International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.7, pp 645-649, 2017

Prevalence of SHV-extended spectrum β-lactamase producing carbapenem –resistant *Klebsiella pneumoniae* among patients with lower respiratory tract infections in Babylon Province-Iraq

***Fatima Moeen Abbas**

Department of Biology, College of Sciences for Women, Babylon University, Iraq.

Abstract: This study was carried out to screen the prevalence of *Klebsiella pneumoniae* isolated from patients with lower respiratory tract infections in Babylon province. From December, 2015 to the end of March, 2016 ,a total of 100 sputum samples were collected from patients visited or hospitalized Merjan Teaching Hospital and Al-Hashimya General Hospital. Fifteenth (65%) isolates were identified as *Klebsiella pneumoniae*. All bacterial isolates were evaluated for extended spectrum β-lactamase (ESBL) production phenotypically using disk combination method. Eleven (73.3%) isolates were detected as ESBL-producers. Kirby-Bauer disk diffusion method was employed to determine resistance profile of ESBLs-positive isolates. Higher rates of resistance were observed for ampicillin and piperacillin antibiotics with (81.8%) and (72.7%) resistance rate, respectively, while the lowest rate was noticed for imipenem antibiotic (14.28%).Carbapenem-resistant isolates were investigated forbla_{SHV} gene by Polymerase Chain Reaction (PCR) method,2(100%) isolates gave positive results.
Keywords : *Klebsiella pneumoniae*, Lower respiratory tract infection, Antibiotics resistance, ESBL,bla_{SHV} gene,PCR.

Introduction

Antibiotic resistance to human pathogenic bacteria is frequently reported all over the world [1].Such bacteria have limited therapeutic options and regarded as multidrug resistant bacteria (MDR),some of these are extended spectrum β-lactamase (ESBL) producers with great variation among different geographical locations [2,3].ESBLs are plasmid mediated enzymes can confer resistance to oxymino-and ureidpencillins, oxyimino-cephalosporins and monobactams. Various types of ESBLs are known ,the most common ones are SHV, TEM, and CTX-M types.All have been increasingly described worldwide [4,5].

ESBL-producing organisms have been shown to cause higher morbidity and mortality and fiscal burden. Long hospital stay,sever underlying disease, age over 60 years and previous antibiotics exposure have been reported as a serious risk to acquire infections with ESBL producing strains [6]. The successful dissemination of these enzymes can be attributed to localization of ESBL-encoding genes onself transmissible or mobilizable broad host range plasmids[7,8,9].

The present work was designed to assess the prevalence of *Klebsiella pneumoniae* isolated from lower respiratory tract infections in Babylonprovince, identify ESBL-producing isolates phenotypically using disk combination method ,determine their resistance pattern and detect bla_{SHV} gene by Polymerase Chain Reaction (PCR)method.

Materials and Methods

Bacterial isolates

Between December, 2015 and March, 2016 ,a total of 100 sputum samples were obtained from patients visited or hospitalized Merjan Teaching Hospital and Al-Hashimy General Hospital / Babylon Province. These included: 65 males and 35 females at age of 28≥ years -old. All samples were cultured on different selective and differential media like Blood agar, MacConkey agar (Himedia , India) and Eosin methylen blue agar (Biolife, Italy). The isolates were identified on the bases of the standard methods described by Holt *et al*.[10],Colleee*t al*.[11] and MacFaddin[12].

Antimicrobial susceptibility testing

ESBL-positive isolates were screened for their antimicrobial susceptibilities testing using Kirby –Bauer disk diffusion method against 12 kinds of antibiotics from 6 different classes on Mueller- Hinton agar medium (Oxiod,England) [13]. The selected antibiotic disks were: ampicillin (10µg), piperacillin (100µg), amoxicillin-clavulanic acid (10 µg) , cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), cefoxitin (30µg),imipenem (10µg), meropenem (10µg), erythromycin (15µg), levofloxacin (5µg) andnorfloxacin(10 µg). After 18 hrs of incubation at 37 C^{o} ,the zones of inhibition were measured and compared with the Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. *Escherchia coli* ATCC 25922 (College of Medicine ,University of Kufa) was used as quality control.

Phenotypic detection of extended spectrum β-lactamase production (Recommended byCLSI,2010)

ESBLs detection was performed phenotypically using disk combination method aspreviously described [15].

