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Original Article

Dissemination of ST101 *bla*$_{OXA-48}$ producing *Klebsiella pneumoniae* at tertiary care setting

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Abstract

Introduction: The worldwide dissemination of the acquired carbapenemases in Gram-negative bacteria is a strongly expressed demand for the emergence of post antibiotic era. The aim of this study was to test the production of carbapenemase by *Klebsiella pneumoniae* strains isolated from hospitalized cancer patients and to investigate the genetic relationship of carbapenemase producing carbapenem resistant *K. pneumoniae* using multilocus sequence typing (MLST).

Methodology: Antibiotic susceptibility testing and phenotypic testing for extended spectrum β-lactamases (ESBL) and carbapenemases production were performed. PCR amplification of ESBL and carbapenemase genes was performed. MLST was done to detect the genetic relatedness of the isolates.

Results: Our data showed all strains were sensitive to colistin. Carba NP test was positive in thirty-one carbapenem resistant *K. pneumoniae* isolates and 26 out of 34 *K. pneumoniae* isolates were metallo-beta-lactamases (MBL) positive. All carbapenemase-positive isolates were ESBL CTX-M-1-like positive. *bla*$_{OXA-48}$ gene was detected in 25 isolates (80.65%) and 21 isolates (67.75%) produced *bla*$_{NDM-1}$ like enzyme. VIM and KPC genes were not identified in this study. Association of *bla*$_{OXA-48}$ like and *bla*$_{NDM-1}$ like was found in 15 (48.39%) isolates, while the coproduction of OXA-48-like and IMP-1 was revealed in only one *K. pneumoniae* isolate. MLST revealed ten distinct sequence types (STs).

Conclusion: Here we have documented the coexistence of NDM-type and OXA-48-like, and the coproduction of OXA-48-like and IMP in carbapenem resistant *K. pneumoniae* in patients with cancer. The dominant clone of the OXA-48-like-producing *K. pneumoniae* isolates from Egypt was ST101 epidemic clone belonging to clonal complex 101, an association that has been reported worldwide. The second most frequent ST was ST383.ST11 was assigned to OXA-48-producing *K. pneumoniae*.

Key words: *K. pneumoniae; bla*$_{OXA48}$; *bla*$_{NDM-1;}$ ST101; ST383; Egypt.

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Introduction

Klebsiella pneumoniae is a significant bacterial pathogen associated with hospital-acquired infections and capable of causing severe morbidity and mortality, especially in immunocompromised patients [1], and the occurrence of carbapenemase expands mortality rates [2]. Acquired carbapenemases are encoded by transferable genes located in mobile elements such as plasmids and transposons, which may disseminate between various isolates, carbapenemases mediated resistance represents the major threat in carbapenem resistant *K. pneumoniae* [3]. The existence of either class B (IMP, VIM, NDM) or classes A (KPC) and class D (OXA-48) carbapenemases have been reported world-wide [3,4].

In the Middle East Northern African countries, such as Morocco, Algeria, Tunisia, Libya, Egypt, or Saudi Arabia, OXA-48-producing *K. pneumoniae* have been predominantly described [5,6]. On the other hand, in some regions of the Arabian Peninsula, NDM-1 was the experienced carbapenemases to the greatest extent followed by OXA-48-like carbapenemases [7]. Association of NDM and other carbapenemases (NDM-1/OXA-48) in *K. pneumoniae* has been detected worldwide [8-11]. ST11, ST14, ST101, ST147, and ST258 are major carbapenemase-producing *K.*

pneumoniae clones. Without regard to carbapenemase types; *K. pneumoniae* clones were observed in diverse carbapenemase-producing *K. pneumoniae* [12]. In this study, the aim was to investigate carbapenemase production among *K. pneumoniae* isolates collected from hospitalized cancer patients in Egypt and to study the genetic relationship of carbapenemase producing carbapenem resistant *K. pneumoniae* clinical isolates using Multi-Locus Sequence Typing.

Methodology

Bacterial strains

Thirty-four consecutive non-duplicate carbapenem-resistant *K. pneumoniae* isolates were obtained from different clinical specimens received at the microbiology lab at the National Cancer Institute (NCI), Cairo, Egypt, from June to August 2015. Ethical Committee of NCI, Cairo University, has approved the study, as the bacterial isolates examined were collected from routine clinical microbiology laboratory tests for patient care and no additional clinical specimens were collected for the purpose of research. VITEK 2 (bioMerieux, Marcy l'Etoile, France) automated machine was used for identification and susceptibility testing of the isolates and further identification was confirmed by 16S rRNA sequencing. The identified isolates were preserved in glycerol broth cultures at -70°C.

