Polish Journal of Microbiology
2021, Vol. 70, No 1, 107–116
https://doi.org/10.33073/pjm-2021-010

ORIGINAL PAPER

Clonal Dissemination of KPC-2, VIM-1, OXA-48-Producing *Klebsiella pneumoniae* ST147 in Katowice, Poland

DOROTA OCHOŃSKA[1], HANNA KLAMIŃSKA-CEBULA[2], ANNA DOBRUT[1], MAŁGORZATA BULANDA[1] and MONIKA BRZYCHCZY-WŁOCH[1]*

[1] Department of Molecular Medical Microbiology, Chair of Microbiology, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland
[2] Department of Bacteriology, Leszek Giec Upper-Silesian Medical Centre of the Silesian Medical University in Katowice, Katowice, Poland

Submitted 27 October 2020, revised 4 February 2021, accepted 8 February 2021

Abstract

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is an important bacterium of nosocomial infections. In this study, CRKP strains, which were mainly isolated from fecal samples of 14 patients in three wards of the hospital in the Silesia Voivodship, rapidly increased from February to August 2018. Therefore, we conducted microbiological and molecular studies of the CRKP isolates analyzed. Colonized patients had critical underlying diseases and comorbidities; one developed bloodstream infection, and five died (33.3%). Antibiotic susceptibilities were determined by the E-test method. A disc synergy test confirmed carbapenemase production. CTX-Mplex PCR evaluated the presence of resistance genes $bla_{\text{CTX-M-type}}$, $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-9}}$, and the genes bla_{SHV}, bla_{TEM}, $bla_{\text{KPC-2}}$, $bla_{\text{NDM-1}}$, $bla_{\text{OXA-48}}$, bla_{IMP}, and $bla_{\text{VIM-1}}$ was detected with the PCR method. Clonality was evaluated by Multi Locus Sequence Typing (MLST) and Pulsed Field Gel Electrophoresis (PFGE). Six (40%) strains were of XDR (Extensively Drug-Resistant) phenotype, and nine (60%) of the isolates exhibited MDR (Multidrug-Resistant) phenotype. The range of carbapenem minimal inhibitory concentrations (MICs, μg/mL) was as follows doripenem (16 to > 32), ertapenem (> 32), imipenem (4 to > 32), and meropenem (> 32). PCR and sequencing confirmed the $bla_{\text{CTX-M-15}}$, $bla_{\text{KPC-2}}$, $bla_{\text{OXA-48}}$, and $bla_{\text{VIM-1}}$ genes in all strains. The isolates formed one large PFGE cluster (clone A). MLST assigned them to the emerging high-risk clone of ST147 (CC147) pandemic lineage harboring the $bla_{\text{OXA-48}}$ gene. This study showed that the *K. pneumoniae* isolates detected in the multi-profile medical centre in Katowice represented a single strain of the microorganism spreading in the hospital environment.

K e y w o r d s: clonal dissemination, carbapenem-resistant *Klebsiella pneumoniae*, hospital, PFGE, MLST

Introduction

Klebsiella pneumoniae is a critical multidrug-resistant (MDR) bacterium in humans responsible for numerous hospital infections linked to high morbidity and mortality since treatment options are limited (Navon-Venezia et al. 2017). *K. pneumoniae* from the family *Enterobacteriaceae*, occurs in the human and animal gastrointestinal tract microbiome. It is a commonly found opportunistic pathogen associated with the hospital environment and, overall, accountable for approximately a third of all Gram-negative infections. It has a role in extraintestinal infections, such as urinary tract infections, pneumonia, surgical site infections, cystitis, and life-threatening infections, including endocarditis and septicemia. It is also a significant cause of severe community-onset infections, such as necrotizing pneumonia, endogenous endophthalmitis, and pyogenic liver abscesses (Podschun and Ullmann 1998).

With the ever-growing antibiotic resistance, *K. pneumoniae* is a pathogen recognized for its antibiotic resistance; hence, it is categorized as an ESKAPE organism, besides other essential MDR pathogens (Boucher et al. 2009). The accumulation of ARGs by *K. pneumoniae*, by de novo mutations, is continuous under antibiotic selective pressure, and through the acquisition of plasmids and transferable genetic elements, it stimulates extensively drug-resistant (XDR) strains harboring a 'super resistome'. In the past twenty years, many high-risk (HiR) MDR and XDR *K. pneumoniae* sequence

* Corresponding author: M. Brzychczy-Włoch, Department of Molecular Medical Microbiology, Chair of Microbiology, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland; **e-mail: mbrzych@cm-uj.krakow.pl**

types have appeared that exhibit great capacity of causing multicontinental outbreaks and continued global dissemination (Navon-Venezia et al. 2017).

Currently, the spread of carbapenem-resistant *K. pneumoniae* (CRKP) has become a severe problem in the molecular epidemiology of hospital infections.

Frequently, carbapenems serve as the last resort in the effective treatment of serious infections caused by multidrug-resistant bacteria. Enzymes that hydrolyze carbapenems, called carbapenemases, are the major cause of carbapenem resistance (Matsumura et al. 2017). The molecular classes A, B, and D carbapenemases are rapidly disseminated worldwide, challenging the treatment of Gram-negative infections (Nordmann and Poirel 2014). Recent reports have demonstrated that various carbapenem-hydrolyzing enzymes are disseminated worldwide in CRKP isolates. The fast evolution of carbapenem resistance quickly evolved in *Enterobacteriaceae* in the past decade and became a developing global threat. The majority of studies on antibiotic-resistant *K. pneumoniae* focus on characterizing carbapenemase producers (KPC, NDM, VIM, and OXA-48), various clonal groups or complexes (e.g., CG15, CG17, CG258, or CC147), and epidemic plasmids (IncA/C, IncFII, IncL/M, and IncN) that have been suggested to participate in their global expansion (Nordmann and Poirel 2014). Carbapenemase co-producers have been reported in distinct geographic locations: European countries (France, Germany, Greece, Italy, and Poland), Israel, the United States, China, and West Asia (Turkey) (Baraniak et al. 2011; Nordmann and Poirel 2014; Baraniak et al. 2015; Guo et al. 2016; Lee et al. 2016; Zautner et al. 2017; Bukavaz et al. 2018).

