International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2736-1756, Vol. 17 (1), pp. 001-007, January, 2023. Available online at www.internationalscholarsjournals.org © International Scholars Journals
Author(s) retain the copyright of this article.

Full Length Research Paper

Molecular Characterization of CTX-M ESBLs among Pathogenic *Enterobacteriaceae* isolated from different regions in Sudan

Hisham N Altayb[1]*, Mohamed A M Siddig[2], Nagwa M El Amin[3], Ahmed Ibrahim Hashim[1] Maowia M. Mukhtar[4]

[1]College of medical laboratory science, Sudan University of science and technology, Sudan.
[2]Botany department, Faculty of Science, University of Khartoum, Sudan.
[3]Department of microbiology, Faculty of medicine, university of Khartoum, Sudan.
[4]Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan.

Accepted 07 November, 2022

This was a cross sectional study conducted to detect and characterize CTX-M genes among extended spectrum β-lactamases (ESBLs) producing *Enterobacteriaceae* isolated from different regions in Sudan. A total of 305 of *Enterobacteriaceae* clinical isolates were collected from different regions in Sudan. ESBLs production was initially screened by cefotaxime, cefepime and Ceftazidime, then confirmed by disk combination method and PCR. DNA sequencing was done to differentiate betwen bla_{CTX-M} genotypes. *Escherichia coli* was the most predominant isolate (58%), followed by *Klebsiella pneumoniae* (26.6%), *Citrobacter freundii* (3.6%), *Enterobacter species* (6.2%) and *Proteus species* (5.6%). ESBLs were detected by disk combination method in 128/305 (42%) of the tested isolates; Khartoum State 23/36 (64%), Gizera State 54/100 (54%), Sinnar State 49/92 (53%) and White Nile State 2/77 (2.6%). Three quarters of the ESBLs producers (96/128) were positive for bla_{CTX-M} genes by PCR. The $bla_{CTX-M-15}$ gene was the most predominant gene 18/23 (78.3%), followed by $bla_{CTX-M-14}$ 3/23(13%), $bla_{CTX-M-27}$ 1/23 (4.3%) and $blaC_{TX-M-98}$ 1/23 (4.3%). There was a transition mutation (substitution of A with G at position 25) in the bla_{CTX-M} gene (ID: KP309815), that affected protein structure. In conclusion $bla_{CTX-M-15}$ was the most commonly encountered gene and widely spread in different Sudanese regions.

Keywords: *blaCTX-M; Beta-Lactamases Genes; Enterobacteriaceae; Sudan.*

INTRODUCTION

Antimicrobial resistance is becoming a chronic problem in the therapeutic intervention of infections. Production of ESBLs is a common mechanism of resistance among Gram negative bacilli. Production of ESBLs by *Enterobacteriaceae* has already had serious clinical implications. Several studies have confirmed an association between ESBL-producing *Enterobacteriaceae* and treatment failure, excess mortality, prolonged hospital

*Corresponding Author's Email: hishamaltayb@gmail.com

stay and increased treatment costs (Schwaber and Carmeli 2007; Pena et al., 1997). Nosocomial and community-associated infections such as urinary tract infections are frequently caused by *Klebsiella pneumoniae* and *Escherichia coli*; infections caused by these species are usually treated with Cephalosporins, so resistance rates to these antimicrobial agents have also been increasing (Murray et al., 2007).

Extended-spectrum β-lactamases (ESBLs) of the CTX-M types have been reported increasingly in gram-negative bacteria, mostly in *Escherichia coli* (Bonnet 2004). All CTX-M-types are ESBLs genes and do not have corresponding non-ESBL progenitors like TEM and SHV-type ESBLs. Based on their amino-acid sequence diversity, there are 172 variants of CTX-M genes identified until August 25, 2016, according to https://www.lahey.org/studies/.

CTX-M-15 is the most important gene among CTX-M types that has been associated with epidemic and mosaic plasmids, and also may play a role in conferring Carbapenem resistance in isolates with outer membrane impermeability (Livermore and Woodford 2006; Gröbner et al., 2009). CTX-M-15 efficiently can hydrolyze both Cefotaxime and Ceftazidime unlike other CTX-M enzymes (Karim et al., 2001). In Sudan, there is paucity of information on CTX-M types; hence, this study was conducted to investigate the prevalence and types of CTX-M types in different Sudanese geographical regions.

