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Emergence of OXA-48 and TEM coproducing *Klebsiella pneumoniae* isolated from Al-Kasr Alaieny hospital, Egypt

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ABSTRACT

This study aims to investigate the co-existence of extended spectrum β lactamse (ESBL) and carbapenemase enzymes (CRE) in *Enterobacteriaceae* isolates. Twenty isolates were collected from patients from Al-Kasr Alaieny hospital. Most patients suffered from urinary tract infection (40%) and respiratory tract infection (15%).The isolates were identified by MALDI-TOF spectroscopy, 80% were identified as *Klebsiella pneumoniae* and 20% as *E. coli*. The isolates were resistant to almost all classes of antibiotics and were regarded as pandemic isolates with the exception of some isolates that showed sensitivity to tetracycline and levofloxacin. ESBL producing isolates were 75%, while 50% of the isolates were CRE. The coexistence of both ESBL and CRE were recorded in 8/20 isolates. PCR revealed that all *K. pneumonia* isolates harbored OXA-48, while 87.5% had TEM gene. This study highlights the emergence of pandemic ESBL-CRE infections between patients in Egyptian hospitals.

Key words: OXA-48 , TEM , *Klebsiella pneumonia*, Al-Kasr Alaieny hospital, Egypt

INTRODUCTION

Enterobacteriaceae are Gram negative bacteria, rod shaped, that included many pathogens: *Klebsiella pneumoniae*, *K. oxytoca*, *E. coli*, *Enterobacter cloacae* and other members. The importance of this family referred to being associated with both community and hospital acquired infections (Lutgring & Limbago, 2016). Extended spectrum β lactamse producing bacteria (ESBLs), and carbapenem resistant *Enterobacteriaceae* (CRE) have been emerged globally leaving no optimal therapy for severe infection (Lynch *et al.*, 2013). Therefore,Center of Disease Control and Prevention (CDC) reported that the antibiotic resistant Gram negative bacteria represent the most threat to human health (CDC, 2013)

Both ESBLs and CRE are able to produce enzymes that hydrolyse β lactams. Several varieties of ESBL determinants (TEM, CTX, SHV and GES) and carbapenemase determinants (KPC, IMI, OXA-48 and SME) spread all over the world (Okeke *et al.*,2005; Nordmann *et al.*, 2012;; Shaikh *et al.*, 2015; Borah *et al.*, 2016). Enzyme mediated resistance is encoded by genes that may be chromosomally or on mobile genetic elements (plasmids or transposons).

This study aims to investigate the existence of carbapenemase and ESBl encoding genes OXA-48 and TEM, respectively in clinical isolates collected from Al-Kasr Alaieny hospital, Cairo University.

MATERIALS AND METHODS

Clinical specimen's collection and patient's data:

The study included 20 isolates which were obtained from Al-Kasr Alaieny hospital. Patient's data were recorded. Clinical isolates were recovered from different sources and different hospital departments.

Screening for ESBL, CRE and identification of clinical isolates:

All clinical isolates were tested for their susceptibility against ceftazidime and imipenem for detection of ESBL and CRE, respectively. Clinical isolates were identified by conventional methods of identification (Health Protection Agency, 2013). Finally the isolates were identified using MALDI-TOF spectroscopy (Zimmermann, 2015).

Antimicrobial susceptibility and MIC determination:

Antimicrobial susceptibility were performed according to Kirby-Bauer disk diffusion method (Bauer*et al.,* 1966) against piperacillin (PRL 100), cephalosporins (CFR 30, CXM 30, FOX 30, FEP 30), amikacin (AK 30), tetracycline (TE 30), levofloxacin (LEV 5), nitrofurantoin (F 300) and amoxicillin/clavulanic acid (AMC 30). The results were interpreted according CLSI, (2017). MIC of clinical isolates against imipenem and cefotaxime were detected by microdilution broth method.