The majority of KPC-producing microorganisms also express β-lactamases and possess genes conferring resistance to other antimicrobials, i.e. aminoglycosides, fluoroquinolones, or co-trimoxazole (Nordmann and Poirel 2014). The resistance rates vary significantly across countries; MDR *K. pneumoniae* is endemic in Mediterranean countries, and Eastern and South-Western Europe. It stems from ESβL production in more than 50–60% strains, and non-susceptibility to third-generation cephalosporins, fluoroquinolones, and aminoglycosides (Navon-Venezia et al. 2017).

In 2011, the National Reference Center for Susceptibility Testing (NRCTS) and the KPC-PL Study Group published the first report from Poland that presented the molecular characteristics of *K. pneumoniae* producing KPC carbapenemases (Baraniak et al. 2011). Between 2011 and 2015, the bacteria caused 1,067 infection outbreaks; among them, 123 were caused by *K. pneumoniae*, and a higher number of outbreaks were reported from Masovian and Silesian voivodships (Baraniak et al. 2011). Poland belongs to the countries of the highest rate of *K. pneumoniae* isolates' resistance to all groups of drugs subjected to monitoring, and these rates are twice as high as elsewhere in the European Union (EU)/European Economic Area (EEA). In these countries, resistant *K. pneumoniae* isolates consist on average 20.5% of all MDR multidrug-resistant strains (Bukavaz et al. 2018).

Given the abovementioned data and the increased frequency of isolation of CRKP strains from the hospital environment, we conducted a microbiological and molecular characterization of carbapenem-resistant *K. pneumoniae* isolates with emphasis on the antibiotic resistance profile, identification of ESβL genes, detection of carbapenemase genes, and the isolates' genetic relationship.

Experimental

Materials and Methods

Hospital settings and sample collection. The study was performed in the Upper-Silesian Medical Centre of the Silesian Medical University in Katowice (GCM), one of the largest multi-profile medical centres, and one of the largest hospitals in Poland. The hospital consists of 24 departments and treats over 160 thousand patients per year. Between February and August 2018, 15 non-duplicate CPKP isolates were collected from fecal samples of 14 patients admitted to three hospital wards characterized below. The Department of Neurology with the Stroke Sub-department (NR) receives 1,750 admissions per year and has 14 rooms with 44 beds; the Department of Internal Medicine and Rheumatology (REU) receives 1,935 admissions per year and has 12 rooms with 37 beds, and the Department of Anaesthesia and Intensive Care (OAIT) receives 1,750 admissions per year and has five rooms with ten beds (Table I). A total of 505 *Enterobacteriaceae* isolates were obtained from the patients in these wards over one-year.

Bacterial identification, antimicrobial susceptibility testing and phenotypic screening. Bacterial identification and preliminary susceptibility testing were performed using the automated VITEK® 2 Compact System (bioMérieux, France). The MICs of the 23 antimicrobial agents were evaluated with E-test strips (AB BIODISK, bioMérieux, France). MIC value results were interpreted according to the EUCAST breakpoints (EUCAST 2019). All isolates were screened phenotypically for the presence of KPC-, OXA-48-, and metallo-β-lactamases (MBL). Double-disc synergy tests (DDST) were carried out to confirm ESβL production (CLSI 2018) and to detect MBL production, as published previously (Matsumura et al. 2017). The phenotypic detection of KPC-producing isolates was assessed

Table I
Demographic data and characteristics of the fourteen patients with *K. pneumoniae* co-producing KPC-2, OXA-48, VIM-1 and CTX-M-15 during the outbreak.

Patient ID/ Isolate no.	Age (years) /sex	Hospital ward(s)	Date of isolation	Type of specimen	Status (type) of colonization/ infection	Duration of hospitalization (days)	Underlying conditions	Antimicrobial used prior to isolation of carbapenemase producer(s)	Antimicrobial used as treatment for infections	Outcome Alive/ Dead
3	74/M	OAIT	08/03/2018	Rectal swab	Colonization	03/01–14/04/2018 (102 days)	Abdominal Aortic Aneurysm (AAA)	Ciprofloxacin + Gentamycin + Itraconazole	Metronidazole	Dead
6965	60/F	OAIT	28/02/2018	Rectal swab	Colonization	08/02–01/04/2018 (53 days)	Tuberculosis, Chronic Obstructive Pulmonary Disease (COPD)	Colistin + Voriconazole	–	Dead
6976/1 6976/2	45/M	OAIT	01/03/2018 12/03/2018	Rectal swab Blood	Colonization Bacteremia	21/02–29/03/2018 (37 days)	Guillain–Barré Syndrome (GBS)	Colistin + Linezolid	Ampicillin/sulbactam + Amikacin	Alive
1	67/F	OAIT	07/03/2018	Rectal swab	Colonization	02/03–29/06/2018 (112 days)	COPD, diabetes, hypertension	Ceftriaxone + Levofloxacin	–	Dead
6968 (index case)	63/M	NR	28/02/2018	Rectal swab	Colonization	16/02-31/03/2018 (44 days)	Hypertension, atherosclerosis	Ceftriaxone + Metronidazole	–	Alive
2	69/M	NR	07/03/2018	Rectal swab	Colonization	23/02-28/03/2018 (34 days)	Post-stroke conditions	–	–	Alive
4	43/M	NR	08/ 03/2018	Rectal swab	Colonization	26/02-12/03/2018 (15 days)	Hypertension	Ceftriaxone + Metronidazole	–	Dead
154/25428	61/M	NR	30/07/2018	Rectal swab	Colonization	04/07-06/08/2018 (34 days)	Stroke	–	–	Dead
7/25804	70/M	NR	04/08/2018	Rectal swab	Colonization	25/07-02/08/2018 (9 days)	Stroke	Amoxicillin/ clavulanic acid	–	Alive
11/25808	50/F	NR	04/08/2018	Rectal swab	Colonization	23/07-03/08/2018 (12 days)	Stroke	–	–	Alive
13/25810	77/M	NR	04/08/2018	Rectal swab	Colonization	22/07-09/08/2018 (19 days)	Hypertension, ischemic heart disease	Amoxicillin/ clavulanic acid	–	Alive
6	70/F	REU	09/03/2018	Rectal swab	Colonization	26/02-14/03/2018 (17 days)	Diabetes, metastatic lung cancer	–	–	Alive
7	78/F	REU	09/03/2018	Rectal swab	Colonization	27/02-16/03/2018(18 days)	Rheumatoid Arthritis (RA), hemorrhagic diathesis, coronary disease, peptic ulcer disease, hypertension, atherosclerosis, gout	Imipenem	Vancomycin	Alive
5	90/F	REU	08/03/2018	Rectal swab	Colonization	03.03-17.03.2018 (14 days)	Atherosclerosis, acute arterial thrombosis of the lower extremity	Meropenem + Amikacin	–	Alive