MATERIALS AND METHODS

Bacterial Isolates

Three hundred and five *Enterobacteriaceae* isolates (*Escherichia coli* = 177, *K. pneumoniae* = 81, *Citrobacter freundii* = 11, *Enterobacter species* = 19 and *Proteus species* = 17) were collected from different non duplicated clinical isolates of patients from different Sudanese regions including Khartoum State (Haj Alsafi hospital), Sinnar State (Wad Alabass hospital), White Nile State (Aburugba village) and Gizera State (Wad Madani hospital) between December 2012 to November 2013. Chromogenic agar media (Liofilchem Co. Italy) and biochemical tests (according to CLSI guidelines) were used for isolation and identification of bacteria (CLSI 2011). The bacterial isolates were obtained from patient's urine, vaginal swab, and wound swab. A written consent was obtained from every patient before collection of the samples.

ESBLs screening and confirmation

Antibiotic sensitivity and the ability of these isolates to produce ESBLs were determined according to Kirby-Bauer disc diffusion method (Wayne 2008). The following antibiotics disks were used; Ceftazidime (CAZ: 30µg), Cefotaxime (CTX: 30µg), Cefepime (CPM: 30µg), Meropenem (MRP: 10µg) Amikacin (AK: 30µg), Ciprofloxacin (CIP: 5µg), Chloramphenicol (C: 30µg), and Nalidixic acid (NX 30µg) (Hi Media Lab Ltd., India). All isolates for which zone diameter ≤ 27 mm for Cefotaxime or ≤ 22 mm for Ceftazidime or ≤ 18 mm for Cefepime were considered to have a positive screening test for ESBLs, and then they were subjected to Clavulanate confirmatory testing. Phenotypic testing of ESBLs production was performed using the combination discs method according to the recommended method of the Clinical and Laboratory Standards Institute (CLSI) (Wayne 2006). We used Cefotaxime alone (CTX 30µg) and with Clavulanic acid (CTL 30µg+ 10 µg), Cefepime alone (FEP 30µg) and with Clavulanic acid (FEL 30µg+ 10 µg), and Ceftazidime alone (CAZ 30µg) and with Clavulanic acid (CAL 30µg+ 10 µg) (Liofilchem Co. Italy).

Polymerase Chain Reaction (PCR)

All positive isolates by confirmatory tests were subjected to molecular screening for β –lactamases genes using polymerase chain reaction for family specific $bla_{CTX\text{-}M\text{-}like}$. DNA extraction was done by the guanidine chloride method as described by (Alsadig et al., 2014). The PCR was carried out using primers; 5′-SCSATGTGCAGYACCAGT-3′ and CTX-MA-2 5′-CCGCRATATGRTTGGTGGTG-3′, (Cao et al., 2002), (where S is G or C, Y is C or T, and R is A or G) (Metabion, Germany) for $bla_{CTX\text{-}M}$-like genes, in a total reaction volume of 25µl; 5µl Master mix (*iNtRON Biotechnology*, Seongnam, *Korea*), 0.6µl of forward primer, 0.6µl of reverse primer, 2µl DNA and 16.8µl deionized sterile water. The PCR mixture was then subjected to initial denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 seconds, primer annealed at 57°C for 45 seconds, followed by a step of elongation at 72°C for 60 seconds, the final elongation was at 72°C for 5 min. PCR products were analyzed by electrophoresis on a 2% agarose gel in TBE 1X that contain 2.5 µl of (20mg/ml) ethidium bromide at 100V for 40 min. Bands were visualized under U.V trans illuminator. A positive control obtained from previously sequenced CTX-M gene and a negative control contains DW, primers and PCR mixtures were used.

DNA sequencing

DNA sequencing was performed for 23 positive CTX-M genes (7 from Sinnar, 7 from Khartoum, 1 from White Nile and 8 from Gizera). DNA purification in addition to standard DNA sequencing was performed for both strands of $bla_{CTX\text{-}M}$ genes (550Bp) by Macrogen Company (Seoul, Korea).

