Trends in *Klebsiella pneumoniae* strains isolated from the bloodstream in a teaching hospital in southern Italy

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SUMMARY

Klebsiella pneumoniae is a common nosocomial pathogen involved in many infectious diseases such as bacteraemia, urinary and respiratory tract infections. It is responsible for the rise in morbidity and mortality rates since most clinical isolates exhibit resistance to several antibiotics. Moreover, the epidemiology of these nosocomial infections is variable across countries and regions. From January 2015 to December 2017 we retrospectively analysed the bloodstream infections caused by *K. pneumoniae* strains in hospitalised patients with the aim of studying the temporal trends of wild type (WT), multi-drug resistant (MDR), extended drug resistant (XDR), pan-drug resistant (PDR) and carbapenem-resistant (CR) strains. In all, 439 *K. pneumoniae* isolates from 356 patients were collected from all units of the Policlinico of Bari. The majority of clinical isolates were collected from the intensive care unit (125, 28.47%), haematology (34, 7.74%), rehabilitation (27, 6.15%) and cardiac surgery wards (25, 5.69%). Moreover, the majority of the isolates were classified as CR (325, 74.03%, 95%CI: 69.61-78.19) and XDR (255, 58.09%, 95%CI: 53.31-62.72). Annual prevalence rates and monthly counts were analysed using the Chi Squared test for trends and the Poisson regression with multiple p-value correction according to Benjamini and Hochberg's procedure. The annual relative frequencies of the XDR and CR *K. pneumoniae* isolates decreased significantly from 63.37% to 48.44% and from 78.48% to 63.28% respectively, while WT *K. pneumoniae* significantly increased from 13.95% to 23.44%. Poisson regression analysis confirmed the presence of a decreasing monthly trend for the XDR and CR *K. pneumoniae* count series. In order to control the spread of antibiotic resistance, more inclusive surveillance data will be needed to either confirm these results or improve antibiotic stewardship measures.

Keywords: epidemiology, *K. pneumoniae,* resistance, temporal trend.

INTRODUCTION

Worldwide, multi-drug resistant bacteria (MDR) are responsible for around 700,000 deaths/year leading to a severe economic burden [1]. Gram negative bacteria are associated to mortality rates rising to around 40% or higher. Currently, the increasing prevalence for the carbapenem-resistant Enterobacteriaceae results in a public healthcare problem [2].

In fact, a considerable number of patients are colonized by carbapenem-resistant *K. pneumoniae*. In particular, *K. pneumoniae* colonizes the different mucosal surface, including the oropharynx and the gastrointestinal tract. Subsequently, the bacterium enters into other tissues, causing severe infections [3].

Several mechanisms related to carbapenem resistance have been described in Enterobacteriaceae. The first described mechanism was the modification of membrane permeability associated to the

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production of extended spectrum β-lactamases (ESBLs) or AmpC-type β-lactamases. Other mechanisms are represented by the presence of efflux systems and the production of specific β-lactamases, mostly KPC, VIM, NDM and OXA-48 types which show an important geographic variability [4-6].
The European Antimicrobial Resistance Surveillance Network reported that the European percentage of carbapenem resistant *K. pneumoniae* strains is around 8% with significant geographic variability, from countries with values of less than 5% to countries with very high rates such as Greece (61.9%), Italy (33.5%) and Romania (24.7%) [7].
The misuse of the antimicrobials is one of the factors responsible for the development and spread of the multi-drug resistant strains. Consequently, both antimicrobial stewardship and epidemiological evaluation are strongly encouraged to control the spread of the multi-drug resistant strains in the community and the hospital settings [8]. In particular, the epidemiological reports provide relevant information to support the clinicians in prescribing an effective empirical antibiotic therapy [9].
Nowadays, a standard protocol of the most appropriate treatment for carbapenem-resistant *K. pneumoniae* strains is not still available. Moreover, because of the increase in the minimum inhibitory concentrations (MIC) and the reported polymyxin resistance, the treatment of the carbapenem resistant *K. pneumoniae* has been modified and some data suggest the use of a triple drug combination [10-12].
On the contrary, other studies suggest that a simpler two drug regimen may be appropriate [13]. Moreover, other studies suggest the clinical utility of including carbapenem while other authors emphasize the need for a reduced use of carbapenems in the context of infection control and antibiotic stewardship [14].
The aim of this study was to retrospectively analyze the bloodstream infections caused by *K. pneumoniae* strains in hospitalized patients at the Policlinico of Bari. In particular, according to the classification system of Magiorakos et al., the strains were classified as wild type (WT), multi drug resistant (MDR), extended drug resistant (XDR), pan drug resistant (PDR) and carbapenem-resistant (CR) [15].

