about risk factor data (i.e. renal dialysis, cancer diagnosis, previous hospitalizations) and the results included in the study were updated to September and not December 2016. This is a study of clinical epidemiology and a cost-efficacy evaluation is beyond the scope of this paper.

In our previous study we showed that the active surveillance in the three different departments increased the level of CPKP cases isolated by SRS in the years 2012-2014. We observed a complex scenario in our hospital: the absolute number of CPKP seemed decrease from 2015 to 2016, a lower percentage of CPKP was identified during the survey in SD and the number of strains identified in SRS is comparable to that of strains isolated in CS in MD. It should be underlined that the detection of the majority of the positive samples only after a previous negative survey suggests the need for an implementation of the SRS performed and posed serious ethical and medico-legal problems.

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Conflict of interest

The authors declare no conflict of interest.

Ethical Approval

The study was approved by the Ethical Committee for Clinical Experimentation, Padua Province (Ethics Review 3418/AO/15).

Acknowledgments

Preliminary data from this study were presented as poster presentation at the 26th European Congress of Clinical Microbiology and Infectious Diseases, Amsterdam, Holland (9–12 April 2016).

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