Table 1. Characteristics of the blaCTX-M-producing Enterobacteriaceae isolates

Species	Antimicrobial resistance, n (%)							
	CTX	**CAZ**	**FEP**	**CIP**	**NX**	**MRP**	**C***	**K**
E. coli (n= 177)	75 (42)	61 (34)	26 (15)	67 (38)	52 (29)	18(10)	46 (26)	33 (19)
K. pneumoniae (n= 81)	34	30 (37)	5(6.1)	16 (20)	15 (19)	0(0)	33(41)	12 (15)
Enterobacter species	(42)							
(n= 19)	7(37)	7 (37)	1 (5.3)	4 (21)	5(26)	9(0)	8(42)	5 (26)
Proteus species (n= 17)								
Citrobacter species (n= 11)	9(53)	8 (47)	5 (3)	5 (29)	7 (41)	3(18)	9(53)	3(18)
	3(27)	3(27)	1 (9)	1 (10)	1 (10)	1(10)	3 (27)	1 (10)
Total (n=305)	**128**	**109**	**38**	**93**	**80**	**22**	**99**	**54**

CTX, Cefotaxime; CAZ, Ceftazidme; FEP, Cefepime; NX, Nalidixic acid; K, Amikacin; CIP, Ciprofloxacin; MRP, Meropenem; C, Chloramphenicol; n, number of isolates; (%), percentage.

** There was a significant association between species and resistant to Chloramphenicol.*

We selected only 23 bla$_{CTX-M}$ positives gene for DNA sequencing due to the limitation of resources.

Sequence alignment

The nucleotide sequences of the bla$_{CTX-M}$ beta-lactamases genes were searched for sequence similarity using nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Atschul et al., 1997). Nucleotide sequences were translated into the amino acid sequence by Gene MarkS,

(John et al., 2001), version 4.25 (http://exon.gatech.edu/genemark/genemarks.cgi). Protein sequence similarity was searched with BLASTp, highly similar sequences from NCBI were subjected to multiple sequence alignment and evolutionary analysis using BioEdit software (Hall 1999). Mutation analysis and prediction of tertiary protein structure were done online by

Project Hope software (http://www.cmbi.ru.nl/hope/report/2064?10) (16).

Statistical Analysis

We used the chi-square tests of the SPSS version 21.0, (IBM SPSS 2012), to check the statistical significance. The p-value that considered significant was less than 0.05.

RESULTS

A total of 305 clinical isolates of *Enterobacteriaceae* were identified; *E. coli* = 58% (177/305), *K. pneumoniae* = 26.6% (81/305), *Citrobacter freundii* = 3.6% (11/305), *Enterobacter species* = 6.2% (19/305) and *Proteus species* = 5.6% (17/305) (Table 1). The presence of ESBLs genes was confirmed through phenotypic confirmatory test where 128/305 (42%) isolates were positive. The most predominant ESBLs positive were *E. coli* 77/128 (60.2%); followed by *K. pneumoniae* 33/128 (25.7%), *Enterobacter* species 7/128 (5.5%), Citrobacter species 3/128 (2.3%) and *Proteus species* 8/128 (6.3%). Out of 128 (42%) positive ESBLs isolates; 63.8% (23/36) were from Khartoum State, 54% (54/100) from Gizera State, 549/92 (53%) from Sinnar State, and 2/77 (2.5%) from the White Nile state.

Identification of blaCTX-M genes in the clinical isolates

Out of 128 ESBLs producing isolate, 96/128 (75%) were positive for bla$_{CTX-M}$ genes (550bp) by PCR (Fig. 1)

DNA sequencing and multiple sequence alignment

DNA sequencing of amplified bla$_{CTX-M}$ (550 bp) genes (= 23) revealed high occurrence of bla$_{CTX-M-15}$ 78.3% (=18, AGI41341.1, AIQ77714.1), bla$_{CTX-M-14}$ (13.1%) (= 3, AAY58238.1), bla$_{CTX-M-27}$ (4.3%) (n= 1, BAI68283.1) and bla$_{CTX-M-98}$ (4.1%) (n= 1, WP_032495798.1) (Table 2). There was a silent mutation (substitution of G with A at position 24), and transition mutation (substitution of A to G at position 25) in isolate-13 (ID: KP309815) from White Nile State. This mutation resulted in a substitution of Lysine with Glutamic acid, as shown in Fig. 1.

Mutation analysis

Project HOPE online software revealed that there are some differences between wild and mutant residues; the

Figure 1. PCR amplification of CTX-M genes on 2% agaarose gel electrophoresis. Lane 1 DNA ladder: MW 100-1500bp. Lane 2 is a positive control. Lane 3 a negative control. Lane 4, 5, 6, 7, 8, 9 and 10 showingg typical bands size of 550bp corresponding to the molecular size of CTX-M genes.