PATIENTS AND METHODS

From January 2015 to December 2017, all clinical isolates of *K. pneumoniae* recovered from blood cultures were collected from patients hospitalized in all the departments of the Policlinico of Bari (Italy). In particular, two datasets were analyzed. The first dataset included the first *K. pneumoniae* isolate per patient per ward per month to better evaluate the diffusion into the hospital. However, this selection criterion may introduce a correlation in the data. For this reason, the analysis was also repeated using a second dataset (patients' dataset) selecting only the first isolate per patient.
The blood culture bottles were transferred to the laboratory of Bacteriology, U.O.C Microbiology and Virology, Azienda Universitaria-Ospedaliera, Policlinico of Bari, where they were analyzed.
All procedures performed in this study involved human participants, and therefore were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Sample information (date of sampling, ward, type of specimen, testing results) together with the data of patients for whom testing was performed (*i.e.*, age and sex) were recorded in an anonymous database by transforming sensitive data into alphanumeric codes. No clinical data associated with these specimens were available.

Bacteria classification
Biochemical identification and antibiotic susceptibility were performed using the automated VITEK 2 system (bioMérieux) according to the manufacturer's instructions. The interpretative breakpoints of the MIC values were based on the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The isolates with the intermediate susceptibility values were classified as resistant.
The classification of the clinical isolates was performed by using the classification system of Magiorakos et al. [15]. In particular, they were classified as:

1. MDR (Multi Drug Resistant): non-susceptible to ≥1 agent in ≥3 antimicrobial categories;
2. XDR (Extensively Drug Resistant): non-susceptible to ≥1 agent in all but ≤2 categories;
3. PDR (Pan Drug Resistant): non-susceptible to all antimicrobial agents listed;

4. WT (Wild Type): susceptible to all antimicrobial categories;
5. CR (carbapenem-resistant): resistant to one or more carbapenems (meropenem, imipenem or ertapenem)

Statistical analysis

To evaluate the independence of the annual percentage of each classification category, the Chi squared test or the Fisher test were performed as appropriate. The five p-values were then corrected for multiple comparisons with the Benjamini and Hochberg's (BH) correction with False Discovered Rate (FDR) <1% [16].

With the aim of evaluating the presence of an annual trend, the Chi Squared test for trend was performed on each classification category and the *p-values* were corrected by BH's procedure with FDR<1%.

Moreover, to verify also the presence of a trend on the monthly time series of the total monthly number of each classification category, the presence of autocorrelation was evaluated by correlogram. Next, a Poisson regression was performed with only time as the independent variable. All the *p-values* obtained were corrected by BH's procedure with FDR<1%.

In addition, to verify the assumption of independence of the variables, all analyses were repeated on the patients' dataset. Finally, the correlation between the XDR and CR time series was evaluated by Pearson's coefficient. Calculations of all statistical tests were performed by the open source environment R 3.3.2 [17].

RESULTS

Globally, 439 *K. pneumoniae* isolates from 356 patients (142 females and 214 males, female to male ratio: 0.66) were collected. 176/356 (49.4%) patients were aged between 60 and 79 years.

From 2015 to 2017, the total number of *K. pneumoniae* isolates from the bloodstream decreased from 172 to 128, respectively. The majority of clinical isolates were collected from Intensive Care Unit (ICU) (125/439, 28.47%), haematology (34/439, 7.74%), rehabilitation (27/439, 6.15%) and cardiac surgery wards (25/439, 5.69%).

