ejpmr, 2020,7(6), 89-93

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

MOLECULAR CHARACTERIZATION OF CARBAPENEMASE-PRODUCING ENTEROBACTERICEAE DOMINANCE OF BLAOXA-48 AND BLAKPC PRODUCERS IN KHARTOUM, SUDAN

Ahmed M. Elsadig[1]*, Mohammed A. Hammad[2], Salahaldeen H. Dahawi[1], Dr Mohammed Ahmed Abd Allah[3], Hisham N. Altayb[4]

[1]Department of Microbiology, Faculty of Medical Laboratory Sciences, Al-Neelain University, Khartoum, Sudan.
[2]Department of Microbiology, Faculty of Medical Laboratory Sciences, Dar Eloum College for Science and Technology, Khartoum, Sudan.
[3]Department Of Pathology ,College of Medicine ,Karary Uuniversity Sudan.
[4]Biochemistry Department, Faculty of Science, King Abdulaziz University, Jeddah, 21452, Saudi Arabia.

***Corresponding Author: Ahmed M. Elsadig**

Department of Microbiology, Faculty of Medical Laboratory Sciences, Al-Neelain University, Khartoum, Sudan.

Article Received on 06/04/2020 **Article Revised on 27/04/2020** **Article Accepted on 17/05/2020**

ABSTRACT

Introduction: Carbapenemase producing bacteria represent a challenging problem for health care providers, particularly in acute-care and long-term-care facilities and more in community- acquired infections. In contrast to other Enterobacteriaceae (e.g., Escherichia coli, Klebsiella pneumoniae and Enterobacter, which are major nosocomial pathogens affecting debilitated and immunocompromised patients, carbapenem resistance is still very rare in non-typhoidal salmonella serovars. **Methods**: 101 enterobacteriaceae clinical isolate were collected at different hospitals and 8 food handlers' clinical samples were received from central laboratory management in Khartoum State. Identification of the isolates was done by using conventional biochemical methods, and antimicrobial sensitivity testing was done according to CLSI guidelines. Isolates were then tested for the presence of carbapenemase encoding genes using multiplex PCR with specific primers for the detection of OXA-48 and KPC genes. **Result:** Out of the 109 isolates Escherichia coli and Klebsiella sp. demonstrated the majority with 43 % and 35.7 % respectively. Carbapenem resistance was demonstrated in 31 (28%) of the isolates. The presences of OXA-48 and KPC genes was confirmed in 40/109 (29%) of the isolates. **Conclusion:** The study concluded the evidence of the presence of the KPC and OXA-48 genes, further studies and a regular monitoring system for early detection of Carbapenemase-producing organisms are recommended with continuous surveillance of resistance to these "last resort" antibiotics.

KEYWORDS: Carbapenem resistant enterobacteriaceae, carbapenemase producing enterobacteriaceae, KPC, OXA, multiplex.

BACKGROUND

Clinical infections acquired by carbapenem-resistant Enterobacteriaceae are increasingly prevalent and have become a major worldwide threat to human health. Carbapenem resistance is driven primarily by the acquisition beta lactamase enzymes, which are able to degrade carbapenem antibiotics (carbapenemases) and result in high levels of resistance and treatment failure. Clinically relevant carbapenemases include both serine beta lactamases (SBLs; e.g., KPC-2 and OXA-48) and metallo beta lactamases (MBLs), such as NDM-1. MBL-producing strains are endemic within the community in many Asian countries, have successfully spread worldwide, and account for many significant CRE outbreaks.[1]

The predominant carbapenemase produced by CP-CRE worldwide is the Klebsiella pneumoniae carbapenemase (KPC) family encoded by the blaKPC gene.[2]

Recently CR strains have been found in variety of Salmonella sp. plenty of these serovars are frequently involved in human and animal infections. For instance S.Typhimurium, (monophasic variant), S. Indiana, and S. Kentucky, which are also characterized by a high level of MDR— particularly associated with the spread of successful clones. These three serovars ranked among the top ten associated with human infections in the European Union. Carbapenem resistance is still very rare in typhoidal and non-typhoidal serovars. Nevertheless, it has already been detected in isolates recovered from humans, companion animals, livestock, wild animals and food.[3]

.