F – Female; M – male; NR – Department of Neurology with the Stroke Subdepartment; OAIT – Department of Anaesthesiology and Intensive Care; REU – Department of Internal Medicine and Rheumatology

by an available protocol (Doi et al. 2008). According to the protocol, the boronic acid combined-disk tests using meropenem (10 μg) as the antibiotic substrate and 3-aminophenylboronic acid (3-APBA) (300 g, Sigma-Aldrich) as the inhibitor of KPC production was used. The detection of OXA-48 was performed in line with the EUCAST guidelines (Shaker et al. 2018). MDR and XDR were defined according to a standardized international document (Magiorakos et al. 2012).

Extended-spectrum β-lactamase genes (ESβLs) identification. Polymerase chain reaction (PCR), as described by Caltagirone et al. (2017) was used to determine bla_{SHV} and bla_{TEM}. CTX-Mplex PCR was used to detect $bla_{CTX\text{-}M\text{-}type}$, $bla_{CTX\text{-}M\text{-}1}$, and $bla_{CTX\text{-}M\text{-}9}$ (Xu et al. 2005).

Carbapenemase genes detection. The existence of carbapenemase encoding genes ($bla_{KPC\text{-}2}$, $bla_{NDM\text{-}1}$, $bla_{OXA\text{-}48}$, bla_{IMP}, and $bla_{VIM\text{-}1}$) was confirmed using the PCR method (Bukavaz et al. 2018).

PFGE. The genetic relatedness of isolates was investigated using the Pulsed Field Gel Electrophoresis (PFGE) method, following genomic DNA extraction and digestion with *Xba*I endonuclease (Fermentas, Lithuania), as described previously (Han et al. 2013). *Salmonella enterica* subsp. *enterica* serovar Braenderup strain H9812 (ATCC® BAA664™) and *K. pneumoniae* ATCC® BAA-1705™ were used as reference markers. PFGE banding patterns were compared using BioNumerics v.6.5 (Applied Maths, Belgium) software. The relatedness was determined by the unweighted pair group method using the average linkages (UPGMA), and the similarity of bands was calculated using the Dice coefficient.

MLST. Multilocus Sequence Typing (MLST) was conducted with the use of seven conserved housekeeping genes (*gap*A, *inf*B, *mdh*, *pgi*, *pho*E, *rpo*B, and *ton*B) (Guo et al. 2016). A complete protocol of the MLST procedure, including the allelic profile and sequence type (ST) assignment techniques, is available in MLST databases.

Results

Patients' characteristics. Patients' characteristics is presented in Table I. During seven months, the 15 *K. pneumoniae* isolates were collected from fourteen patients aged 65.5 years on average. The average duration of hospitalization was 37 days. All patients were found to be colonized by similar CRKP isolates. One patient developed a bloodstream infection (only blood isolate available) and was successfully treated with linezolid and colistin. Two of the improved patients were treated with carbapenem. Five patients died; their deaths were not associated with any particular etiology, though. CRKP strains were isolated at various stages of patients' hospital stay. An average time from patient admission to the microorganism isolation was 16 days; in patient no. 3 the corresponding period was 64 days, while in patients nos. 1 and 5 – the period was only five days. None of the patients had traveled abroad shortly before their hospital admissions, so any travel experience could explain the colonization.

Epidemiologic investigation. The studied *K. pneumoniae* isolates' epidemic curve exhibited a bimodal distribution of cases with two peaks separated by 148 days (Fig. 1). It could indicate double discrete periods (February-March 2018 and July-August 2018) with unobserved cases in January, April, May, and June. The NR ward was involved in these distinct periods. Following the index case, four new cases were registered in the OAIT, six cases in the NR, and three in the REU (Table I).

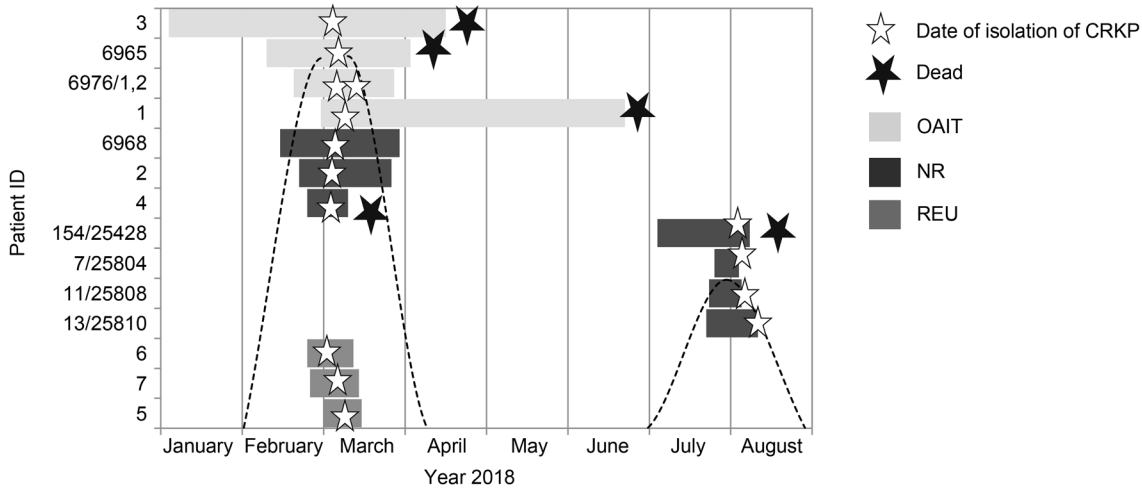

Fig. 1. Gantt diagram depicting a timeline of patients' admissions in relationship with the three wards and the length of stay in each ward. Epidemic curve based on the number of *K. pneumoniae* isolated in the time frame of the study. Abbreviations: OAIT-Department of Anaesthesia and Intensive Care, NR-Department of Neurology with the Stroke Subdepartment, REU-Department of Internal Medicine and Rheumatology.