Three hundred twenty-five out of 439 (74.03%, 95% Confidence Interval [95%CI]: 69.61-78.19) *K. pneumoniae* isolates were CR while 255/439 (58.09%, 95%CI: 53.31-62.72) isolates were XDR. Two hundred forty-eight out of 255 XDR (97.25%) strains were also CR. The other CR isolates were also MDR (45/79, 56.96%) and PDR (32/32, 100%). The majority of the XDR (176/439, 40.09%) and the CR (219/439, 49.89%) isolates were collected from patients aged between 50 to 79, respectively. On the other side,

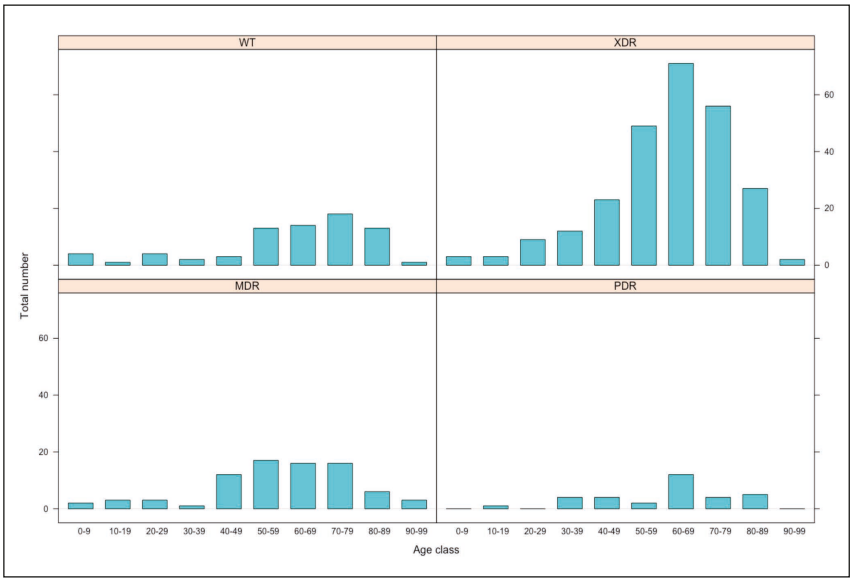


Figure 1 - Total number of clinical isolates of *K. pneumoniae* per resistance category and per age class of the patients.

Table 1 - Evaluation of independence and presence of a trend of the 439 isolates of *K. pneumoniae* from blood.

K. pneumoniae, Isolates					
Year (Total isolates)	*WT*	*MDR*	*XDR*	*PDR*	*CR*
	Number (% per year)	*Number (% per year)*	*Number (% per year)*	*Number (% per year)*	*Number (% per year)*
2015 (172)	24 (13.95%)	25 (14.53%)	109 (63.37%)	14 (8.14%)	135 (78.48%)
2016 (139)	19 (13.67%)	27 (19.42%)	84 (60.43%)	9 (6.47%)	109 (78.42%)
2017 (128)	30 (23.44%)	27 (21.09%)	62 (48.44%)	9 (7.03%)	81 (63.28%)
Total number (%, 95%CI)	73 (16.63, 13.34-20.52)	79 (18.00, 14.58-21.98)	255 (58.09, 53.31-62.72)	32 (7.28, 5.11-10.24)	325 (74.03, 69.61-78.19)
Independence test (*p value*)	$0.048^{Chi, S}$	$0.298^{Chi, NS}$	$0.028^{Chi, S}$	$0.871^{Fi, NS}$	$0.004^{Chi, S}$
Chi squared test for trend (*p value*)	0.038^{S}	0.133^{NS}	0.011^{S}	0.688^{NS}	0.004^{S}

95%CI: 95% Confidence Interval
Chi: Chi Squared test
Fi: Fisher's test
S: Significant after BH's correction
NS: Non-significant after BH's correction

K. pneumoniae was less frequently detected in the other class ages. In particular, younger and older age groups were less involved in *K. pneumoniae* detection (Figures 1 and 2). The majority of the XDR isolates were collected in the ICU (75/255, 29.41%).