Data collection

The data recorded for each patient were age, sex, clinical diagnosis, ward of isolation, department (medical or surgical), hospitalization, prior carbapenem therapy, clinical site of infection, duration of episode and the outcome one month following infection.

Susceptibility testing

The VITEK 2 system and disc diffusion assays (Oxoid ltd., Basin Stoke, Hants, England) on Mueller Hinton agar were used to perform antibiotic susceptibility testing and results were explained as stated in Clinical and Laboratory Standards Institute (CLSI) recommendations [13]. Colistin susceptibility was detected by using the breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) as there is no breakpoint available for polymyxins for *Enterobacteriaceae* according to CLSI guidelines [14]. The following antimicrobial agents were used: amikacin, ampicillin, ampicillin-sulbactam, cefazolin, cefepime, cefoxitin, ceftazidime, ceftriaxone, ciprofloxacin, colistin, gentamicin, levofloxacin, meropenem, piperacillin-tazobactam, sulfamethoxazole / trimethoprim, and tobramycin.

Phenotypic detection

The Carba NP test (RAPIDEC, bioMérieux, Marcy l'Etoile, France) were in accordance with the manufacturer's instructions to seek the occurence of carbapenemases. MBL production was tested using a combined disk assay [15]. Ceftazidme/ceftazidme+clavulanate E test according to manufacturer's instructions was performed to test ESBL production.

Detection of carbapenemases and ESBL genes

An in-house multiplex PCR test using previously described primers was used to test the presence of carbapenemase genes ($bla_{\text{OXA-48}}$, $bla_{\text{NDM-1}}$, bla_{IMP}, bla_{VIM}, and bla_{KPC}) and ESBL CTX-M genes [16,17]. All PCR experiments were done in presence of Negative and positive controls. Analysis of reaction mix containing PCR product (5 microliters) were analyzed by electrophoresis in 1 % (w/v) agarose (Fermentas, Waltham,US). The amplicons of $bla_{\text{OXA-48}}$were purified using a QIAquick PCR Purification Kit (Qiagen, Crawley, UK) and sequenced in both directions using the ABI Prism 3700 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). The sequence of the gene was compared with sequences in the GenBank nucleotide database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) [18].

Genotyping of OXA-48 producing K. pneumoniae isolates by MLST analysis

Genotyping was determined by MLST analysis. MLST with seven housekeeping genes (*gapA, infB, mdh, pgi, phoE, rpoB and tonB*) was performed on the OXA-48 producing *K. pneumoniae* isolates according to institute Pasteur's MLST scheme (www.pasteur.fr/mlst) [19]. Alleles and sequence types (STs) were assigned by using the MLST database (www.pasteur.fr/mlst/Kpneumoniae.html).

Results

A total of (n = 34) non-duplicate carbapenem-resistant *K. pneumoniae* isolates were obtained from different patient samples. Susceptibility testing results are presented in Table 1. Of the 34 carbapenem resistant *K. pneumoniae* isolates, 31 (91.2%) produced carbapenemase. Twenty-six (83.87%) out of 31 of carbapenemase-positive *K. pneumoniae* isolates were MBL producers. All carbapenemase positive isolates were ESBL positive.

Table 1. Phenotypic and genotypic characteristics of OXA-48-producing *K. pneumoniae* isolates.