Antimicrobial resistance. The antimicrobial resistance profiles for the isolates are listed in Table II. We have classified nine (60%) strains as MDR and six (40%) as XDR. All isolates showed resistance to penicillins, cefalosporins, carbapenems (doripenem, ertapenem, meropenem), quinolone (ciprofloxacin), aminoglycosides (amikacin, gentamycin, netilmycin, tobramycin), and other antibiotics (aztreonam, trimethoprim/sulfamethoxazole, fosfomycin). Eleven isolates (73.3%) were sensitive to amikacin, two isolates (13.3%) were in the intermediate range with the MIC values equal to 12 μg/ml, and two isolates (13.3%) were resistant. Moreover, seven strains (42.8%) were resistant to tigecycline, five (35.7%) were in the intermediate range with the MIC values above 2 μg/ml. Interestingly, two isolates (13.3%) showed susceptibility to imipenem, and the resistance of one isolate (6.7%) to imipenem was within the intermediate range.

Phenotypic screening of carbapenemases and associated β-lactamases. In all *K. pneumoniae* isolates (n = 15, 100%), the ESβL mechanism was not found in phenotypic tests. Also, MBL production was not phenotypically detected in these isolates. However, all strains were positive for *K. pneumoniae* carbapenemase (KPC) production by the modified Hodge test (n = 15, 100%). In addition, phenotyping detection of carbapenem-resistant class D using the carbapenemase detection set with temocillin disc (30 μg) revealed that all isolates were positive for OXA-48 (n = 15, 100%).

β-lactamase genes detection. PCR amplification and sequencing analysis confirmed the presence of bla_{KPC-2}, bla_{OXA-48}, bla_{VIM-1} in all 15 of the *K. pneumoniae* isolates. In addition, the only identified bla_{CTX-M} variant in all 15 strains was $bla_{CTX-M-15}$. The results are presented in Fig. 2. No isolate was found to be positive for bla_{SHV}, bla_{TEM}, bla_{NDM-1}, and bla_{IMP}.

Molecular epidemiology. Out of fifteen isolates concerned, all belonged to a single PFGE cluster (clone A) (Fig. 2).

Moreover, an MLST analysis allowed us to classify all isolates as the sequence type ST147 (allelic profile: 3-4-6-1-7-4-38) belonging to the CC147 clonal complex. It confirmed clonal relationships between the isolates.

Discussion

The presented work stems from the problem of clonal dissemination of KPC-2, VIM-1, and OXA-48-producing *K. pneumoniae* ST147 noticed in one hospital in Katowice, the Silesian Voivodeship, Poland.

K. pneumoniae belongs to Gram-negative bacteria of the family *Enterobacteriaceae*. The pathogens can easily cause hazardous epidemic outbreaks and spread globally in the form of clones with increased virulence and epidemicity. These features are associated with the widespread presence of *Enterobacteriaceae* in humans (gastrointestinal carriage) and their great importance as frequent infection etiologic agent in facilities that carry out inpatient and outpatient treatment (Navon-Venezia et al. 2017). Studies that focus on *K. pneumoniae* are therefore desired, as they provide vital scientific

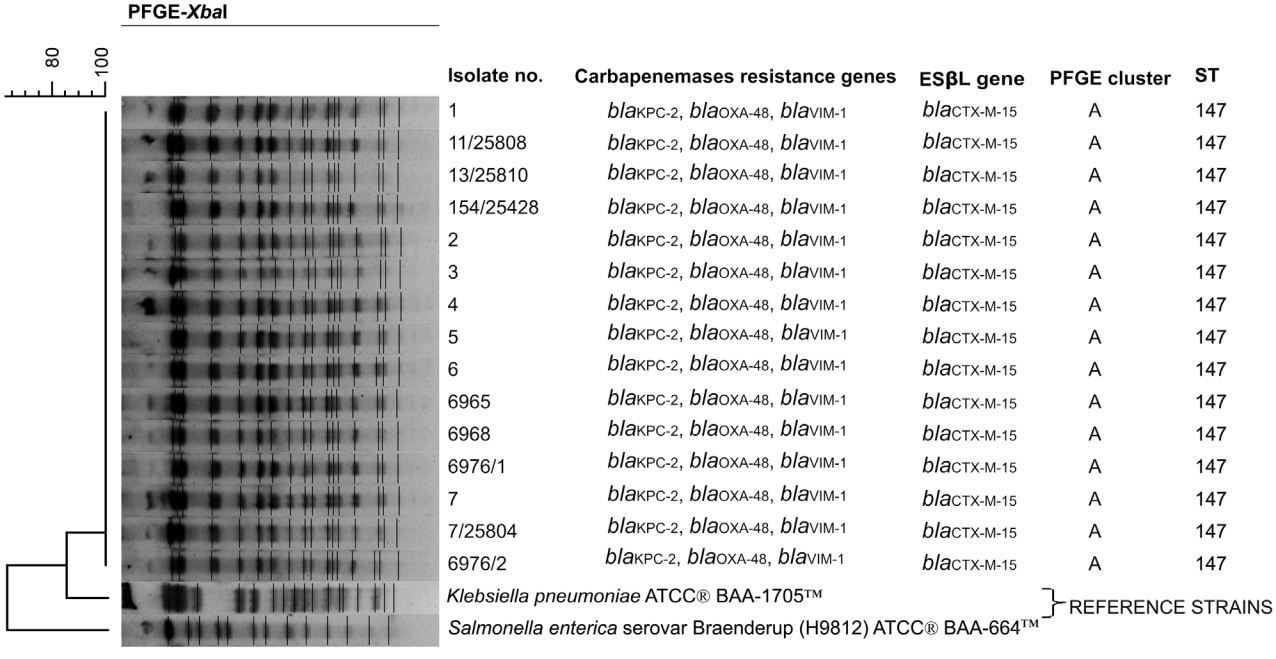

Fig. 2. Dendrogram of *Xba*I-digested genomic DNA from carbapenemase-co-producing *K. pneumoniae* isolates, additionally presenting the results of PCRs for genes encoding carbapenemases and other β-lactamase. PFGE settings: similarity coefficient, Dice; optimization, 1%; tolerance, 1%; clustering method, UPGMA.