Oxacillinases also known as Class D β-lactamases, have the capability to breakdown oxacillin. OXA producing

bacteria are typically unsusceptible to carbapenems, reflecting concurrent resistance mechanisms, such as permeability alteration or the production of other β-lactamases[4] Activity of OXA-48 enzymes is found to be 10-fold higher than that of other OXA enzymes, being intially identified in K. pneumoniae, from which it has spread to other Enterobacteriaceae.[5] KPC enzymes exists on transferable plasmids, their substrate hydrolysis spectrum contain the aminothiazoleoxime cephalosporins, such as cefotaxime. Although the 11 KPC β-lactamases are mostly found in K. pneumoniae, there have been reports of these enzymes in Enterobacter species and in Salmonella species.

Numerous studies were carried out in Sudan regarding carbapenems resistant. In 2012 Abdelrazig et al. found 57 IMP family genes (IMP-7 and IMP-10) among 74 clinical isolates of Pseudomonas aeruginosa[6] Mudathir et al. studied the prevalence of MBL genes in Khartoum state, 2018, among 200 Gram-negative clinical isolates 72(36.1%) were MBL positive. MBL positive genes among 100 carbapenems sensitive and 100 resistant isolates were 27(27%) and 45(45%) respectively. There was a statistically significant association between the antimicrobial susceptibility and the presences of MBL genes (P.value = 0.008). E.coli was the predominant species possessing MBL genes 26(36.1%), with 22(30.7%) species having a combination of MBL genes.[7] In a study carried out in Khartoum, 2016. New Delhi Metallo-Beta-Lactamase- Producing Gram Negative Bacilli causing Pyogenic Infections were studied phenotypically**,** regarding carbapenem sensitivity, 67 Gram-negative isolates (74.4%) were sensitive and (25.6%) were found to be resistant, strains were mostly K. pneumoniae, P. aeruginosa, and Acinetobacter.[8]

The global spread of carbapenem-resistant Enterobacteriaceae (CRE) has been fostered by the lack of preemptive screening of patients in healthcare facilities that could prevent patient-to- patient transmission.[9]

METHODS

Bacterial isolates

A total of 103 Enterobactericeae isolates were obtained from culturing of different clinical samples (Urine = 71, wound swab = 20, stool=2, vaginal swab= 1, and pleural aspirate =3 and pus=6). In collaboration with Khartoum central laboratory Headquarters, we were able to extract (n=6) Salmonella samples collected from food handlers in different restaurants in Khartoum.

Biochemical tests and gram stain were carried out, after which it was incubated for 24 hours aerobically. Different tests were carried out according to different enterobacteriaceae isolates.[10]

Antimicrobial Susceptibility Testing

All identified Enterobactericeae were tested for their antimicrobial susceptibilities by disc diffusion technique according to the Clinical Laboratory Standards Institute (CLSI) guidelines. The antibiotic discs were used: Meropenem (10µg) and Imipenem (10 µg) (Bioanalysis Co. Italy). However new CLSI recommendations lowered the breakpoints of carbapenems and removed the requirement for testing for carbapenemase to determine susceptibility.[11]

ATCC 25922 was used as control for evaluation the disk activity. All isolates for which the zone diameter ≤ 18 mm for Meropenem and impenem disks were considered to have a positive screening test for a carbapenem resistance.[11]

Molecular detection of carbapenemase genes

Molecular techniques, primarily based on PCR, has been the reference standard for the identification and differentiation of resistance genes depending on its excellent specificity, sensitivity, accuracy and rapidity. DNA was isolated from bacterial colonies using the boiling lysis method, strains were streaked onto Nutrient agar (Himedia, India) and grown overnight at 37°C. A loopfull of bacterial growth was suspended in 400 µl of sterile distilled water, incubated at room temperature for 5min, and then boiled for 10 min. After centrifugation at 13200 rpm for 10 minutes, the pellet was discarded and the supernatant containing DNA was checked by gel electrophoresis and then use for PCR or stored at -20°C.[12] PCR amplification was performed using published primer pairs which are as shown, in (Table 1). For 100 pmol/ml from each primer we dissolved them in DW as instructed by manufacture, then for 10 pmol/ml we dissolved 10 µl of each primer in 90 µl DW.