From 2015 to 2017 the annual relative frequencies of the XDR and CR *K. pneumoniae* isolates decreased from 63.37% to 48.44% and from 78.48% to 63.28%, respectively (*p*-values significant after BH's correction). On the contrary, WT isolates of *K. pneumoniae* increased from 13.95% to 23.44% (*p*-value significant after BH's correction) (Table 1). Those results were also confirmed in the patients'dataset (data not shown).

The five monthly time series for each resistance category (WT, MDR, XDR, PDR, CR) are plotted in Figure 3. The XDR and CR times series are very similar, and both seem to exhibit a decreasing trend. On the contrary, the MDR and PDR time series do not suggest the presence of a trend. The correlograms of the five monthly time series

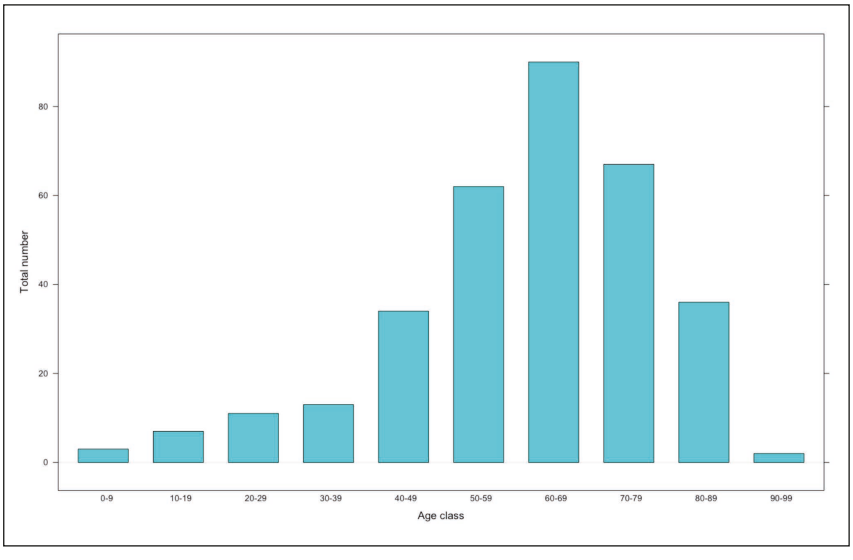


Figure 2 - Total number of clinical isolates of *K. pneumoniae* resistant to carbapenems per age class of the patients.

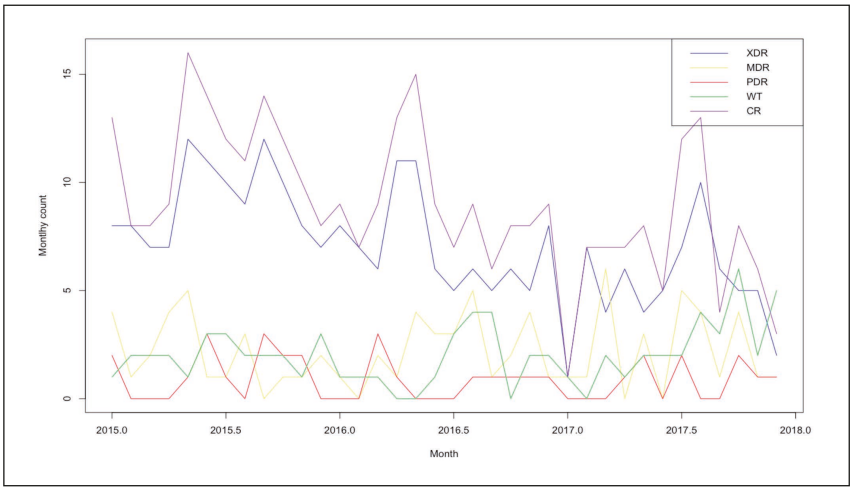


Figure 3 - Monthly time series of the five resistant category of *K. pneumoniae* isolates.

showed the presence of autocorrelation in the XDR and in the CR series (Figure 4).
The five Poisson regressions on the five-time series confirmed the presence of a decreasing monthly trend for the XDR and CR series but failed to detect any monthly trend in the WT series (Table 2).