Isolate	Sample ID	Duration of episode in days	Source	OXA-48	NDM-1	IMP-1	AMP	A/S	P/T	CFZ	FOX	CAZ	CTX	FEP	MEM	AMK	GN	TOB	CIP	LEV	TMP/SMX
1	K17	12	Pus	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	32	≥ 16	16	≥ 16	≥ 16	2	2	≥ 320
2	K60	30	Oral	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 16	≥ 64	≥ 16	≥ 16	≥ 4	≥ 8	≥ 320
3	K64	20	Blood	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 16	16	≥ 16	≥ 16	≥ 4	≥ 8	≥ 320
4	K94	6	CVP	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 16	≥ 64	≥ 16	≥ 16	≥ 4	≥ 8	40
5	K47	18	Blood	+	-	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 16	16	≤ 1	≥ 16	≥ 4	≥ 8	≥ 320
6	K87	18	Blood	+	-	-	≥ 32	≥ 32	≥ 128	≥ 64	8	16	≥ 64	2	≥ 16	≤ 2	≤ 1	8	≥ 4	≥ 8	≥ 320
7	K1	6	Blood	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	16	≥ 64	≥ 64	≥ 16	≥ 64	≥ 16	8	≥ 4	≥ 8	≥ 320
8	K27	25	Blood	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	16	≥ 64	≥ 64	≥ 16	≥ 64	≥ 16	≥ 16	≥ 4	≥ 8	≥ 320
10	K32	4	Blood	+	-	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	16	≥ 64	≥ 64	≥ 16	≥ 64	≥ 16	≥ 16	≥ 4	≥ 8	40
11	K35	9	Blood	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	16	≥ 64	≥ 64	≥ 16	≥ 64	≥ 16	≥ 16	≥ 4	≥ 8	≥ 320
12	K77	22	Blood	+	-	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	16	≥ 64	≥ 64	≥ 16	≥ 64	≥ 16	8	≥ 4	≥ 8	≥ 320
13	K79	32	Blood	+	-	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	16	≥ 64	≥ 64	≥ 16	≥ 64	≥ 16	8	≥ 4	≥ 8	≥ 320
14	K88	20	Blood	+	-	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	16	≥ 64	≥ 64	≥ 16	≥ 64	≥ 16	8	≥ 4	≥ 8	≥ 320
15	K91	10	nephrostomy	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 16	≥ 64	≥ 16	≥ 16	≥ 4	≥ 8	≥ 320
16	K98	7	Blood	+	-	+	≥ 32	8	≤ 4	≤ 4	≤ 4	≤ 1	≤ 1	≤ 1	≥ 16	≤ 2	≤ 1	≤ 1	≤ 0.25	≤ 0.12	≥ 320
17	K44	1	Blood	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	4	≥ 64	≥ 64	≥ 16	≥ 64	2	≥ 16	≥ 4	≥ 8	≥ 320
18	K45	14	Blood	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	4	≥ 64	≥ 64	≥ 16	≥ 64	≤ 1	≥ 16	≥ 4	≥ 8	≥ 320
19	K46	9	wound	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	4	≥ 64	≥ 64	≥ 16	≥ 64	≤ 1	2	≥ 4	≥ 8	≥ 320
20	K51	2	Blood	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	4	≥ 64	≥ 64	≥ 16	≥ 64	2	≥ 16	≥ 4	≥ 8	≥ 320
21	K11	20	Blood	+	-	-	≥ 32	≥ 32	≥ 128	≥ 64	≤ 4	≤ 1	≤ 1	≤ 1	≥ 16	≤ 2	≤ 1	≤ 1	≤ 0.25	≤ 0.12	≤ 20
22	K23	12	Blood	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 16	≥ 64	≥ 16	≥ 16	≥ 4	≥ 8	≥ 320
23	K81	7	Oral	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 16	≥ 64	4	8	≥ 4	≥ 8	≥ 320
24	K86	7	Blood	+	+	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 16	≥ 64	4	8	≥ 4	≥ 8	≥ 320
25	K2	23	Drain	+	-	-	≥ 32	≥ 32	≥ 128	≥ 64	≥ 64	≥ 64	≥ 64	≥ 64	≥ 16	≥ 64	≥ 16	≥ 16	≥ 4	≥ 8	≥ 320

The recommended breakpoints for resistance according to CLSI [14]. AMP: ampicillin; A/S: ampicillin/sulbactam; P/T: piperacillin/tazobactam; CFZ: cefazoline; FOX: cefoxitin; CAZ: ceftazidime; CTX: ceftriaxone; FEP: cefepime; MEM: meropenem; AMK: amikacin; GN: gentamycin; TOB: tobramycin; CIP: ciprofloxacin; LEV: levofloxacin; TMP-SMX: trimethoprim-sulfamethoxazole.

Table 2. *K. pneumoniae* harbouring *bla*$_{OXA-48,}$ with isolation details, patient history& outcome, and sequence type.