Table II
Antimicrobial resistance profiles of the studied *K. pneumoniae* isolates.

Patient ID/Isolate no.		3		6965		6976/1		6976/2		1		6968		2		4		154/25428		7/25804		11/25808		13/25810		6		7		5	
Antibiotics:		MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S	MIC (µg/ml)	R/I/S
Penicillins	Amoxicillin/ Clavulanic acid	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R
	Ampicillin	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R
Cephalosporins	Cefaclor	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R
	Cefuroxime	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R
	Cefotaxime	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R
	Cefotaxime/ Cefotaxime + clavulanic acid	>16/>1	R	>16/>1	R	>16/>1	R	>16/>1	R	>16/>1	R	>16/>1	R	>16/>1	R	>16/>1	R	>16/>1	R	>16/>1	R	>16/>1	R	>16/>1	R	>16/>1	R	>16/>1	R	>16/>1	R
	Ceftazidime	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R
	Ceftazidime/ Ceftazidime + clavulanic acid	>34/>4	R	>34/>4	R	>34/>4	R	>34/>4	R	>34/>4	R	>34/>4	R	34/>4	R	>34/>4	R	>34/>4	R	>34/>4	R	>34/>4	R	>34/>4	R	>34/>4	R	>34/>4	R	>34/>4	R
	Cefepime	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R
Carbapenems	Doripenem	>32	R	16	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R
	Ertapenem	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R
	Imipenem	>32	R	>32	R	16	R	16	R	>32	R	>32	R	>32	R	>32	R	>12	R	4	S	4	S	8	I	>32	R	>32	R	>32	R
	Meropenem	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R
Quinolone	Ciprofloxacin	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R
Aminoglycosides	Amikacin	4	S	8	S	32	R	32	R	8	S	12	R	6	S	4	S	8	S	8	S	8	S	8	S	12	R	6	S	6	S
	Gentamycin	64	R	64	R	64	R	64	R	64	R	64	R	64	R	64	R	64	R	64	R	64	R	64	R	64	R	64	R	64	R
	Netylmycin	128	R	4	I	64	R	64	R	128	R	32	R	16	R	16	R	12	R	16	R	16	R	16	R	32	R	16	R	16	R
	Tobramycin	64	R	64	R	32	R	32	R	64	R	64	R	64	R	64	R	32	R	64	R	64	R	64	R	64	R	64	R	64	R
Other	Aztreonam	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R
	Colistin	0.50	S	0.75	S	0.125	S	0.125	S	0.50	S	0.50	S	0.50	S	0.50	S	0.50	S	0.50	S	0.75	S	0.50	S	0.50	S	0.50	S	0.50	S
	Tetracycline	32	R	128	R	8	R	8	R	32	R	32	R	32	R	8	R	>256	R	>256	R	>256	R	>256	R	8	R	8	R	128	R
	Tigecycline	2	R	8	R	4	R	4	R	2	R	2	R	2	R	4	R	0.75	R	0.75	R	1.0	R	2	R	4	R	4	R	8	R
	Trimethoprim/ Sulfamethoxazole	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R	>32	R
	Fosfomycin	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	>256	R	R>256	R	>256	R	>256	R	>256	R	>256	R

MIC – Minimum Inhibitory Concentration; R – Resistant; I – Susceptible, increased exposure; S – Susceptible

grounds for the relevant action to be taken in the field of public health in respect of molecular epidemiology.

This study's subject was a particular group of microorganisms classified as bacterial alarm agents (BCA) of particular virulence or resistance, i.e., carbapenemase-producing *K. pneumoniae* strains belonging to the order *Enterobacterales*. All *K. pneumoniae* isolates were derived from intestinal colonization cases in the patients studied. This work elaborated epidemiological opinions on the BCA epidemic threat occurrence in the multiprofile medical center in Katowice. It was also based on suspicion of an epidemic outbreak since we noticed clonal dissemination of several CRKP isolates and one case of symptomatic hospital infection with a blood CRKP strain. This strain was isolated in a given department 48 hours after the patient's admission. The colonization of fourteen consecutive hospitalized patients with the same strain of the CRKP was also reported. CRKP isolates had unique properties such as the accumulation of numerous antibiotic resistance mechanisms and the capability to persist in a hospital environment. It resulted in special attention being paid to CRKP strains' health care risk (Navon-Venezia et al. 2017). The genes encoding KPC, MBL, and OXA-48 are located on the so-called mobile genetic elements (plasmids, transposons) that allow effective spreading within bacterial populations. The genes encoding KPC carbapenemases (bla_{KPC}) are located on the Tn4401 transposon located on plasmids with different types of replicons (IncF, IncL/M, ColE1, IncR, and IncX3). These plasmids show the ability to conjugate and propagate the bla_{KPC} genes to new bacterial populations (Baraniak et al. 2011).

On the other hand, the most important families of the MBL acquired are the enzymes IMP and VIM occurring both in non-fermenting and intestinal bacilli. The bla_{IMP} and bla_{VIM} genes always exist as cassettes inserted into integrons. In turn, integrons can be located on transposons and move with them between DNA molecules (Izdebski et al. 2018b). ESβL enzymes exist mainly as acquired, plasmid-encoded β-lactamases. ESβL-encoding genes are often located on conjugation plasmids (IncFII, IncI), including those with a broad host range (IncA/C, IncL/M). It allows them to spread rapidly, also between strains belonging to different species. Frequently noted are encoded by bla_{CTX-M} genes ESβL enzymes, active against cefotaxime CTX-M and localized on plasmids in *Enterobacterales* (Izdebski et al. 2018a; 2018b).