Detection of OXA-48 and TEM by PCR:

Polymerase chain reaction (PCR) was carried on isolates resistant to imipenem and third generation of cephalosporins. DNA was extracted using colony PCR method (Tsuchizaki *et al.*, 2000). PCR primers are shown in Table (1). PCR reaction used for carbapenemase and ESBL genes were as follow: 95 °C initial denaturation for 5 min., 30 cycles of (denaturation 95 °C for 40 sec, annealing for 40 sec as recommended in Table (1), extension at 72 °C for 40 sec.) and a final extension step at 72 °C for 7 min. Gel electrophoresis of PCR products were performed.

Table (1): Primers used for amplification of OXA-48 and TEM genes.

Primers	Sequence (5'-3')	Ta	Product size (bp)	References
OXA-48 F	GCGTGGTTAAGGATGAACAC	53º C	438	**Poirel *et al.*(2011)**
OXA-48 R	CATCAAGTTCAACCCAACCG			
TEM-F	ATGAGTATTCAACATTTCCG	51 º C	851	**Schmiedel *et al.*(2014)**
TEM-R	TTAATCAGTGAGGCACCTAT			

RESULTS

Theclinical isolates were recovered from different sources. The patient's data: gender, age, specimens, date of isolation and type of infection were recorded in Table (2). Most specimens were recovered from urine (55%, n= 11), followed by sputum (20% , n=4) and from wound (10% , n=2), one from blood and one from bile. About 15% of the patients (n=3/20) were hospitalized at ICU. Most patients suffered from urinary tract infection (40% , n= 8) and respiratory tract infection (15%, n=3).The investigated specimens were obtained from 65% males (n=13/20) and 35% females (n=7/20).

The identification of clinical isolates revealed that *Klebsiella spp.* represented 80% (n= 16) and *E. coli* was 20% (n=4).Out

of the 20 isolates 75% (n=15) were ESBLs while 40% (n=8) were ESBL and CRE.

The clinical isolates that exhibited ESBL and CRE activity were 8 isolates (Table 3) and they were selected for identification using MALDI-TOF. The isolates were identified as *Klebsiella pneumoniae* with score value>2. The antimicrobial susceptibility of the isolates against different antibiotics was detected. *K. pneumonia* isolates were100 resistant to all antibiotics. Only 37.5% were sensitive to tetracycline and 12.5% were sensitive to levofloxacin.

MIC values confirmed high resistance against carbapenem and third generation of cephalosporins as shown in Table (4).

PCR assay revealed that all the isolates produced both OXA-48 and TEM, while one isolate had OXA 48 and lack TEM gene as shown in Table (4).

Table (2): Patients and specimens data.

Patients No.	Age	Gender	Infection/ Dep.	Date of isolation	specimens
1	N	M	ICU	July 2015	Urine
2	30	M	UTI	July 2015	Urine
3	77	M	BTI	Dec. 2015	Bile culture
4	31	F	UTI	Dec. 2015	urine
5	N	M	Post-surgery	July 2016	Wound
6	N	M	UTI	June 2016	Urine
7	44	F	BSI	March 2015	Blood
8	24	F	RTI	May 2015	Sputum
9	N	M	RTI	Nov 2016	Sputum
10	68	M	Burning infection	Aug. 2015	Pus
11	<1y	F	NICU	Dec. 2015	Sputum
12	35	F	UTI	Aug. 2015	Urine
13	47	M	UTI	Aug. 2015	Urine
14	28	F	UTI	Aug. 2015	Urine
15	50	M	Wound infection	Aug. 2015	Wound
16	26	M	UTI	Aug. 2015	Urine
17	40	M	ICU	Aug. 2015	Urine
18	58	M	RTI	July 2015	Sputum
19	86	M	ICU	Aug. 2015	Urine
20	N	F	UTI	Aug. 2015	Urine

N: non confirmed, ICU: Intensive care unit, UTI: urinary tract infection, BTI: biliary tract infection, BSI: blood stream infection, RTI: respiratory tract infection.

Table (3): Screening of ESBL and CRE associated clinical isolates.