Isolate number	Age	Sex	Site of isolate	Location	Date of sample	Diagnosis (SOT-HM)	Fever	Hospitalized	Previous AB intake (prior one month)	Chemotherapy intake	Co-morbidity	Outcome	Sequence type
K17	65	F	Pus	4th floor	24-June-15	SOT	No	Yes	Yes	No	Yes	Discharged	22
K60	26	F	oral	3rd floor	17-August-15	HM	Yes	Yes	Yes	Yes	Yes	Discharged	11
K64	4	M	Blood	4th floor	17-August-15	HM	Yes	Yes	Yes	Yes	Yes	Discharged	11
K94	63	F	CVP	4th floor	01-September-15	SOT	No	No	No	Yes	No	Discharged	2193
K47	51	F	Blood	5th floor	02-September-15	HM	Yes	Yes	Yes	Yes	No	Discharged	16
K87	51	F	Blood	5th floor	26-August-15	HM	Yes	Yes	Yes	Yes	No	Discharged	16
K1	6	F	Blood	5th floor	15-June-15	HM	Yes	Yes	Yes	Yes	Yes	Discharged	101
K27	17	M	Blood	4th floor	04-July-15	HM	Yes	Yes	Yes	Yes	Yes	Discharged	101
K32	54	M	Blood	4th floor	06-July-15	SOT	No	Yes	Yes	No	Yes	Discharged	101
K35	17	M	Blood	4th floor	06-July-15	HM	Yes	Yes	Yes	Yes	Yes	Discharged	101
K77	2	F	Blood	3rd floor	24-August-15	SOT	Yes	Yes	Yes	Yes	Yes	Discharged	101
K79	7	F	Blood	4th floor	24-August-15	HM	Yes	Yes	Yes	Yes	Yes	Discharged	101
K88	2	F	Blood	3rd floor	26-August-15	SOT	Yes	Yes	Yes	Yes	Yes	Discharged	101
K91	65	M	nephrostomy w.s.	3rd floor	31-August-15	SOT	No	Yes	Yes	No	Yes	Dead	101
K98	6	M	Blood	4th floor	05-September-15	HM	Yes	Yes	Yes	Yes	No	Discharged	37
K44	4	M	Blood	4th floor	10-August-15	HM	No	Yes	Yes	Yes	yes	Dead	383
K45	5	M	Blood	4th floor	10-August-15	HM	Yes	Yes	Yes	Yes	Yes	Dead	383
K46	45	M	wound	5th floor	13-August-15	SOT	No	Yes	Yes	No	No	Discharged	383
K51	4	M	Blood	4th floor	08-August-15	HM	No	Yes	Yes	Yes	Yes	Dead	383
K11	27	F	Blood	4th floor	21-June-15	HM	Yes	Yes	Yes	Yes	No	Dead	785
K23	61	F	Blood	4th floor	27-June-15	HM	Yes	Yes	Yes	Yes	Yes	Dead	147
K81	45	F	Oral	3rd floor	26-August-15	HM	Yes	Yes	Yes	Yes	No	Dead	147
K86	45	F	Blood	4th floor	26-August-15	HM	Yes	Yes	Yes	Yes	No	Dead	147
K2	59	F	Drain	7th floor	16-June-15	SOT	Yes	No	Yes	Yes	Yes	Dead	1399
K16	60	F	Sputum	7th floor	23-June-15	HM	Yes	Yes	Yes	Yes	Yes	Discharged	1399

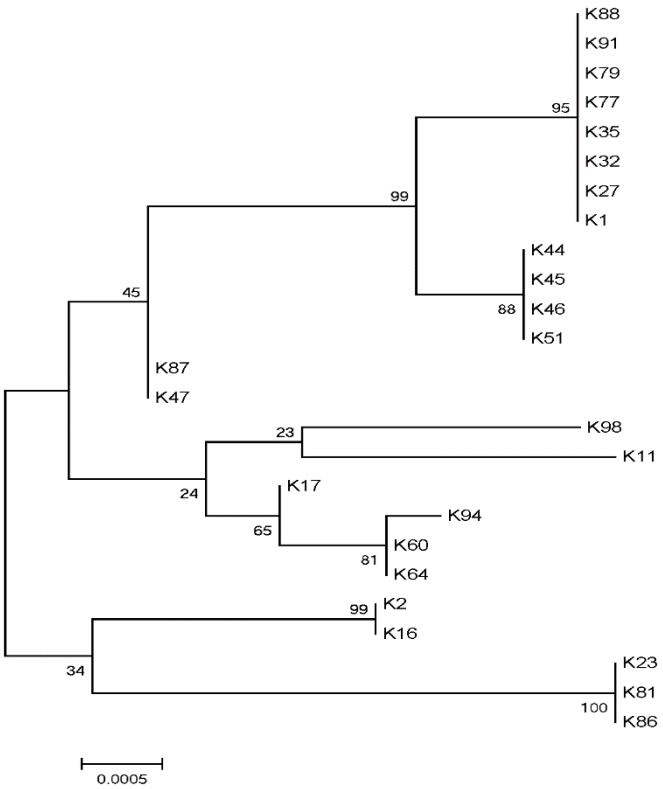
SOT: solid tumors, HM: haematology malignancy.