The isolates' antibiotic susceptibility testing confirmed the β-lactamase antibiotics resistance profile typical for carbapenemase producers, as presented elsewhere (Zacharczuk et al. 2011; Guo et al. 2016; Izdebski et al. 2018a). All strains expressed high-level resistance to doripenem, ertapenem, meropenem, although they showed varying levels of resistance to imipenem. Ertapenem is the carbapenem that has been suggested as most suitable for detecting the presence of bla_{KPC}, which was confirmed by high MIC values for ertapenem (> 32 μg/ml) in the present study. The presence of KPC does not always result in expression of *in vitro* resistance to imipenem or meropenem, as this carbapenemase shows limited efficiency of β-lactam ring hydrolysis in carbapenem particles. In this study, E-test showed resistance to ertapenem of 7/25804, 11/25808, 13/25810 strains, and susceptibility or medium susceptibility to imipenem (MICs 4–8 μg/ml), while PCR confirmed the presence of the bla_{KPC-2} gene in all three isolates. Imipenem and meropenem resistance of other strains might have resulted from decreased outer membrane permeability or other β-lactamases providing a synergistic effect with KPC (Protonotariou et al. 2018). The strains' resistance to third-generation cephalosporins confirmed this hypothesis similarly to the various β-lactamases production by *K. pneumoniae*. The variability in CRKP strains' carbapenem susceptibility illustrates the difficulties encountered while trying to identify strains producing carbapenemases only with the E-testing of resistance profiles. To reliably identify such strains, additional and more specific techniques should be applied (Xu et al. 2005; Magiorakos et al. 2012; Han et al. 2013; Caltagirone et al. 2017; Bukavaz et al. 2018).

The bla_{KPC-2}, bla_{OXA-48}, and bla_{VIM-1} carbapenemase encoding genes and an additional $bla_{CTX-M-15}$ cefotaximase encoding genes have been found to coexist in all the strains concerned. Lee et al. (2016) found that *K. pneumoniae* can carry multiple β-lactamase genes in the same strain, which could be partly responsible for the pathogen's selective success. All types of the *bla* genes were reported in combinations for this species (Lee et al. 2016). So far, *K. pneumoniae* that produce KPC-2 and KPC-3 carbapenemases have been frequently found in Europe, including Poland (Baraniak et al. 2011; Zacharczuk et al. 2011; Baraniak et al. 2015; Grundmann et al. 2017). On the other hand, Matsumura et al. (2017) presented the global dissemination of *Enterobacteriaceae* strains carrying the bla_{VIM} genes. The VIM-producing *Enterobacteriaceae* are generally present in Europe, particularly in Greece, Spain, Hungary, and Italy (Matsumura et al. 2017). Significantly, an increasing problem with VIM-producing *K. pneumoniae* has been recorded in Greece, where the strains have accounted for the deaths of 48% of patients over the last two years (Protonotariou et al. 2018). In Poland, Izdebski et al. (2018b), collected one hundred and nineteen cases of VIM/IMP-positive *Enterobacteriaceae* in the period from 2006 to 2012, and showed many specific or entirely new features of these microorganisms, undoubtedly related to the properties of pathogens isolated in the abovementioned central-southern countries Europe, including several likely imported from abroad (e.g., from Greece) (Izdebski et al. 2018b).

The strains containing OXA-48 β-lactamases provide another example of rapid immigration of harmful microorganisms to Europe from their endemic regions, mostly eastern and southern Mediterranean countries (Egypt, Morocco, and Turkey). OXA-48-like-positive strains cause hospital outbreaks of epidemics in Belgium, France, Netherlands, Germany, Spain, and other countries (Nordmann and Poirel 2014; Grundmann et al. 2017). In the most recent report developed by OXA-48-PL Study Group monitoring the *Enterobacteriaceae* OXA-48-positive strains' spread, the authors demonstrate that these strains have been isolated relatively rarely in Poland (Izdebski et al. 2018a). It was confirmed by other European and international research teams (Nordmann and Poirel 2014; Grundmann et al. 2017). Conjugative transfer of a specific plasmid group is regarded to be the central mechanism in the spread of OXA-48 among the *Enterobacteriaceae* populations (Nordmann and Poirel 2014).

The high MIC values (> 256 μg/ml) for cefotaxime may suggest that the strains produced CTX-M β-lactamases. Multiplex PCR and sequencing have confirmed $bla_{\text{CTX-M-15}}$ presence in all isolates. The presence of various CTX-M β-lactamases in *K. pneumoniae* in Poland has been confirmed by other research teams (Baraniak et al. 2011; Izdebski et al. 2018a; 2018b). No bla_{SHV} or bla_{TEM} have been identified among the isolates. These results are in line with the persisting trend of diminished prevalence of hospital-associated *K. pneumoniae* strains producing SHV or TEM β-lactamases, which has also been noted by other scientists (Rodrigues et al. 2014). The type of ESβLs present in *K. pneumoniae* shifted in the 2000s, which caused outbreaks in hospitals due to the acquisition of plasmids and transposons encoding $bla_{\text{CTX-M-type}}$ ESβLs, consequently resulting in the predominance of CTX-M-producing strains (Calbo and Garau 2015).

Among non-β-lactam antibiotics, colistin has shown the highest activity towards the strains concerned. Similar results have been published by other authors (Zacharczuk et al. 2011). Among aminoglycosides, amikacin showed the highest activity. The vast majority of strains (92.8%) were susceptible to amikacin, unlike in China, where total *K. pneumoniae* resistance to amikacin was demonstrated (Guo et al. 2016). The other aminoglycosides (gentamicin, netilmicin, and tobramycin) were nonreactive to the CRKP isolates concerned, in line with data reported by other authors (Zacharczuk et al. 2011; Guo et al. 2016). On the other hand, Bukavaz et al. (2018) presented other aminoglycoside susceptibility profiles in CRKP strains. These discrepancies are most likely due to different quantities or origins of the strains concerned, though they might as well be related to particular hospitals' or ward's antibiotic policies.