Amount of .5 mg of agarose powder was dissolved in 25 ml 1X TE buffer and heated until it became clear. Then the mixture was cooled to 55°C. 2.5 µl of (20mg/ml) ethidium bromide were added, mixed well and poured in a casting tray, any bubbles were removed and left to solidify at room temperature. The following reagents were used for the multiplex reaction, each gene in the following volumes (total reaction volume was 20 µl) in 0.2 ml PCR tube;

- 8 µl deionized sterile water.
- 8 µl Master mix
- 0.5 µl forward primer (KPC)
- 0.5 µl reverse primer (KPC)
- 0.5 µl forward primer (OXA-48)
- 0.5 µl reverse primer (OXA-48)
- 2 µl DNA (template DNA).

Protocol used for amplifications of OXA48 and KPC genes (multiplex)

The PCR was done by using a thermocycler with the following conditions: initial activation at 94^{O}C for 5 minutes, followed by 35 cycles at 94^{O}C for 1 minute,

56^{O}C for 1 minute and 72^{O}C for 1 minute and a final extension at 72^{O}C for 5 minutes.

Data Analysis

The statistical packages for social sciences (SPSS version 23) was used to clean, describe and analyze the data. All tests will be considered as statistically significant when p-value <0.05.

Ethical Consideration

the study received ethical clearance from the Ethical Research Committee at the Faculty of Medical laboratory Sciences, Alneelain University and administration of the different hospitals in Khartoum state.

Table 1: Primer sets for amplification of carbapenem resistance determine genes.

Gene name	Primer sequence (5/ → 3/)	Amplicons size (bp)
bla-OXA-48	Forward:AACGGGCGAACCAAGCATTTT Reverse:TGAGCACTTCTTTTGTGATGGCT	597
KPC	Forward: TGTTGCTGAAGGAGTTGGGC Reverse: ACGACGGCATAGTCATTTGC	340

RESULTS

Characteristics of isolates

A total of 109 clinical sample were collected, the most isolated bacteria was E.coli 43% (47/109) followed by Klebsiella 35.8% (39/109). Urine demonstrated the highest isolate clinical source by 65% (71/109), frequency of different isolated species and their clinical sources are shown in (Table 2).

Antimicrobial susceptibility testing

Carbapenem resistant was demonstrated in more than third (31%) of the bacterial isolates, the highest rate was seen in Escherichia coli 15.5% followed by K.pneumoniae 8.3%, Proteus, Enterococcus and Salmonella species share the same resistance rate 2%, finally Serratia at 1%, (Table 3) shows the frequency of the enterobacteriaceae species included in the study and their resistant pattern.

Genotypic detection of carbapenemase genes using polymerase chain reaction (PCR)

Out of the 109 isolated enterobacteriaceae, carbapenemase resistance genes was found in more than third of the samples 36% (40/109): 15% (17/109) were positive for blaKPC genes and also the same number 15% (17/109) were positive for blaOXA-48 genes, 5.5% (6/109) were positive for both blaKPC genes and blaOXA-48 genes, details are shown in (Table 4).

Table (2) Frequency of different isolated species and their clinical sources.

		Sample type								
		High Vaginal Swab	Pleural aspirate	Pus	Stool	Urine	Wound swab	Food handlers	Total	P value
Bacterial isolates	E.coli	0	1	4	2	28	12	0	47	
	Enterococcus	0	0	0	0	3	0	0	3	
	Klebsiella	1	1	1	0	29	7	0	39	
	Proteus	0	1	1	0	8	1	0	11	0.000*
	Serratia	0	0	0	0	3	0	0	3	
	Salmonella	0	0	0	0	0	0	6	6	
Total		1	3	6	2	71	20	6	109	

Table 3: Frequency of the enterobacteriaceae species included in the study and their resistant pattern.

		Carbapenem resistant					
		Intermediate	Resistant Both	Sensitive Mer Resistant Imp	Sensistive	Total	P-value
Bacterial isolate	E.COLI	2	17(15.5%)	0	28	47	
	ENTEROCOCCUS	0	2(1.8%)	0	1	3	0.000*
	KLEBSIELLA	1	9 (8.25%)	0	29	39	
	PROTEUS	1	2(1.8%)	1	7	11	
	SERRATIA	0	1(1.1%)	0	2	3	
	Salmonella	0	2(1.8%)	0	4	6	
Total		4	31	1	67	109	

Table 4: Distribution of carbapenemases among various bacterial isolates.