The class D bla_{OXA-48} gene was found in 25 (80.65%) carbapenemase producing *K. pneumoniae* isolates. While 21 (67.74%) isolates expressed MBL NDM-type. The MBL bla_{IMP-1} gene was observed in one *K. pneumoniae* isolate only. No KPC- or VIM-type *β*-lactamases were detected. Carbapenemase association of OXA-48 like and NDM-type was identified in 15 (48.39%) isolates, while the coproduction of OXA-48 like and IMP-1like was revealed in one *K. pneumoniae* isolate (Table 1). CTX-M-1-like gene was present in 100% of carbapenemase producing *K. pneumoniae* isolates.

Table 2 and figure 1 show the results of MLST for OXA-48 producing *K. pneumoniae* isolates. MLST results presented ten distinct STs which were detected among 25 OXA-48-producing *K. pneumoniae* isolates. Fifteen out of 25 isolates were clustered in three major STs [(ST101 (n = 8), ST383 (n = 4) and ST147 (n = 3)]. The predominant ST was ST101 which was detected in 8 (32%) isolates; 4 of them were retrieved from the same 4th. floor at the same day and 2 days apart; the other 3 isolates were recovered from the same 3rd. floor with 2 days apart, the remaining one from different 5th. floor. The second ST was ST383, which were detected in four isolates followed by ST147, which was detected in three isolates.

Two isolates of each of ST11, ST16, and ST1399 were detected, while only one isolate of each of ST22, ST37, ST785and ST2193 were detected (Table 1 and 2).

Figure 1. Phylogenetic tree of 25 *K. pneumoniae* strains built based on the maximum likelihood algorithm in the MEGA6 software [30] and the concatenated alleles of 7 housekeeping genes with bootstrap values. The phylogenetic analysis identified ST101, ST383, ST147, ST1399, ST11, ST16, ST 2193, ST 785, ST 37 and ST 22. The numbers in the branches depict the sample ID of *K. pneumoniae*.



Discussion

The increase in carbapenem-resistant *K. pneumoniae* prevalence may augment the healthcare and economic burden on the society. The current study was carried out to detect carbapenemase production among carbapenem resistant *K. pneumoniae* collected from Egyptian hospitalized patients with cancer and to examine the genetic relationship of carbapenemase producing carbapenem resistant *K. pneumoniae* isolates using MLST. The results of this work clearly demonstrates the occurrence of OXA-48 among carbapenemase resistant *K. pneumoniae* isolates from various origins recovered from patients with cancer having solid tumors and hematology malignancy. The dominant carbapenemase was bla_{OXA-48}, identified in 25 isolates (80.65%) followed by bla_{NDM-1} type, observed in 21 isolates (67.74%). In a different way, bla_{IMP} was detected in single isolate only. Reports of OXA-48 carbapenemases have been published worldwide, including Middle East and North Africa countries, for example, Saudi Arabia, Sultanate of Oman, Qatar, United Arab Emirates, Turkey, Egypt, Tunisia, Morocco, Libya and Lebanon [6,7,10,11,20-22]. The results presented here provide the first insight into the coproduction of the OXA-48 like carbapenemase encoding gene and NDM-type representing MBL production in *K. pneumoniae* as well as the association of OXA-48 like and IMP-1 like among hospitalized cancer patients in Cairo, Egypt. Our results revealed that carbapenemase association with OXA-48 like and NDM-type was identified in 15 isolates, while the coproduction of OXA-48 and IMP-1 like was revealed in only one *K. pneumoniae* isolate. The coexistence of OXA-48 like with NDM-type producers in *K. pneumoniae* is alarming and demands further investigation regarding its possible endemicity in the region.