Patients No.	Isolate code	Isolate ID	IMP	CAZ	ESBL+CRE
1	KUE-1	*Klebsiella* spp.	R	R	+ve
2	KUE-2	*Klebsiella* spp.	S	S	-ve
3	KBE-3	*Klebsiella* spp.	S	R	-ve
4	EUE-4	*E. coli*	S	R	-ve
5	KWE-5	*Klebsiella* spp.	R	R	+ve
6	KUE-6	*Klebsiella* spp.	R	R	+ve
7	KBE-7	*Klebsiella* spp.	I	R	+ve
8	KSE-8	*Klebsiella* spp.	R	R	+ve
9	KSE-9	*Klebsiella* spp.	R	R	+ve
10	KPE-10	*Klebsiella* spp.	S	R	-ve
11	KSE-11	*Klebsiella* spp.	R	R	+ve
12	KUE-12	*Klebsiella* spp.	S	S	-ve
13	KUE-13	*Klebsiella* spp.	S	S	-ve
14	KUE-14	*Klebsiella* spp.	S	R	-ve
15	KWE-15	*Klebsiella* spp.	S	S	-ve
16	EUE-16	*E. coli*	S	S	-ve
17	KUE-17	*Klebsiella* spp.	R	R	+ve
18	KSE-18	*Klebsiella* spp.	R	R	+ve
19	EUE-19	*E. coli*	S	R	-ve
20	EUE-20	*E. coli*	S	R	-ve

Table (4): MIC of *K. pneumoniae* isolates against imipenem, cefotaxime.

Isolate code	**MIC (µg/ ml)**		**Genotyping**	
	IMP	CTX	OXA-48	TEM
KUE-1	256	>128	+ve	+ve
KWE-5	128	>128	+ve	+ve
KUE-6	64	>128	+ve	+ve
KBE-7	≥128	>128	+ve	+ve
KSE-8	>128	>128	+ve	+ve
KSE-9	256	>128	+ve	+ve
KUE-17	128	>128	+ve	-ve
KSE-18	256	>128	+ve	+ve

DISCUSSION

The multidrug resistance in Gram-negative bacteria represents global cries that threaten public human health. As a result of ESBLs evolution in last decades, physicians described carbapenems to treat severe infections caused by ESBLs. Consequently, Carbapenem resistance has been arise leaving limited alternative antibiotics (Ruppé *et al.*, 2015).

In the present study 20 isolates were obtained from patients with different age stages and types of infections. About 35% of patients were ≥ 40 years old. Many studies reported that MDR and CRE affect patients that stay prolonged times in hospitals and suffered from immunodeficiency (CDC, 2015).The most prevalent type of infection was urinary tract infections (40%) followed by respiratory tract infections (15%). van Duin *et al.* (2014) demonstrated that urinary tract infections (UTI) were the most commonly observed infection associated with CRE.

In this study, *Klebsiella* spp. was more predominant than other *Enterobacteriaceae* isolates (80%). Screening for ESBL and CRE activity revealed that there were high incidence of ESBLs and CRE. By using phenotypic detection methods, it was found that 75% of *Klebsiella spp* was ESBL , while 50% was CRE. There was no carbapenemase activity detected in *E. coli* isolates. Moreover, it was found that all *Klebsiella* spp. that had carbapenemase activity were capable of producing ESBL in conjugation with carbapenemase activity. Similarly, Sievert *et al.*(2013) stated that *Klebsiella pneumonia* was the most prevalent member of *Enterobacteriaceae* that exhibit carbapenem resistance. Myat *et al.* (2017) demonstrated that in a study including 42 clinical isolates belonged to *Enterobacteriaceae,* 47.6% (n=20/42) of *E. coli* and 16.6% (n=7/42) of *K. pneumoniae* had both ESBL and carbapenemase activity.

Antimicrobial susceptibility showed high resistance to almost all classes of antibiotics except for tetracycline and levofloxacin, this could be explained as both ESBLs and carbapenemase encoding genes are known to be carried on mobile genetic elements (plasmids or transposons) that had additional resistance genes against other classes of antibiotics (Zarfel *et al.*, 2011; Shaikh *et al.* 2015).