Table 2. Phenotypic and genotypic detection of bla$_{CTX-M}$ genes from different Sudanese cities

Isolate	State	Sample	Screening tests			Phenotypic tests			CTX-M genes
			CTX	CAZ	FEP	CTL	CAL	FEL	
E. coli	Sinnar	Urine	+	+	-	+	+	+	CTX-M 15
K. pneomoniae	Sinnar	Urine	+	+	-	+	+	+	CTX-M 15
K. pneumoniae	Sinnar	Urine	+	+	-	+	+	+	CTX-M 15
K. pneumoniae	Sinnar	Urine	+	+	-	+	+	+	CTX-M 15
E.coli	Sinnar	Urine	+	+	-	+	+	+	CTX-M 15
E.coli	Sinnar	Urine	+	+	-	+	+	+	CTX-M 14
K. pneumoniae	Sinnar	Urine	+	+	-	+	+	+	CTX-M 15
E.coli	Khartoum	Urine	+	+	-	+	+	+	CTX-M 15
E.coli	Khartoum	Wound swwab	+	+	+	+	+	+	CTX-M 15
E.coli	Khartoum	Urine	+	+	-	+	-	+	CTX-M 15
E.coli	Khartoum	Urine	+	+	+	+	+	-	CTX-M 14
E.coli	Khartoum	Wound swwab	+	+	-	-	-	+	CTX-M 15
E.coli	Khartoum	Urine	+	+	+	+	+	+	CTX-M 15
E.coli	Khartoum	Wound swwab	+	+	+	-	-	+	CTX-M 15
E.coli	White Nile	Wound swwab	+	+	-	+	+	+	CTX-M 15
E.coli	Gizera	Urine	+	-	-	+	+	+	CTX-M 98
E.coli	Gizera	Urine	+	+	+	+	-	+	CTX-M 15
E.coli	Gizera	Urine	+	-	-	+	+	+	CTX-M 15
E.coli	Gizera	Urine	+	-	-	+	+	+	CTX-M 15
E.coli	Gizera	Urine	+	-	-	+	+	+	CTX-M 27
E.coli	Gizera	Urine	+	-	-	+	-	+	CTX-M 14
E.coli	Gizera	Urine	-	-	-	-	-	+	CTX-M 15
E.coli	Gizera	Urine	+	+	-	+	+	+	CTX-M 15

Abbreviations: CAZ= Ceftazidime, CTX= Cefotaaxime, FEP= Cefepime, CTL= *Cefotaxime+ Clavulanicc acid, CAL= Ceftazidime + Clavulanic acid and FEL= Cefepime+ Clavulanic acid.*

Figure 2. Comparison of amino acid sequences of the mutant CTX-M gene (isolate-13), with normal CTX-M-15 isolates and known CTX-M-15 genes that obtained from NCBI. The alignment was performed usingg Bio Edit multiple sequence alignment.

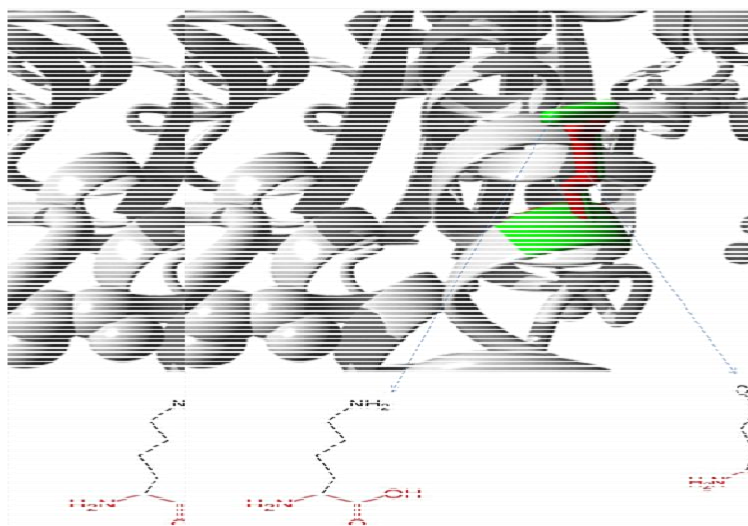

Figure 3. The 3D-structure of the mutant CTX-M-15 genne as compared to CTX-M-15 (AIQ77714). The protein is colored gray, the side chains of both the wild-type and the mutant residue are shown and colored green and red respectively.

wild type residue forms a hydrogen bond withh the Glutamic acid at position 20. The size difference betwween wild-type and mutant residue makes the new residue inn an in correct position preventing the same hydrogen bondd from binding at the same position of the original wild-typee residue. The wild type residue forms a hydrogen bond withh the Histidine at position 74 (Figure 2 and 3).

