WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 145-154. Research Article ISSN 2277– 7105

PREVALENCE OF *BLATEM* AND *BLASHV* GENES IN MULTIDRUG RESISTANT *KLEBSIELLA PNEUMONIAE* ISOLATED FROM HOSPITAL'S PATIENTS WITH BURNS INFECTIONS IN AL-NAJAF GOVERNORATE – IRAQ.

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Article Received on
27 April 2015,

Revised on 22 May 2015,
Accepted on 14 June 2015

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ABSTRACT

The aim of the present study was to investigate the prevalence of *blaTEM* and *blaSHV* genes in *Klebsiella pneumoniae* isolates that isolated from 90 hospital's patients with burns infection in AL-Najaf Governorate – Iraq. The results of the current study demonstrated that out of 90 burns swabs there were 12 isolates were diagnosed as *Klebsiella pneumoniae* The susceptibility test for 12 isolates of *Klebsiella pneumoniae* proved that there was highly resistance to antibiotic; their pattern of antimicrobial susceptibility showed that 100% was resistant to amoxicillin , amoxiclav and nitrofurantoin , 83.33% was resistant to cefotaxime , 66.66% was resistant to ceftriaxone, 91.66% was resistant to and ceftazidime, 41.66% was resistant to gentamicin , amikacin , tobramycin and tetracycline. *blaTEM* and *blaSHV* genes in multidrug resistant *Klebsiella pneumoniae* were detected by using monoplex PCR method, the results demonstrated that there were 12 isolate (100 %) was positive for *blaTEM* gene and 11 isolates (91.66%) was positive for *blaSHV* gene.

KEYWORDS: *K.pneumoniae*, MDR, *blaTEM* , *blaSHV*, Iraq.

INTRODUCTION

Klebsiella pneumoniae its gram negative, non-motile and capsulated bacteria, recognized as a common opportunistic pathogen, and cause many infections, it accounts for a significant proportion of healthcare-associated, or nosocomial, infections that are frequently caused by gram-negative enterobacteria.[1] In many reports, it is one of the

four most common gram-negative pathogens, together with *Pseudomonas aeruginosa*, *E. coli*, and *serratias spp.*[2] Many reports have been published worldwide on outbreaks caused by *K. pneumoniae* in different healthcare settings, like neonatal wards, nursing homes, and intensive care units.[3,4,and5] *K. pneumoniae* is inherently resistant to penicillins, including semi-synthetic broad-spectrum penicillins. Therefore, the drug of choice for empirical treatment is often a cephalosporin. However, the use of cephalosporins is known to select for resistant *K. pneumoniae* strains.[6] This is of great concern in human healthcare around the world. The number of *K. pneumoniae* strains producing ESBL variants of the widespread plasmid-encoded beta-lactamases belonging to the enzyme families *TEM*, *SHV*, and *CTX-M* are constantly.[7] *Klebsiella pneumoniae* is inherently resistant to penicillins and early cephalosporins due to constitutive production of a chromosomally encoded class a group 2b beta-lactamase.[8] In addition to this enzyme, many *K. pneumoniae* strains produce one or more plasmid-mediated beta-lactamases. The most common belong to the enzyme families *TEM*, *SHV*, and *CTX-M* .[9, 10]

MATERIALS AND METHODS

Isolation and identification of *Klebsiella pneumoiae* isolates

Klebsiella pneumoniae isolates were isolated from patients with burns infections admitted to the AL-Sadder Medical City in AL-Najaf Governorate-IRAQ. From the period between August to January 2015. All bacterial isolates were identified by culture, microscopic and biochemical characteristics tests[11] and by using the Vitek®2 system (bioMe´rieux, France).

Antimicrobial susceptibility testing

Susceptibility testing was done for 10 types of antibiotics were performed by using the disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute 2012.[12]

DNA extraction

A total DNA was extracted by using DNA extraction kit (Bioneer-Korea) According to the manufacturer's instructions.