The present analysis has shown a relatively high percentage of isolates resistant to tigecycline (42.8%), a synthetic minocycline analogue with a broad spectrum of activity. The antibiotic is successfully applied to treat infections caused by multi-resistant bacterial strains, including *K. pneumoniae.* During tigecycline treatment, *Enterobacteriaceae* representatives, including CRKP strains, may become resistant to the antibiotic, as evidenced by Pfarrell et al. (2018). They demonstrated cross-resistance between tigecycline and minocycline in resistant *Enterobacteriaceae* strains due to the MDR pumps, which remove drugs from cells. The distribution of acquired tigecycline resistance can vary depending on the geographic location or time; therefore, it is recommended to concern data on local resistance, particularly while treating severe infections (Pfarrell et al. 2018).

Genetic similarity of CRKP isolates was determined by comparing PFGE profiles (Han et al. 2013). This method has been widely used by European and non-European research teams that study epidemic outbreaks of *K. pneumoniae* and evidence global spread of this species' epidemic clones (Guo et al. 2016; Bukavaz et al. 2018; Protonotariou et al. 2018). All *K. pneumoniae* strains analyzed in this study belonged to ST147 (CC147). Navon-Venezia et al. (2017) reported that ST147 *K. pneumoniae* clone spread globally. The involvement of this pandemic clone in the spread of CTX-M-15 and other carbapenemases in European, Asian, and Middle East countries was confirmed by others (Rodrigues et al. 2014; Guo et al. 2016; Zautner et al. 2017). The data found in the literature on *K. pneumoniae* clonal organization has been confirmed by research focused on the molecular epidemiology of infections due to antibiotic-resistant *Enterobacteriaceae* (Guo et al. 2016; Protonotariou et al. 2018).

Because of the clonal nature of the isolates studied, the clinical data about their origin was reviewed to examine whether the same clonal type was circulating in the three various hospital wards. The study focused mainly on stroke patients or patients suffering from cardiovascular complications who were transferred to multiple-bed hospital rooms in Stroke Recovery Units, which were the only places where they were in touch. Moreover, it is plausible that the neurology ward played a major role in disseminating the bacteria throughout the hospital. The spread of the pathogen isolates across three different hospital wards could imply that the personnel who can access all hospital wards freely, unlike the patients and their relatives, might have transmitted the infections. Scientific literature emphasizes the role of hospitalization (and its duration) in the process of bacterial colonization. The frequency of gastrointestinal colonization with *K. pneumoniae* strains in hospitalized patients was high in our study. Gastrointestinal

colonization with CRKP is an important risk factor for infections with strains of the same phenotype due to the easy transfer of the genes and strains' persistence in hospital environments, which hinders their eradication. This research evidences *K. pneumoniae* adaptation typical of endemic hospital strains, which are corroborated by the accumulation of locally spreading resistance mechanisms to the antibiotics most commonly used for therapeutic purposes.

ORCID
Monika Brzychczy-Włoch https://orcid.org/0000-0002-7415-0154

Ethics approval

The study was approved by the Bioethics Committee of the Jagiellonian University in Krakow, Poland (KBET/1072.6120.264.2019).

Authors' contributions

DO collected the data, performed the molecular analysis and drafted the manuscript; HK-C collected the isolates with clinical data, performed the hospital laboratory analysis and coordinated the microbiological analysis; MB consulted the cases and edited the manuscript; MBW supervised the research and analysis, coordinated and edited the manuscript.

Funding

The present work was financially supported by Jagiellonian University Medical College in Krakow (Poland), Grant No: K/ZDS/007829.

Acknowledgments

The authors are thankful to Karolina Winiewicz and Renata Wrona-Moczurad, the employees of the Department of Bacteriology of the GCM for their cooperation and assistance in conducting the study.

Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Baraniak A, Grabowska A, Izdebski R, Fiett J, Herda M, Bojarska K, Żabicka D, Kania-Pudło M, Młynarczyk G, Żak-Puławska Z, et al.; KPC-PL Study Group. Molecular characteristics of KPC-producing *Enterobacteriaceae* at the early stage of their dissemination in Poland, 2008–2009. Antimicrob Agents Chemother. 2011 Dec;55(12):5493–5499 https://doi.org/10.1128/AAC.05118-11

Baraniak A, Izdebski R, Fiett J, Herda M, Derde LP, Bonten MJ, Adler A, Carmeli Y, Goossens H, Hryniewicz W, et al.; MOSAR WP2, WP3, and WP5 Study Groups. KPC-Like Carbapenemase-Producing *Enterobacteriaceae* Colonizing Patients in Europe and Israel. Antimicrob Agents Chemother. 2015 Dec 28;60(3):1912–1917. https://doi.org/10.1128/AAC.02756-15

Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009 Jan 1;48(1):1–12. https://doi.org/10.1086/595011

Bukavaz S, Budak M, and Celik AD. bla_{KPC-2} and bla_{OXA-48} producing *Klebsiella pneumoniae* found in a Turkish hospital in the Balkans. Afr J Microbiol Res. 2018;12(16):370–379. https://doi.org/10.5897/AJMR2018.8841

Calbo E, Garau J. The changing epidemiology of hospital outbreaks due to ESBL-producing *Klebsiella pneumoniae*: the CTX-M-15 type consolidation. Future Microbiol. 2015;10(6):1063–1075. https://doi.org/10.2217/fmb.15.22

Caltagirone M, Nucleo E, Spalla M, Zara F, Novazzi F, Marchetti VM, Piazza A, Bitar I, De Cicco M, Paolucci S, et al. Occurrence of extended spectrum β-lactamases, KPC-type, and MCR-1.2-producing *Enterobacteriaceae* from wells, river water, and wastewater treatment plants in Oltrepò Pavese area, Northern Italy. Front Microbiol. 2017 Nov 10;8:2232. https://doi.org/10.3389/fmicb.2017.02232

CLSI. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100: 282. Wayne (USA): Clinical and Laboratory Standards Institute; 2018.