DISCUSSION

The production of ESBLs is one of the most important mechanisms of antimicrobial resistance. The recent increasing number of ESBLs producing *Enterobacteriaceae* is attributed to the emergence of CTX-M beta-lactamase (Livermore annd Hawkey 2005). CTX-M ESBLs are the most prevalent ESBLs worldwide (Paterson and Bonomo 2005). In Sudan there were many studies conducted to investigate the pr evalence of ESBLs genes (Mekki et al., 2010; Omar et al., 2013, Abdelmoneim et al., 2005; Hamedelnil and Eltayeb 2012). In this study *E. coli* was the most predominant ESSBLs producer presenting 58% of all isolates. This may be due to the fact that *E. coli* producing CTX-M beta-lactamasses is present as common intestinal flora being harmless to the host disseminating in

the community and hospital set up where they initiate infection (Munday et al., 2004).

In this study the resistance to Cefotaxime (45.6%) was higher compared to Ceftazidime (39.3%) and Cefepime (13 %). A high rate of resistance to Cefotaxime (67.8%) has also been reported by (Mobaiyen et al., 2007). after studying 786 ESBL-producing *E. coli* strains isolated in Tabriz hospitals between 2006 to 2007. Moreover, there was a high rate of Ceftazidime resistance (39.3%) among blaCTX-M-producing isolates, this could be due to high production of $bla_{CTX-M-15}$ in these isolates. $bla_{CTX-M-15}$ was reported by (Poirel et al., 2002) to have some hydrolytic activity against Ceftazidime, this may explain the high Ceftazidime resistance rate observed in this study. Furthermore, Cephalosporins resistance rate and production of bla_{CTX-M} genes in this study was higher in urban cities than rural areas. In Khartoum (Capital of Sudan) 23/36 (64%) of the isolates were resistant to Cephalosporins, followed by 54/100 (54%) in Madani (Gizera State) the second city in Sudan, and 49/92 (53%) in Wad Alabass Locality (Sinnar State), while lower resistance rate was observed in rural areas such as Abu Rugba village 2/77 (2.6 %). These findings could be due to the fact that cities are more crowded than rural areas that facilitate ESBLs spread, in addition to the increase in antibiotics consumption in cities where the antibiotics are available and affordable in contrast to rural areas where these antibiotics are either unavailable or very expensive, and this is clearly observed in the results of Abu Rugba village where they were neither hospitals nor pharmacies. In Sudan, unfortunately, the Cephalosporins and other antibiotics are over the counter medication which explains the overuse of the antibiotics (Hamedelnil and Eltayeb 2012).

Bla_{CTX-M} genes were detected in 96/128 (75%) of the ESBLs producers in this study, these findings are similar to those reported in Argentinean public hospitals where CTX-M accounted for roughly 70% of all ESBLs (Quinteros et al., 2003). The high prevalence rate of ESBLs is largely explained by the uncontrolled use of antibiotics and the absence of active infection control programs with good antibiotic policies. The ESBLs producers which were negative for bla_{CTX-M} genes 32/128(25%) in this study may harbor other ESBLs genes which confirms the need for further research in this field.

$bla_{CTX-M-15}$ and $bla_{CTX-M-14}$ are by far the most important ones, virtually invading all human and animal compartments as well as the environment all over the world (Cantón 2008; Hawkey and Jones 2009). This study highlighted the alarming explosive spread of $bla_{CTX-M-15}$-producing *Enterobacteriaceae* in different Sudanese regions; even in a remote village e.g.,(Abu Rugba). $bla_{CTX-M-15}$ was the most predominant one that representing 18/23 (78.3%) of all $bla_{CTX-M-like}$ types. This could be probably one reason for the growing problem of antibiotic resistance. This novel study in Sudan elaborates the presence of $bla_{CTX-M-14}$ (13%), $bla_{CTX-M-98}$ (4.3%) and $bla_{CTX-M-27}$ (4.3%). The transition mutation in isolate-13 made a difference in charge between the wild-type and mutant amino acid, which introduced the opposite charge at this position. This possibly disrupts contacts with other molecules. The wild-type and mutant amino acids differ in size. The mutant residue is smaller than the wild-type residue, this will cause a possible loss of external interactions.