		Genes				Total
		KPC	NO GENE	OXA- 48	BOTH GENES	
Isolates	E.coli	7	29	8	3	47
	Enterococcus	0	1	2	0	3
	Klebsiella	8	24	5	2	39
	Proteus	1	7	2	1	11
	Salmonella	0	6	0	0	6
	Serratia	1	2	0	0	3
Total		17	69	17	6	109

DISCUSSION

Carbapenems are critical "last resort" antibiotics reserved for the treatment of serious infections caused by MDR Gram-negative bacteria, particularly in patients with prolonged hospital stays.[13]

It as well represents a major threat to the ongoing usefulness of carbapenem antibiotics to treat severe, often life-threatening, gram-negative bacterial infections. In the present study 31% of the clinical isolates were found to be carbapenem resistant which is quite enough to spread awareness, the lack of effective drugs targeting these resistance mechanisms is an urgent medical priority.

This study attempted to assess the Enterobactericeae carbapenem resistant rates at Khartoum State, Sudan. The study revealed that the prevalence of E.coli (43%) was the highest among the clinical isolates followed by Klebsiella. These results are almost similar to the findings published by Mudathir et al.[7] previously reported in Khartoum hospital in which the prevalence was 36.1%. The clinical isolates were extracted mostly from urine and less frequently from blood, wound secretion or swab and lower respiratory tract secretions. The data showed difference regarding the phenotypic and genotypic carbapenemases existence in which 29% (32/109) showed phenotypic resistance to meropenem and imipenem discs, while 36% (40/109) showed genotypic resistance by possessing OXA or KPC genes. Schechner et al., justifies the phenomena by indicating to the presence of metallo-beta lactamases, in addition to that two possibilities exist that may explain these IMP/MER resistant while PCR-negative isolates. Firstly as PCR could be falsely negative due to inhibitory substances in the reaction, secondly due to technical inexperience of the laboratory workers. However, the most probable reason could be the existence of other carbapenemases genes not used in this studiy such as the ESBL and the member of the Serratia marcescens (SME) family of carbapenemhydrolyzing beta-lactamases.[14]

However new CLSI recommendations lowered the breakpoints of carbapenems and removed the requirement for testing for carbapenemase (e.g.MHT) to determine susceptibility. The data also showed resistance among Salmonella species 2 %, extracted from food handlers, the results contradicts with numerous studies in Sudan but agrees with Javier et al in Spain. Continuous surveillance of resistance to these "last resort" antibiotics is required to establish new methods to detect the main sources.[15]

The study revealed the prevalence of OXA-48 and KPC by 15.5% for each, these findings agrees with a study conducted in Sudan by Salma Elnour et al.[16] regarding OXA-48 but contradicts with the KPC gene prevalence in a study in Khartoum by Salma Satir et al.[17] 5.5% (6/109) of the isolates were found possessing 2 carbapenamase genes (OXA/KPC), those finding agrees with the finding of Abdelhakam Hassan et al[18], and also agree with results from Turkey by Koksal et al., 2016.[34]

Among the limitations of the study was the difference in diagnostic methods routinely used globally for detection of carbapenemase production. Another limitation was the low number of samples collected, this could have been addressed if more years of data were available for the research and as well more funding sources. The lack of data on cases of food handlers also the number of cases studied, the lack of information on other comorbid diseases, such as diabetes and HIV coinfection, limited further stratification of the carbapenem resistant population into different risk groups and more detailed results.

CONCLUSION

In conclusion, gene transfer together with co- and cross-selection mechanisms can be involved in the development of carbapenem resistance especially Salmonella species. More global epidemiological surveillance of carbapenem resistance is required to eradicate more possible infections. Determination of OXA-48 and KPC genes by molecular techniques in carbapenemase producing bacteria may give useful data about their epidemiology and risk factors associated with these infections.[15] Therefore, carbapenemase producing bacteria should be promptly identified for appropriate antibiotic prescription and proper implementation of infection control measures.

What is known about this topic

- Resistance to critical "last resort" antimicrobials.

What this study adds

- High prevalence of Carbapenem resistant

enterobacteriaceae in Sudan

- Salmonella resistant carabapenems

Competing interests

The authors declare no competing interests.

ACKNOWLEDGMENT

Ahmed Elsadig, Mohamed Hammad: designed the study; Ahmed Elsadig, Salahaldeen Dahawi, Hisham Altayb: carried out the data collection, and laboratory work, participated in the statistical analysis; All authors coordinated and helped to draft the manuscript, read and approved the final manuscript.

