International Journal of Infectious Diseases 59 (2017) 103–109

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

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

Andrea Bartolini[a], Monica Basso[a], Elisa Franchin[a], Nicola Menegotto[a], Anna Ferrari[a], Ettore De Canale[b], Samantha Andreis[a], Renzo Scaggiante[c], Stefania Stefani[d], Giorgio Palù[a], Saverio Giuseppe Parisi[a,*]

[a] Department of Molecular Medicine, University of Padova, Via Gabelli 63, 35100 Padova, Italy
[b] Microbiology and Virology Unit, Padova Hospital, Via Giustiniani, 2, 35121 Padova, Italy
[c] Infectious Diseases Unit, Padova Hospital, Via Giustiniani, 2, 35128 Padova, Italy
[d] Department of Bio-Medical Sciences, University of Catania, Via Androne 81, 95124 Catania, Italy

ARTICLE INFO

Article history:
Received 25 February 2017
Received in revised form 4 April 2017
Accepted 5 April 2017
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
carbapenemases
Klebsiella pneumoniae
colistin
longitudinal survey
multilocus sequence typing

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

* Corresponding author at: Department of Molecular Medicine, University of Padova, Via Gabelli 63, 35100 Padova, Italy. Tel: +00390498272344; Fax: +00390498272355.
E-mail address: saverio.parisi@unipd.it (S.G. Parisi).

http://dx.doi.org/10.1016/j.ijid.2017.04.007

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.