The fitted lines of the Poisson regressions on the XDR and CR time series are reported in the Figure 5. Despite the presence of autocorrelation in the XDR time series, the correlograms of the residuals of the five Poisson regressions did not detect any autocorrelation (data not shown).

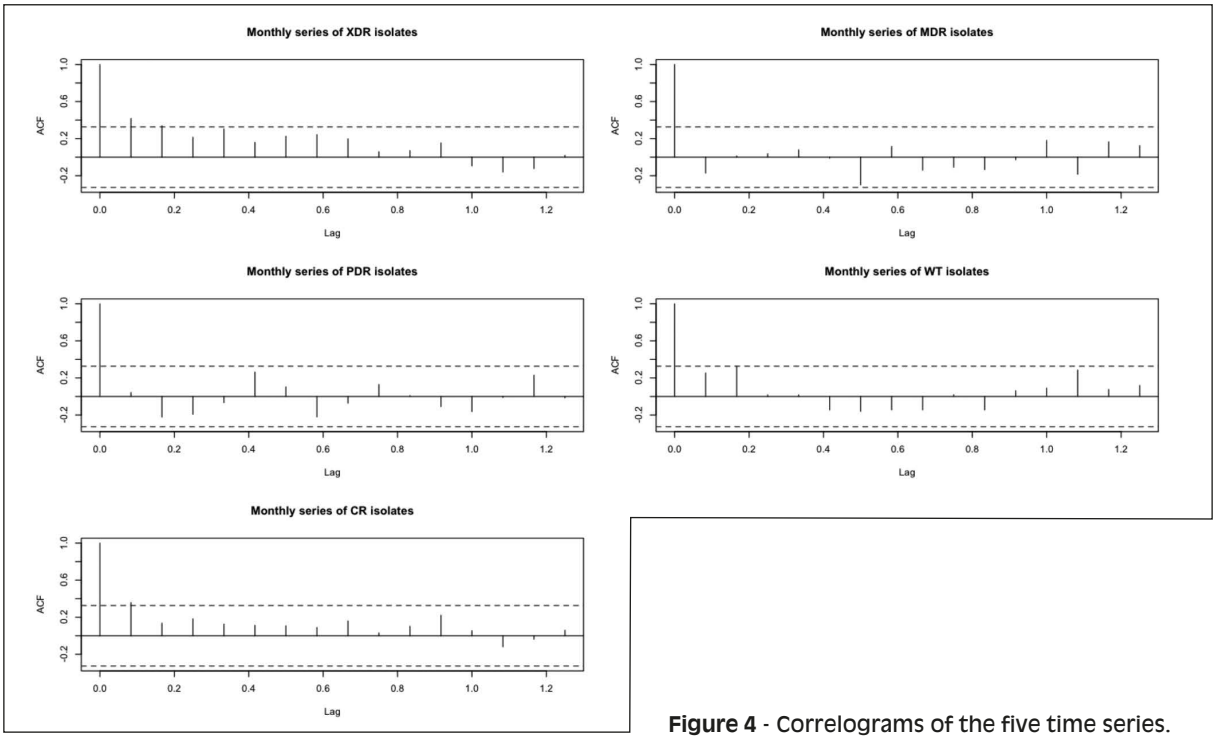


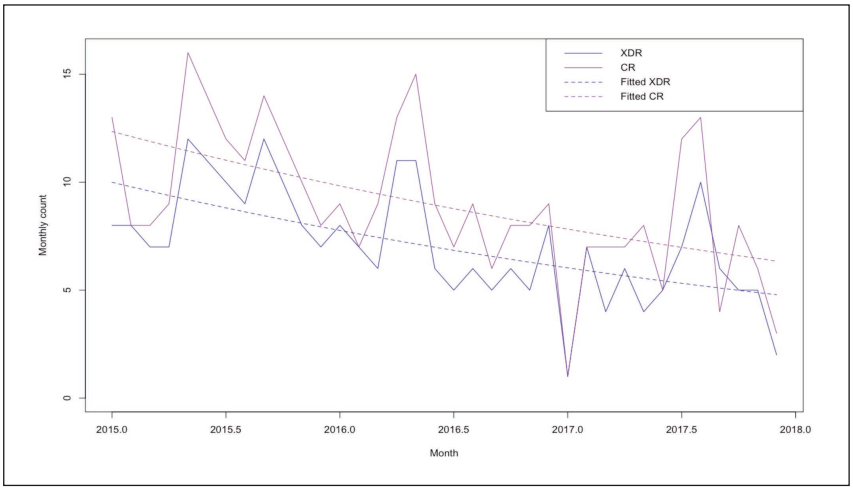
Figure 4 - Correlograms of the five time series.