Primers

Table 1 shows the primers used the current study (Ensor *et al*., 2009.).[13]

Table 1: Primers used in the current study

gene	Oligo Sequence (3′→5′)	Product size (bp)
SHV	F- GGCCGCGTAGGCATGATAGA R-CCCGGCGATTTGCTGATTTC	714
TEM	F- CAGCGGTAAGATCCTTGAGA R-ACTCCCCGTCGTGTAGATAA	643

Programs of monoplex PCR Thermocycling conditions

Table 2 shows the programs of monoplex PCR thermocycling conditions used the current study (Ensor *et al*., 2009.).[13]

Table 2: Programs of monoplex PCR Thermocycling conditions used the current study

	Initial Denaturation C^{o} / Time	Cycling condition C^{o} / Time				Final extension C^{o} / Time
Gene		Denaturation	Annealing	Extension	Number of Cycles	
blaTEM	95 C^{o} /5 min	94 C^{o} /30 sec	52C^{o}/45 sec	72C^{o}/45sec	30	72C^{o}/7min
blaSHV	95 C^{o} /5 min	94 C^{o} /30 sec	55C^{o}/60 sec	72C^{o}/45sec	30	72C^{o}/7min

Agarose Gel preparation and DNA Loading

This method was carried out according to Bartlett and Stirling (1998).[14]

Statistical analysis

Statistical analysis was performed with GraphPad Prism version 5software, percentages was used for the comparison between samples of the study.

RESULTS

Total specimens

The result proved that the total numbers of *k.pneumoniae* isolates were 12 from total 90 specimens isolated from patients with burns infection, with percentage 13.333%. (Table3).

Table 3: Total and percentage of *K.pneumoniae* isolates that isolated from burns infections

Total of burns specimens	*k.pneumoniae*	%
90	12	13.333

Antimicrobial susceptibility testing

The results of the present study proved that all twelve isolates of *K. pneumoniae* were highly resistance to 3rd generation of cephalosporins and other antibiotics (Table 4). Their pattern of antimicrobial susceptibility showed that 100% was resistant to amoxicillin, amoxiclav and nitrofurantoin, 83.33% was resistant to cefotaxime, 66.66% and 91.66% was resistant to ceftriaxone and ceftazidime, respectively, 41.66% was resistant to gentamicin, amikacin, tobramycin and tetracycline.

Table 4: Antibiotics sensitivity test for 12 clinical isolate of *Klebsiella pneumoniae*

Antibiotics	Concentration	Sensitive	Intermediate	Resistant	Percentage of resistance
Amoxicillin	25 µg	0	0	12	100%
Amoxiclav	30 µg	0	0	12	100 %
Cefotaxime	30 µg	2	0	10	83.33%
Ceftriaxone	30 µg	4	0	8	66.66%
Ceftazidime	30 µg	1	0	11	91.66%
Gentamicin	15 µg	7	0	5	41.66%
Amikacin	30 µg	4	3	5	41.66%
Tobramycin	10 µg	6	1	5	41.66%
Tetracycline	30 UI	7	0	5	41.66%
Nitrofurantoin	30 µg	0	0	12	100 %

Molecular detection of *blaTEM* and *blaSHV* genes in *K. pneumoniae* isolates

blaTEM and *blaSHV* genes were molecular detected in 12 isolates of *K. pneumoniae* by using monoples PCR. The result of the current study demonstrated that there was 12 isolates (100%) were positive for *blaTEM* gene and 11 isolates (91.66%) were positive for *blaSHV*. (Figures 1 and 2), respectively.