In conclusion this study showed high prevalence of $bla_{CTX-M-15}$ producing bacteria in different Sudanese regions. These findings necessitate the need for epidemiological monitoring and prudent use of antimicrobial agents to limit the spread of $bla_{CTX-M-15}$ producing isolates. These results presents an alarming situation in Sudan which indicates the needs for further research in the field accompanying prompt control measures to prevent infections with theses resistant isolates.

Ethics statement

This study was specially approved by Biotechnology park-African City of Technology, Sudan (http://www.act.sd), since the samples from diagnostic centers, not directly from a human.

Disclosure

All authors have approved the final article and declared no conflicts of interest.

Availability of data and material

All sequences in this study were submitted to the NCBI database (http://www.ncbi.nlm.nih.gov/), and their accession as follows; **KP317181, KP317185, KP317182, KP317183, KP317186, KP317198, KP317184, KP317187, KP317188, KP317189, KP317199, KP317190, KP309815, KP317201, KP317191, KP317192, KP317193, KP317202, KP317200, KP317194, KP317195, KP317196 and KP317197**.

Author's contribution

HNA carried out the molecular genetic studies, doing the sequence alignment. MAS and AIH drafted the manuscript and supervised this research. NMA participated in experiments design and PCR. MMM conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.

Competing interests

None declared.

Funding

None.

ACKNOWLEDGMENTS

The authors kindly thank Hassuna Eltayeb from Abu Dhabi Media Co. for language editing of the manuscript. Also, we thank the staff and students of the Institute of endemic diseases, University of Khartoum, Sudan. We acknowledge Dr. Hiba Salah-Eldin, Dr. Saher M Bakhiet for their support, Ms. Haifa from Wad Madani Hospital and Mr. Riham from Haj Alsafi Hospital for their assistance in sample collection.

REFERENCES

Abdelmoneim Awad, Idris Eltayeb, Lloyd Matowe, Lukman Thalib (2005). Self-medication with Antibiotics and Antimalarials in the community of Khartoum State, Sudan. *J Pharm Pharmaceut Sc*. 2005; 8(2): 326-331.

Alsadig G, Mohamed A. Arbab, Sawsan AH Aldeaf (2014). Allele Frequency of P53 Gene Arg72Pro In Sudanese Meningioma Patients And Controls. *In.J. of Scientific and Technology Res*. 2014; 3 (6). 2014; 2277-8616.

An e-Science approach with life scientist friendly interfaces (2010). “Protein structure analysis of mutations causing inheritable diseases” BMC Bioinformatics. 8; 11(1):548; PMID: 21059217; DOI: 10.1186/1471-2105-11-548. Sites in protein structures*”. J MolBiol*: 2010; 339(3):607-33.

Atschul SF, Madden TL, Schaffer AA (1997). Gapped BLAST and PSI-BLAST. A new generation of protein database search programmes”. *Nucleic Acid Res* 25: 3389-3402.

Bonnet R (2004). Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. *Antimicrob. Agents Chemother*. 2004; 48, 1-14.

Cantón R (2008). “Epidemiology and evolution of β-lactamases,”in *Evolutionary Biology of Bacterial and Fungal Pathogens*, eds F.Baquero, C. ombela, G.H. Casslel, and J. A. Gutierrez-Fuentes (Washington: ASM Press). 2008;249–270.

Cao V, Lambert T, Courvalin, P (2002). ColE1-like plasmid pIP843 of *Klebsiella pneumoniae* encoding extended-spectrum ß-lactamases CTX-M-17. *Antimicrob Agents Chemother*. 2002;46, 1212–1217.

Clinical and Laboratory Standards Institute (2011). Performance Standards for Antimicrobial Susceptibility Testing; Twenty First informational Supplement, CLSI document M 100-S21. Wayne, PA; USA: Clin and Lab standards Institute; 2011.

Gröbner S, Linke D, Schutz W, Fladerer C, Madlung J (2009). Emergence of carbapenem-non-susceptible extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates at the university hospital of Tübingen, Germany. *J Med Microbiol* 2009, 58(Pt 7): 912– 922.