REFERENCE

1. Munoz-Price LS, Poirel L, Bonomo RA, Schwaber MJ, Daikos GL, CormicanM, Cornaglia G, Garau J, Gniadkowski M, Hayden MK, et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. Lancet Infect Dis, 2013; 13: 785-96; PMID:23969216.
2. Hugas, M.; Beloeil, P. Controlling Salmonella along the food chain in the European Union—Progress over the last ten years. Euro Surveil, 2014; 19: 20804.
3. Everett M, Sprynski N, Coelho A,Castandet J, Bayet M, Bougnon J, Lozano C,Davies DT, Leiris S, Zalacain M, Morrissey I, Magnet S, Holden K, Warn P, De Luca F, Docquier J-D, Lemonnier M. Discovery of a novel metallo-β-lactamase inhibitor that potentiates meropenem activity against carbapenem-resistant Enterobacteriaceae. Antimicrob Agents Chemother, 2018; 62: e00074-18.
4. Queenan AM, and Bush K. Carbapenemases: the versatile betalactamases. Clin Microbiol Rev, 2007; **20**: 440–458.
5. Zhao WH, and Hu ZQ. Acinetobacter: a potential reservoir and dispenser for β- lactamases. Crit Rev Microbiol, 2012; 38: 30-51.
6. Abdelrazig MD, HamedElnil YF. Molecular characterization and sensitivity pattern of Pseudomonas aeruginosa among patients with different diseases in Khartoum, Sudan. J Sci Technol, 2012; 13(12): 32–6.
7. Adam MA, Elhag WI. Prevalence of metallo-β-lactamase acquired genes among carbapenems susceptible and resistant Gram-negative clinical isolates using multiplex PCR, Khartoum hospitals, Khartoum Sudan. BMC infectious diseases, 2018 Dec; 18(1): 668.
8. Reham M. Basher. Phenotypic Detection of New Delhi Metallo-Beta-Lactamase Producing Gram Negative Bacilli causing Pyogenic Infections in Khartoum State (Sudan). African Journal of Medical Sciences, 2016; 1(2): ajmsc.info.
9. Richter SS, Marchaim D. Screening for carbapenem-resistant Enterobacteriaceae: who, when, and how? Virulence, 2017 May 19; 8(4): 417-26.
10. Ibrahim EH, G Sherman, S Ward, VJ Fraser, and MH Kollef. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting. Chest, 2000; 118**:** 146-155.
11. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI document M100- S25 (ISBN 1-56238-989-0 [Print]; ISBN 1-56238-990-4 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2015. CLSI, 2008.
12. Chen L, Mediavilla JR, Endimiani A, Rosenthal ME, and Zhao Y.Multiplex real-time PCR assay for detection and classification of Klebsiella pneumoniae carbapenemase gene (bla KPC) variants. J Clin Microbiol, 2011; 49: 579-585.
13. Tzouvelekis, L.S.; Markogiannakis, A.; Psichogiou, M.; Tassios, P.T.; Daikos, G.L. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: An evolving crisis of global dimensions. Clin. Microbiol. Rev, 2012; 25: 682–707.
14. Schechner V, Kotlovsky T, and Kazma M**.** Asymptomatic rectal carriage of blaKPC producing carbapenem-resistant Enterobacteriaceae: who is prone to become clinically infected? Clin Microbiol Infect, 2013; **19**(5): 451–456.
15. Javier Fernández, Beatriz Guerra and M. Rosario Rodicio. Resistance to Carbapenems in Non-Typhoidal Salmonella enterica Serovars from Humans, Animals and Food. Vet. Sci, 2018; 5: 40.
16. Mohamed SE, Alobied A, Saeed MI, Hussien WM. New Delhi Metallo-β-lactamase (NDM)-mediated Carbapenem-Resistant Pseudomonas aeruginosa Clinical Isolates in Sudan. South Asian Journal of Research in Microbiology, 2018 May 29: 1-5.
17. Elsir MA, Bakheit MA, Satir SB, Elkhalifa AI, El Hussein AR. Molecular Detection of Carpabenem Resistant Gene (blaKPC) among Different Gram Negative Bacteria using Loop Mediated Isothermal Amplification (LAMP) Assay, Khartoum State, Sudan.
18. Abdelhakam H Ibrahim, AliAl Fadhil A Omer. Molecular Characterization of Carbapenemase Producing Klebsiella Pneumoniae Dominance of OXA-48, KPC, VIM and NDM Producers in Khartoum, Sudan. J Clin Rev Case Rep, 2017; 1: 3.
19.