The coproduction of carbapenemases producing genes could be described as NCI is a tertiary hospital receiving patients from different regions of Egypt with the high possibility that patients receive treatment before presenting to NCI in other hospitals. Patients with Cancer are hospitalized for long periods, in

addition to being immunocompromised thus are predisposed to easily acquiring highly resistant isolates from hospital environment. VIM and KPC gene expression were not identified in our study, indicating that during the period of the study carbapenemases didn't have any role in our hospital.

Our MLST results illustrated ST101 to be the most predominant ST among the carbapenem-non-susceptible OXA-48- producing *K. pneumoniae* isolates investigated in the present study, which was detected in 8 isolates. The results of molecular epidemiologic study of OXA-48 in Europe and North Africa in an 11-year (2001–2011) showed that ST101 was the most commonly observed, followed by ST395 and ST15 in *K. pneumoniae* [23]. The high widespread of these STs illustrates their epidemic potential. The second and third most common STs in our study were ST383 and ST147, which were detected in 4 and 3 isolates respectively. ST383 was previously reported for the first time in study in China in 2016 which documented the outbreak of OXAKp ST383 worldwide [24]. In this study we document the occurrence of OXA-48-producing *K. pneumoniae* isolates involving an ST101 and ST383 clones in NCI Hospital, Egypt. Clonal dissemination was identified in OXA-48-producing *K. pneumoniae* isolates in the present study where ST101 was detected in 8 isolates ; 4 of them were detected in the same floor (4th) at the same day and 2 days apart; the other 3 isolates were recovered from the same floor (3^{rd}) with 2 days apart, the remaining one from different floor (5^{th} floor), also out of the 4 ST383 isolates; 3 of them were recovered from the same floor (4^{th} floor) and one recovered from the fifth floor; However, the dissemination of bla_{OXA-48} in our hospital is attributed to diverse sequence types of *K. pneumoniae* in which 10 distinct sequence types were identified. It is noteworthy to recognize the impact of clonal dissemination on the widespread of OXA-48-producing *K. pneumoniae,* in this study 9/25(36%) patients died (Table 2), most of patients were hospitalized and all of them having solid tumors or hematology malignancy.

Three isolates were assigned to ST147 OXA-48-producing *K. pneumoniae* which is the first time to be detected worldwide; ST147 is a significant clone of VIM-producing *K. pneumoniae* isolates in Hungary [25], KPC-producing *K. pneumoniae* isolates in Greece and Canada [26,27] and NDM producing *K. pneumoniae* isolates in Canada, India, Sweden and the UK [27,28]. In addition, also an association of ST147 with IMP-8-producing *K. pneumoniae* isolates in a Far East country was observed [29].

ST11 was found in 2 isolates in this study; *K. pneumoniae* ST11 was identified in France for the first time in 1997 and it has subsequently been detected across the world involving in the USA, Sweden, Norway, Finland, the Netherlands, Hungry, Poland, the Czech Republic, Portugal, Spain, Italy, Brazil, China and South Korea [30]. Only one isolate of each of ST22, ST37, ST785 and ST2193were detected in our study.

Conclusion

In Conclusion, OXA-48 and NDM- 1 were the most common carbapenemases, and the most frequently detected sequence types were ST101 and ST383 for OXA-48-producing *K. pneumoniae* in Cancer patients in Egypt. ST383 was the second most common ST which has previously only been described in China. ST11 was assigned to OXA-48-producing *K. pneumoniae* for the first time worldwide. Association of NDM-type and OXA-48-like, and the coproduction of OXA-48-like and IMP in carbapenem-resistant *K. pneumoniae* were also identified in our hospital. A useful addition to classical investigation methods was proved in the study by implementing the molecular epidemiology. The main mode of spread in nosocomial outbreaks might be attributed to reservoirs in healthcare workers, patients, or the hospital environment.

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Ethical statement

A written informed consent was not obtained from patients because the bacterial isolates studied were collected from the routine work of clinical microbiology laboratory for patient care and no additional clinical isolates were obtained for the reason of the study. It is a standard practice not to get written informed consent for use of bacterial isolates unlinked to patient identity from the routine clinical laboratory. Therefore, the Ethics Committee of Microbiology Department, NCI, Cairo University has approved the study.

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