Molecular detection of bla_{SHV} gene

DNA preparation

Bacterial DNA was prepared by salting out method as described by Pospiech and Neuman[16]. with some modification and used directly for PCR as DNA template.

PCR amplification

The SHV ESBL was amplified by Polymerase Chain Reaction (PCR) by using the following sets of designed primers (Bioneer,Korea):SHV/F (5¯ATGCGTTATATTCGCCTGTG3¯) and SHV/R (5¯-TGCTTTGTTATTCGGGCCAA¯3¯) (753bp), in a 25 µl reaction volume using 12.5 µl Go Taq Green Master Mix 2X (Promega), 5 µl DNA template, 2.5 µl of 10 pmol/ µl of specific up stream primers and, 2.5 µl of 10 pmol/ µl of specific down stream primers and 2.5 µl nuclease-free water. Thermal cycler was used under the following conditions: an initial denaturation at 94/30sec, followed by 35 cycles of denaturation at 94 C˚ for 30 sec, anneling at 60 C˚ for 1min, extension at 72 C˚ for 1 min. and a final extension step of 72 at 10 min [17,18]. Theampilification product was analyzed on 1.5% agarose gel at 70 volts for 2-3hrs , after staining with ethidium bromide, then the product was visualized under UV-Transilluminator and photographed with Geldocumentation system. 100 bp DNA Ladder (Bioneer,Korea) was used to assess PCR product size.

Results and Discussion

Out of 100 sputum samples,15 (65%) isolates were detected as *K.pneumoniae*. This result higher than that reported by Chiunnusamy*et al.* who identified 26(31.71%) *K.pneumoniae* from sputum samples [19].Other study characterized 30(23%) isolates as *K.pneumoniae* from sputum samples in a tertiary care hospital,India [1].

Phenotypic identification of ESBL production was carried out by disk combination method,results revealed that 11(73.3%) isolates were inferred as ESBL producers.(Table-1). Prevalence of ESBL-producing *K.pneumoniae* is recorded to be high 35 (%92.1),in Iran[5]. Other study from Nepal reported 4(5.8%) *K.pneumoniae* isolates as ESBL producers recovered from different clinical samples [20].Thus early detection of ESBL-production is of great clinical relevance because it is recommended that any strain that is confirmed as

ESBL producer should be considered as a resistant to all extended –spectrum beta lactam antibiotics regardless of susceptibility testing results [21].

Table (1):Numbers and percentages of extended spectrum beta lactamase producing*K.pneumoniae*using disk combination method.

No(%) of *K.pneumoniae* isolates	No. (%) of ESBL-positive isolates	No. (%) of ESBL-negative isolates
15	11(73.3%)	4(26.7%)

Antimicrobial susceptibility testing of ESBL-producing isolates revealed that the highest rate of resistance was observed for ampicillin antibiotics (81.8%), the next most resistant antibiotics was piperacillin (72.7%) .(Table-2).In a research work achieved by Romanus and Egwushowed that (94%) of*K.pneumoniae*isolates were resistant to ampicillin [22].A previous report demonstrated (100%) resistance rate for ampicillin by *K.pneumoniae*, all were ESBL-producers [23].Such higher resistance may be attributed to the excessive use of these drugs in Babylon hospitals .

Table(2):Antibiotics resistance pattern of ESBL -producing *Klebsiella pneumonia* against various antibiotics(n=11).

Antibiotic class	Agent used	No.(%) of resistant ESBL-producing isolates
Penicillins	ampicillin	9 (81.8%)
	piperacillin	8 (72.7%)
β –lactams /β- lactamase inhibitor combinations	amoxicillin-clavulanic acid	6 (54.5%)
Cephems	cefotaxime	7 (63.6%)
	ceftazidime	7 (63.6%)
	cefriaxone	8(72.7%)
	cefoxitin	6(54.5%)
Penems	imipenem	2(18.1 %)
	meropenem	4(36.3%)
Macrolides	erythromycin	5 (45.4%)
Quinolones	levofloxacin	4 (36.3%)
	norfloxacin	4 (36.3%)

However, the lowest rate of resistance was detected with imipenem antibiotic (18.1%).(Table -2). While Olowe*et al*. recorded zeroresistance to imipenem among *K.pneumoniae* recovered from different clinical samples in south western Nigeria [21].