Molecular assay for OXA-48 and TEM revealed that 87.5 of *K. pneumoniae* had TEM, while all isolates harbored OXA-48.Clinical isolates may carry other ESBLs or CRE genes. Pitout *et al.* (2015) demonstrated that OXA-48 is considered the most prevalent encoding gene in the world.North Africa, Morocco, and Tunisia were reported as having endemic situation for OXA-48 producing *K.pneumoniae* (Manenzhe *et al*., 2015).Poirel *et al.* (2004) detected OXA-48-producing *K. pneumonia* that harbored ESBLs genes, TEM-1, SHV-2a and OXA-47. Myat *et al.* (2017) also studied the prevalence of ESBL and CRE and reported that about 50% *K. pneumonia* produced both ESBL (CTX-M) and (NDM).

In conclusion, this study revealed the coexpression of CRE and ESBLs in *K. pneumonia* and that can give a warning alarm of the emergence of pandrug resistant bacteria in some Egyptian hospitals.

REFERENCES

Bauer, A.W.; Kirby, W. M.; Sherris, J. C., and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45(4):493-6.

Borah, V.; Saikia, K.; Chandra, P.; Hazarika, N. and Chakravarty, R.

(2016). New Delhi metallo-β-lactamase and extended spectrum β-lactamases co-producing isolates are high in community-acquired urinary infections in Assam as detected by a novel multiplex polymerase chain reaction assay. Indian J. Med. Microbiol., 34(2): 173.

Centers for Disease Control and Prevention (CDC) (2013). Antibiotic Resistance Threats. Available at : https://doi.org/CS239559-B

Centers for Disease Control and Prevention (CDC) (2015). Carbapenem-resistant Enterobacteriaceae (CRE) Infection. Available at: https://www.cdc.gov/hai/organisms/cre/cre-patientfaq.html

Clinical Laboratory Standard Institutes, CLSI (2017). Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI supplement M100. Wayne, PA*:* Clinical and Laboratory Standards Institute.

Health Protection Agency (2013). UK Standards for Microbiology Investigations Identification of Campylobacter species. Standards Unit, Microbiology Services, ID23, 1–22.

Lutgring, J.D. and Limbago, B.M. (2016). The Problem of Carbapenemase-Producing-Carbapenem-Resistant *Enterobacteriaceae* detection. J. Clin. Microbiol., 54(3): 529–534.

Lynch, J. P. ; Clark, N. M. and Zhanel, G.G. (2013). Evolution of antimicrobial resistance among *Enterobacteriaceae* (focus on extended spectrum beta-lactamases and carbapenemases). Expert Opinion on Pharmacotherapy, 14(2), 199–210.

Manenzhe, R.I.; Zar, H.J.; Nicol, M.P. and Kaba, M. (2015). The spread of carbapenemase-producing bacteria in Africa: A systematic review. J. Antimicrob. Chemotherapy, 70(1): 23–40.

Myat, T. O.; Hannaway, R.F.; Zin, K.N.; Htike, W.W.; Win, K.K.; Crump, J.A. and Ussher, J.E. (2017). ESBL- and Carbapenemase - Producing *Enterobacteriaceae* in Patients with Bacteremia, Yangon, Myanmar, 2014. Emerging Infectious Diseases, 23 (5): 857-859.

Nordmann, P.; Dortet, L. and Poirel, L. (2012). Carbapenem resistance in *Enterobacteriaceae*: Here is the storm. Trends Mol. Med., 18(5): 263–272.

Okeke, I. N., Laxminarayan, R., Bhutta, Z. A., Duse, A. G., Jenkins, P., O'Brien, T. F., Pablos-Mendez, A. and Klugman, K. P. (2005). Antimicrobial resistance in developing countries. Part I: recent trends and current status. The Lancet Infectious Diseases, 5(8): 481–493.