Table 2 - Evaluation of the presence of a trend by Poisson regression on the five time series.

	Poisson regressions (full dataset)				
	WT	*MDR*	*XDR*	*PDR*	*CR*
Intercept	-484.17	-43.21	511.02	231.56	462.12
p value	0.080^{NS}	0.869^{NS}	<0.001^{S}	0.575^{NS}	<0.001^{S}
Time (month)	0.24	0.02	-0.25	-0.11	-0.23
p value	0.079^{NS}	0.867^{NS}	<0.001^{S}	0.575^{NS}	<0.001^{S}
	Poisson regressions (patients' dataset)				
	WT	*MDR*	*XDR*	*PDR*	*CR*
Intercept	-566.56	59.29	646.30	328.30	593.28.00
p value	0.047^{S}	0.844^{NS}	<0.001^{S}	0.484^{NS}	<0.001^{S}
Time (month)	0.28	0.03	-032	-0.16	-0.29
p value	0.047^{S}	0.842^{NS}	<0.001^{S}	0.483^{NS}	<0.001^{S}

S: Significant after BH's correction
NS: Non-significant after BH's correction

Figure 5 - Fitted lines obtained by the Poisson regressions on the XDR and CR time series.



The XDR and CR time series showed evidence of correlation (Pearson's coefficient=0.90, *p* value<0.001) that was maintained after the detrending of the two series (Pearson's coefficient=0.855, *p* value<0.001).
Finally, the Poisson regressions on the patients'dataset confirmed both the presence of a decreasing trend on the XDR and CR time-series and the increasing trend of the WT time-series.

DISCUSSION

Although the total number of *K. pneumoniae* isolates from blood has decreased in the last few years, the temporal analysis has detected a decrease in both XDR and CR *K. pneumoniae* isolates and an increase in WT *K. pneumoniae* isolates. This finding has been quite interesting since the important burden is carried by *K. pneumoniae* in terms of morbidity and mortality.
In the last few years, *K. pneumoniae* has developed resistance to several antibiotic classes leading to an increase in the life-threatening infections and to a major interest towards the most appropriate treatments [2, 18]. In Europe, more than a third (34.5%) of the *K. pneumoniae* strains were resistant to at least one of the antimicrobials under surveillance. In particular, the resistance rates in *K. pneumoniae* isolates were 25.7%, 24.6%, 19.0% and 6.1% for the third generation cephalosporins, fluoroquinolones, aminoglycosides and carbapenems,

respectively. In Italy, carbapenem-resistant *K. pneumoniae* isolates showed a constant trend, ranging from 34.3% in 2013 to 33.9% in 2016 [19]. On the other hand, an Italian study has reported an increase in carbapenem-resistant *K. pneumoniae* rates [20]. However, despite the decrease in the proportion of XDR and CR *K. pneumoniae*, in our study the carbapenem-resistance rates (48.44% and 63.28%, respectively) are higher when compared to other Italian rates.

Alicino et al. have reported that crude 30-day mortality was higher in patients with carbapenem-resistant *K. pneumoniae* (36.1%) when compared to those with carbapenem-susceptible strains (23.5%) [20]. Also, Xu et al. have confirmed these results [21]. In fact, the patients with carbapenem-resistant *K. pneumoniae* showed a higher mortality compared to carbapenem-susceptible *K. pneumoniae* (pooled crude Odds ratio: 2.80, 95%CI: 2.15-3.65). Moreover, the reported mortality was 54.30% (95% CI 47.51-61.02) in bloodstream infections, 13.52% (95% CI 7.50–20.92) in the urinary tract infections, 48.9% (95% CI 44.47–53.46) in intensive care unit-admission and 43.13% (95% CI 32.40-54.16) in solid organ transplantation patients [21].