PCR analysis revealed that 2(100%) carbapenem-resistant *K.pneumoniae* isolates harbored *bla* SHVgene.Fig (1).An *et al*.detected 120 (75.9%) ESBL-producing *K.pneumoniae* harbored *bla*SHV in multiple medical centers in China [24]. Also,*bla*SHV gene was identified in 8 (32%) *K.pneumoniae* isolated from clinical specimen including sputum samples in Nigeria[21].

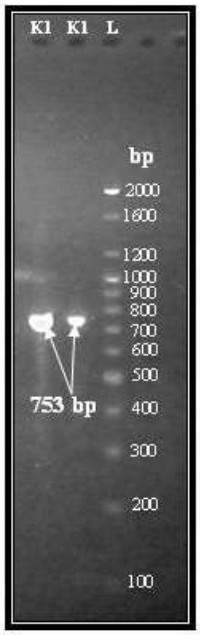

Figure (1): Agarose gel electrophoresis showing PCR product of*bla*SHV gene at 753bpin 2 isolates of carbapenem resistant *K.pneumoniae* (lane 1,2) .The DNA moleculer size marker (100- bp Ladder) is shown in lane L.

Conclusions

SHV-type ESBL gene was detected in *K.pneumoniae* recovered from lower respiratory tract infections in Babylon province.Further surveillance of multiple ESBL-harboring organisms with MDR phenotype is of great issue in the treatment and selection of the drug of choice.

Acknowledgement

Special thanks goes to medical staff in Merjan Teaching Hospital and Al-Hashimya General Hospital for their support to carry out thisstudy.

References

1. Shilpa, K1; Thomas,R. and Ramyashree, A.(2016).Isolation and antimicrobial sensitivity pattern of *Klebsiella pneumoniae* from sputum samples in a tertiary care hospital.Int. J. Curr. Res. Med. Sci. 2(1): 58-64.
2. Behrooozi, A.;Rahbar,M.andYousefi, J.V.(2010) Frequency of extended spectrum betalactamase (ESBLs) producing *Escherichia coli* and *Klebseilla pneumonia* isolated from urine in an Iranian 1000-bed tertiarycare hospital. Afric. J.Microb.Res. 4: 81-84.
3. Xie, L.;Xu, M.; Yang, T.; Zhu, C. Zhu,B.and Hu, Y. (2010) Studies on amino acid replacement and inhibitory activity of aβ-lactamase inhibitory peptide. Biochem. 75: 336-341.
4. Grobner, S.; Linke, D.; Schutz, W.; Fladerer, C.; Madlung, J. Autenrieth, I. B.; Witte, W. and Pfeifer, Y. (2009). Emergence of carbapenem non- susceptible extended- spectrum β - lactamase- producing *Klebsiella pneumoniae* isolates at the university hospital of Tubingen, Germany. J. Med. Microbiol. 58:912-922.
5. Mansury,D.;Motamedifar،M.;Sarvari،J.;Babak Shirazi,B. andKhaledi, A.(2016).Antibiotic susceptibility pattern and identification of extended spectrumβ-lactamases (ESBLs) in clinical isolates of *Klebsiella pneumonia* from Shiraz, Iran.Iran. J. Microbiol. 8 (1): 55-61.
6. Silva ,N.;Oliveira,M.;Bandeira,A.C.;andBrites ,C.(2006).Risk factors for infection by extended – spectrum beta-lactamase producing *Klebsiella pneumoniae* in a teriary hospital in Salvador. Braz.J.Infect.Dis.10:191-192.