Pitout, J.D.D.; Nordmann, P. and Poirel, L. (2015). Carbapenemase-Producing *Klebsiella pneumoniae*, a Key Pathogen Set for Global Nosocomial Dominance. Antimicrob. Agents and Chemotherapy, 59(10): 5873–5884.

Poirel, L.; Heritier, C.; Tolun, V. and Nordmann, P. (2004). Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrobial Agents and Chemotherapy, 48(1):15–22.

Poirel, L.; Walsh, T.R.; Cuvillier, V. and Nordmann, P. (2011). Multiplex PCR for detection of acquired carbapenemase genes. Diagn. Microbiol. Infect. Dis., 70 (1): 119-123.

Ruppé, É.; Woerther, P.-L. and Barbier, F. (2015). Mechanisms of antimicrobial resistance in Gram-negative bacilli. Annals of Intensive Care, 5(1): 21.

Schmiedel, J.; Falgenhauer, L.; Domann, E.; Bauerfeind, R.; Prenger-Berninghoff, E.; Imirzalioglu, C. and Chakraborty, T. (2014). Multiresistant extended-spectrum ??-lactamase-producing *Enterobacteriaceae* from humans, companion animals and horses in central Hesse, Germany. BMC Microbiol., 14 (1):187-200.

Shaikh, S.; Fatima, J.; Shakil, S.; Rizvi, S.M.D. and Kamal, M.A. (2015). Antibiotic resistance and extended spectrum beta-lactamase: Types, epidemiology and treatmnet. Saudi J. Biolog. Sci., *22*(1): 90–101.

Sievert, D. M.; Ricks, P.; Edwards, J. R.; Schneider, A.; Patel, J.; Srinivasan, A. and Fridkin, S. (2013). Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009-2010. Infection Control and Hospital Epidemiol., 34(1): 1–14.

Tsuchizaki, N.; Ishikawa, J. and Hotta, K. (2000). Colony PCR for rapid detection of antibiotic resistance genes in MRSA and enterococci. Japanese J. Antibiotics, 53(6): 422–429.

van Duin, D.; Perez, F.; Rudin, S.D.; Cober, E.; Hanrahan, J.; Ziegler, J. and Bonomo, R.A. (2014). Surveillance of carbapenem-resistant *Klebsiella pneumoniae*: tracking molecular epidemiology and outcomes through a regional network. Antimicrobial Agents and Chemotherapy, 58(7): 4035–4041.

Zarfel, G.; Hoenigl, M.; Würstl, B.; Leitner, E.; Salzer, H.J.F.; Valentin, T. and Grisold, A.J. (2011). Emergence of carbapenem-resistant Entero-bacteriaceae in Austria, 2001-2010. Clin. Microbiol. Infect., 17(11): 5–8.

Zimmermann, S. (2015). MALDI-ToF. In Modern Techniques for Pathogen Detection, 221-252.

ظهور عزلات كلبسيلا نيمونيا منتجة لكل من أوكسا 48 وتيم من مستشفى القصر العينى- مصر

امل محمد شوقى[2،1] ، سحر طلبه محمد طلبه[2] ، نوران حامد عسار[1] ، هيام محمود حمودة1

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المستخلص

تهدف هذه الدراسة لتحديد قدرة عائلة البكتيريا المعوية على انتاج كل من الانزيمات ESBL ، carbapenemase. فى هذه الدراسة تم تجميع 20 سلالة بكتيرية من مرضى يتم علاجهم فى مستشفى القصر العيني. أغلب المرضى فى هذه الدراسة يعانون من عدوى الجهاز البولى وعدوى الجهاز التنفسى بنسب 40% و 15% بالترتيب. أظهرت نتائج الدراسة ان 50% من بكتيريا *Klebsiella spp.* قادرة على انتاج الكاربابينميز (carbapenemase) بينما 75% قادرة على انتاج الانزيمات الخاصة بال ESBL.أكد تفاعل البلمرة المتسلسل (PCR) على وجود كل من الجينات TEMو OXA-48 بالنسب 87.6% و 100% بالترتيب فى آن واحد.