K. pneumoniae is generally considered the main representative among the carbapenemase producing strains. Ferranti et al. have reported a high prevalence of CR *K. pneumoniae* strains (87.8%), followed by *Enterobacter cloacae* (5.9%), *Citrobacter freundii* (2.35%) and *Escherichia coli* (1.4%), mainly collected by urine, respiratory tract and blood samples. More generally, carbapenem resistance in Italy has risen to hyper-endemic levels in both *Enterobacteriaceae* and non-fermenting Gram negative bacteria (*Pseudomonas aeruginosa* and *Acinetobacter baumannii*) [22,23].

KPC producing *K. pneumoniae* in Italy accounts for almost 96% of carbapenemase producing *K. pneumoniae* strains [22]. In general, KPC represent the most frequent carbapenemase isolates in Italy. Despite this, some recent data reported the detection of several carbapenem resistant *K. pneumoniae* clinical isolates with OXA-48 and NDM suggesting, therefore, a possible epidemiological change [24].

The high rate of CR strains in our study (63.28% during 2017) is quite worrisome because the majority of the CR isolates are also XDR (76.31%) with the subsequent reduced availability of therapeutic options. The strict association between the XDR and the CR strains was also confirmed by the high Pearson's coefficient value of the XDR and CR detrended time-series (0.855). As a consequence of the increased carbapenem resistance, the data of recent years in Europe have demonstrated an increase in the consumption of polymyxins, mainly colistin, leading to increased resistance to colistin in CR Enterobacteriaceae over time [22].

It is possible that the decrease in the drug resistant *K. pneumoniae* in the past three-years might be due to the intensification of hospital-based infection measures. In fact, these measures are known to reduce the incidence of infections due to MDR gram negative bacteria, including CR *K. pneumoniae* [25, 26]. However, despite the decrease in XDR and CR isolates, the rates of the MDR and PDR isolates remained quite constant over time (18.00% and 7.28%, respectively). In particular, the percentage value of the PDR *K. pneumoniae* strains (7.28%) raises some important questions regarding the proper therapeutic approach (combination therapy, new compounds) [27].

For this reason, an intensification of hospital infection control measures may be considered desirable. However, a study of Gentile et al. raised concerns regarding the knowledge of the basics of an appropriate antibiotic treatment of Italian physicians therefore suggesting the need to improve such knowledge [28].

This study has some important limitations. Firstly, the data are presented as rates, but it was not possible to evaluate either the incidence or mortality rates. Moreover, clinical data and antibiotic consumption data regarding the patients were unavailable. For this reason, it was not possible to correlate the decrease in the XDR and CR *K. pneumoniae* strains to specific clinical conditions and antibiotic-based treatments. The retrospective nature of this study did not allow for an investigation of the compliance to specific infection control measures as well as the efficacy of antibiotic stewardship. More data will be needed in the future to associate such results to specific infection control and antibiotic stewardship measures. Moreover, this is a single centre study and the results cannot be generalized to other settings. Finally, the mechanism of carbapenem-resistance was not confirmed by specific phenotypic or molecular assays, and there is also a lack of information about the clonal type of the *K. pneumoniae* isolates.

Despite these limitations, the data suggest that resistant *K. pneumoniae* isolates (XDR and CR) are decreased. However, a major concern is represented by the fairly constant presence of PDR isolates. In fact, PDR isolates are able to cause fatal nosocomial outbreaks with high mortality rates [29]. Further infection control efforts will be necessary to prevent the spread of these strains.

Funding
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Ethical approval
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.
This study was approved by Ethical Committee of Azienda Ospedaliero-Universitaria "Consorziale Policlinico", Bari (N. 1929). The study followed the local ethical guidelines of the Azienda Ospedaliero Universitaria Policlinico of Bari.

Conflict of interest
The authors declare that they have no conflict of interest to disclose.

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