7. Szabo, D.;Melan, M.A.;Hujer, A.M.;Bonomo, R.A.;Hujer, K.M.; Bethel, C.R.;Kristof,K.and Paterson, D.L.(2005) Molecular analysis of the simultaneous production of twoSHV-type etended-spectrum Beta-lactamases in a clinical isolate of *Enterobacter cloacae* by using single-nucleotide polymorphism genotyping. Anti-Age. Chemo.; 49: 4716-4720.
8. Jean ,S.S.and Hsueh,P.R. (2011) High burden of antimicrobialresistance in Asia. Int.J.Antimicrob. Agents 37: 291–295.
9. Dhillon, R.H.andClark, J. (2012) ESBLs: a clear and presentdanger. Crit.Care Res.Pract. 625170.
10. Holt, J. G.; Krieg, N. R.; Sneath, H. A.; Stanley, J. T. and Williams, S. T. (1994). Bergeys manual of determinative bacteriology. 9th. Ed., Baltimore; Wiliams and Wilkins, USA.
11. Collee, J. G.; Fraser, A. G.; Marmiom, B. P.; and Simmon, A. (1996). Mackie and McCarteny Practical Medical Microbiology. 4th ed. Churchill Livingstone Inc., USA.
12. MacFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria. 3rd ed. Lippincott Williams and Wilkins, USA.
13. Bauer, A. W.; Kirby, W.M.M.; Sherris, J.C and Track, M. (1966). Antibiotic susceptibility testing by standardized single disc method .Am.J. Clin.Pathol.45: 493-496.
14. Clinical and Laboratory Standards Institute (CLSI). (2010). Performance standards for antimicrobial susceptibility testing; 20th. Informational Supplement. Approved standard M07-A8. Clinical and Laboratory Standards Institute.
15. Wadekar,M.D.;Anuradha,K .andVenkatesh,D.(2013). Phenotypic detection of ESBL and MBL inclinical isolates of *Enterobacteriaceae* .Int. J. Curr. Res. Aca . Rev. 1 (3):89-95.
16. Pospiech, T. and Neumann, J. (1995). In genomic DNA isolation Kieser eds. JohnInnes Center. Norwich NR4 7UH. U.K.
17. Paterson, L. D.; Hujer, K. M.; Hujer, A. M.; Yeiser, B. and Michael, D. (2003). Extended- spectrum β - lactamases in *Klebsiella pneumoniae* bloodstream isolates from seven countries: dominance and widespread prevalence of SHV and CTX-M type β - lactamases. J. Antimicrob. Agents Chemother. 47: 3554-3560.
18. Hujer, K. M.; Hujer, A. M.; Hulten, E. A.; Bajaksouzian, S.; Adams, J. M.;Donskey, C.J.;Ecker, D.J.;Massire,C.;Eshoo,M.W.;Sampath,R.;Thomson,J.M.;Rather,P.N.;Craft,D.W.;Fishbian,J.T.;Ewell,A. J.; Jacobes,M.R.;Paterson,D.L. and Bonomo,R.A. (2006). Analysis of antibiotic resistance genes in multidrug resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the walter reed army medical center. Antimicrob. Agents Chemother. 50(12): 4114-4123.
19. Chinnnusamy, N.; Vedachalam,D. and Arumugam,V.(2016). A study on bacteriological profile and antimicrobial susceptibility pattern of sputum samples in patients with lower respiratory tract infections a tertiary care hospital. Indian .J .Microbiol. Res. 3(1):27-30.
20. Poudyal,S.;Bhatta,D.R.;Shakya,G.;Upadhyaya,B.; SP Dumre,S.P.; Buda, G.andKandel,B.p.(2011). Extended Spectrum â-lactamase producing multidrug resistant clinicalbacterial isolates at National Public Health Laboratory, Nepal.Nepal Med . Coll. J. 13(1): 34-38.
21. Olowe,O.A.; Oladipo, G. O.; Makanjuola, O.A. and Olaitan, J. O.(2012).Prevalence of extended spectrum beta-lactamass (ESBLs) carrying genes in *Klebsiella*spp. from clinical samples at ile-ile, south western Nigeria. Int. J. Pharm. Med. and Bio.Sc.1(2):129-138.
22. Romanus, I.I. and Egwu,O.A.(2011).Analysis of antibiotic susceptibility of *Klebsiella pneumoniae* isolated from different clinical specimen in Enugu state.Cana.J.pure and appl.Sci.5(3):1609-1614.
23. Huang,S.Y.; Pan, K.Y.; Liu,X.Q. Xie,X.Y. Dai, X.L. ; Chen, B.J. ; Wu , X.Q. and Li, H.Y. (2015). Analysis of the drug-resistant characteristics of *Klebsiella pneumoniae* isolated from the respiratory tract and CTX-M ESBL genes.Gen. and Mol. Research. 14 (4): 12043-12048.
24. An,S.;Chen,J.;Wang,Z.;Wang,X.;Yan,X.;Li,J.; Chen, Y.;Wang,Q.;Xu, X.;Li,J.;Yang,J.;Wang,H.and Gao,Z. (2012).Predominant characteristics of CTX-M-producing *Klebsiella pneumonia* isolates from patients with lower respiratory tractinfection in multiple medical centers in China. FEMS Microbiol.Lett. 332 :137–145.

****