Hall TA (1999). “Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT”. *Nucl. Acids*. Symp. Ser. 1999; 41:95-98.

Hamedelnil FY, Eltayeb HN (2012). Molecular detection of Extended Spectrum β-lactamases (ESBLs) genes in *E. coli* isolated from urine specimens. *Int . J of advanced and scientific research.* October, 2012, ISSN 2249-9954.

Hawkey PM, Jones AM (2009). The changing epidemiology of resistance. *J. Antimicrob. Chemother.* 2009; 64, i3–i10.

IBM SPSS (2012). Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp, Released 2012. www.ibm.com/software/analytics/spss/ IBM Corp.

John B, Alexandre L, Mark B (2001). Gene Mark S: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions”. *Nucleic Acids Research* 29. 2001; 2607-2618.

Karim A, Poirel L, Nagarajan S, Nordmann P (2001). Plasmid-mediated extended-spectrum β-lactamase (CTX-M-3 like) from India and gene association with insertion sequence ISEcp1. *FEMS Microbiol Lett* 2001, 201(2): 237-241.

Livermore DM, Hawkey PM (2005). CTX M changing the face of ESBLs in UK. *J Antimicrob Chemother*. 2005; 56:451–4.

Livermore DM, Woodford (2006). The β-lactamase threat in *Enterobacteriaceae, Pseudomonas* and *Acinetobacter*. *Trends Microbiol* 2006, 14(9): 412-420.

Mekki AH, Hassan AN, Elsayed DM (2010). Extended Spectrum Beta Lactamases Among Multi Drug Resistant *Escherichia Coli* and Klebsiella Species Causing Urinary Tract Infections In Khartoum. *J Bact Res*.2010; 2(3):18-21.

Mobaiyen H, Nahaei MR, Amirmozafari N, Moaddab SR, Hoseni F (2007). First report of CTX-M type β -lactamase producing clinical *Escherichia coli* isolates from intensive care units in Tabriz hospitals. Abstr. 1st *Iranian Cong of clinical Microbio,* Shiraz, Iran, Abstracts' Book, 2007; 152.

Munday CJ, Whitehead GM, Todd NJ (2004). *J Antimicrob Chemother*. 2004; 54:628–33.

Murray PR, Baron EJ, Jorgensen, Landry JH, Michael ML (2007). *Manual of Clinical microbiology. 9th ed*. Washington: American Society for Microbiology; 2007.

Omar BA, Alfadel OO, Atif HA, Mogahid ME (2013). Prevalence of TEM, SHV and CTX-M genes in *Escherichia coli* and *Klebsiella* spp. in urinary isolates from Sudan with confirmed ESBL phenotype, *Life Science Journal* 2013;10(2) http://www.lifesciencesite.com.

Paterson DL, Bonomo RA (2005). Extended-spectrum beta-lactamases a clinical update. *Clin Microbiol Rev* 2005, 18(4):657-686.

Pena C, Pujol M, Ricart A, Ardanuy C, Ayats J (1997). Risk factors for faecal carriage of *Klebsiella pneumoniae* producing extended spectrum beta-lactamase (ESBL-KP) in the intensive care unit. *J. Hosp. Infect*. 1997;35:9–16.

Poirel L, Kampfer P, Nordmann P (2002). Chromosome-encoded Ambler class A beta-lactamase of *Kluyvera georgiana*, a probable progenitor of a subgroup of CTX-M extended-spectrum beta-lactamases. *Antimicrob Agents Chemother.* 2002;46(12):4038-40.

Quinteros M, Radice M, Gardella N, Rodriguez MM, Costa N, Korbenfeld D (2003). Extended-spectrum Beta lactamases in *Enterobacteriaceae* in Buenos Aires, Argentina, public hospitals. Antimicrob Agents Chemother 2003; 47: 2864-7.

Schwaber MJ, Carmeli Y (2007). Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. *Journal of Antimicrobial Chemotherapy* 2007;60(5):913-20.

Wayne PA (2006). Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. 7th ed. M7–A7. *Clinical and Laboratory Standards Institute*; 2006.

Wayne Pa, USA (2008). Performance standards for antimicrobial susceptibility testing, eighteenth informational supplement,” Tech. Rep. M100-S18, Clinical and Laboratory Standards Institute; 2008.