Doi Y, Potoski BA, Adams-Haduch JM, Sidjabat HE, Pasculle AW, Paterson DL. Simple disk-based method for detection of *Klebsiella pneumoniae* carbapenemase-type β-lactamase by use of a boronic acid compound. J Clin Microbiol. 2008 Dec;46(12):4083–4086. https://doi.org/10.1128/JCM.01408-08

EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 9.0. Basel (Switzerland): The European Committee on Antimicrobial Susceptibility Testing; 2019.

Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasević AT, Cantón R, Carmeli Y, Friedrich AW, Giske et al.; European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. Lancet Infect Dis. 2017 Feb;17(2):153–163. https://doi.org/10.1016/S1473-3099(16)30257-2

Guo L, An J, Ma Y, Ye L, Luo Y, Tao C, Yang J. Nosocomial outbreak of OXA-48-producing *Klebsiella pneumoniae* in a Chinese hospital: clonal transmission of ST147 and ST383. PLoS One. 2016 Aug 4; 11(8):e0160754. https://doi.org/10.1371/journal.pone.0160754

Han H, Zhou H, Li H, Gao Y, Lu Z, Hu K, Xu B. Optimization of pulse-field gel electrophoresis for subtyping of *Klebsiella pneumoniae*. Int J Environ Res Public Health. 2013 Jul 1;10(7):2720–2731. https://doi.org/10.3390/ijerph10072720

Izdebski R, Baraniak A, Zabicka D, Machulska M, Urbanowicz P, Fiett J, Literacka E, Bojarska K, Kozinska A, Zieniuk B, et al.; OXA-48-PL Study Group. Enterobacteriaceae producing OXA-48-like carbapenemases in Poland, 2013-January 2017. J Antimicrob Chemother. 2018a Mar 1;73(3): 620–625. https://doi.org/10.1093/jac/dkx457

Izdebski R, Baraniak A, Zabicka D, Sekowska A, Gospodarek-Komkowska E, Hryniewicz W, Gniadkowski M. VIM/IMP carbapenemase-producing Enterobacteriaceae in Poland: epidemic *Enterobacter hormaechei* and *Klebsiella oxytoca* lineages. J Antimicrob Chemother. 2018b Oct 1;73(10):2675–2681. https://doi.org/10.1093/jac/dky257

Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbapenemase-producing *Klebsiella pneumoniae*: epidemiology, genetic context, treatment options, and detection methods. Front Microbiol. 2016 Jun 13;7:895. Available from: https://doi.org/10.3389/fmicb.2016.00895

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for

interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012 Mar;18(3):268–281.
https://doi.org/10.1111/j.1469-0691.2011.03570.x

Matsumura Y, Peirano G, Devinney R, Bradford PA, Motyl MR, Adams MD, Chen L, Kreiswirth B, Pitout JDD. Genomic epidemiology of global VIM-producing Enterobacteriaceae. J Antimicrob Chemother. 2017 Aug 1;72(8):2249–2258.
https://doi.org/10.1093/jac/dkx148

Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol Rev. 2017 May 1;41(3):252–275.
https://doi.org/10.1093/femsre/fux013

Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among *Enterobacteriaceae* worldwide. Clin Microbiol Infect. 2014 Sep;20(9):821–830.
https://doi.org/10.1111/1469-0691.12719

Pfaller MA, Huband MD, Streit JM, Flamm RK, Sader HS. Surveillance of tigecycline activity tested against clinical isolates from a global (North America, Europe, Latin America, and Asia-Pacific). Int J Antimicrob Agents. 2018 Jun;51(6):848–853.
https://doi.org/10.1016/j.ijantimicag.2018.01.006

Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev. 1998 Oct;11(4):589–603.
https://doi.org/10.1128/CMR.11.4.589

Protonotariou E, Poulou A, Politi L, Sgouropoulos I, Metallidis S, Kachrimanidou M, Pournaras S, Tsakris A, Skoura L. Hospital outbreak due to a *Klebsiella pneumoniae* ST147 clonal strain coproducing KPC-2 and VIM-1 carbapenemases in a tertiary teaching hospital in Northern Greece. Int J Antimicrob Agents. 2018 Sep;52(3):331–337. https://doi.org/10.1016/j.ijantimicag.2018.04.004

Rodrigues C, Machado E, Ramos H, Peixe L, Novais Â. Expansion of ESBL-producing *Klebsiella pneumoniae* in hospitalized patients: a successful story of international clones (ST15, ST147, ST336) and epidemic plasmids (IncR, $IncFII_K$). Int J Med Microbiol. 2014 Nov;304(8):1100–1108.
https://doi.org/10.1016/j.ijmm.2014.08.003

Shaker OA, Gomaa HE, ElMasry SA, Halim RMA, Abdelrahman AH, Kamal JS. Evaluation of combined use of temocillin disk and mastdisks inhibitor combination set against polymerase chain reaction for detection of carbapenem-resistant *Enterobacteriaceae*. Open Access Maced J Med Sci. 2018 Feb 1;6(2):242–247.
https://doi.org/10.3889/oamjms.2018.090

Xu L, Ensor V, Gossain S, Nye K, Hawkey P. Rapid and simple detection of bla_{CTX-M} genes by multiplex PCR assay. J Med Microbiol. 2005 Dec;54(Pt 12):1183–1187.
https://doi.org/10.1099/jmm.0.46160-0

Zacharczuk K, Piekarska K, Szych J, Zawidzka E, Sulikowska A, Wardak S, Jagielski M, Gierczynski R. Emergence of *Klebsiella pneumoniae* coproducing KPC-2 and 16S rRNA methylase ArmA in Poland. Antimicrob Agents Chemother. 2011 Jan;55(1):443–446.
https://doi.org/10.1128/AAC.00386-10

Zautner AE, Bunk B, Pfeifer Y, Spröer C, Reichard U, Eiffert H, Scheithauer S, Groß U, Overmann J, Bohne W. Monitoring microevolution of OXA-48-producing *Klebsiella pneumoniae* ST147 in a hospital setting by SMRT sequencing. J Antimicrob Chemother. 2017 Oct 1;72(10):2737–2744.
https://doi.org/10.1093/jac/dkx216