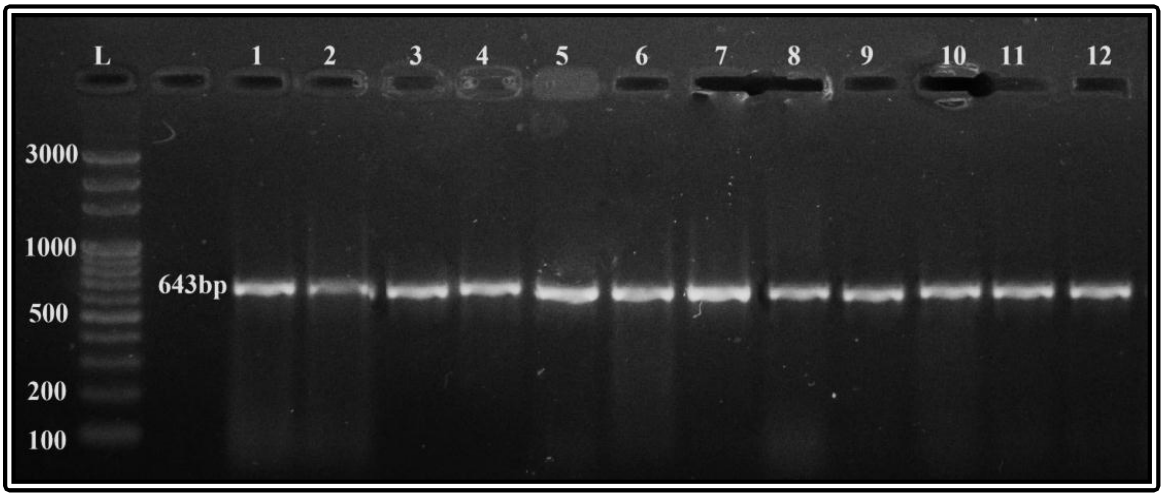

Figure 1: Ethidium bromide-stained agarose gel electrophoresis of monoplex PCR amplified products from extracted total DNA of *klebsiella pneumoniae* isolates isolated from different clinical specimens. Lane: (1 to 12 isolates) amplified with diagnostic *blaTEM* gene, show positive results at 643 bp . The electrophoresiswas performed at 80 volt for 95 Minutes. (L): DNA molecular size marker (100bp ladder , 100 to 3000 bp).

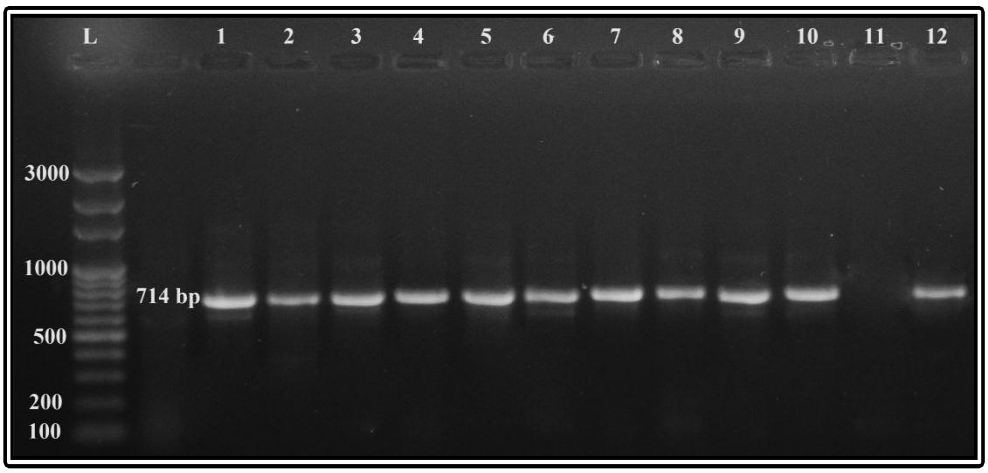

Figure 2: Ethidium bromide-stained agarose gel electrophoresis of monoplex PCR amplified products from extracted total DNA of *klebsiella pneumoniae* isolates isolated from different clinical specimens. Lane: (1 to 12 isolates) amplified with diagnostic *blaSHV* gene, show positive results at 714 bp . The electrophoresis was performed at 80 volt for 95 Minutes. (L): DNA molecular size marker (100bp ladder , 100 to 3000 bp).

DISCUSSION

Klebsiella pneumoniae it's one of the most important multidrug resistant pathogenic bacteria that cause several diseases such as, burns infections, urinary and respiratory tract infections, wounds and blood infections and liver abscess, because it has several important genes such

as *blaTEM* and *blaSHV* its remain the most important extended spectrum beta-lactamase producing microorganisms isolated from different clinical courses from hospital's patients worldwide. Burns infections considered is one of the most dangerous infections caused by highly multidrug resistance gram negative bacteria like *K.pneumoniae* , *P. aeruginosa* and *S.aureus*.

In the present study proved that out of 90 burns swabs there were 12 isolates were identified as *K.pneumoniae*. This result is in agreement with Amna *et al*., (2014)[15] and Majid *et al.,* (2014)[16] who they are reported that *klebsiella pneumoniae* and *Pseudomonas* were the most gram negative bacteria that causing burns , wounds , urinary tract infection , respiratory tract infection and blood with percentage range 12% , 10% and 15%, respectively. So burns injury considered a main problem in the world because of destroying the skin barriers that avoid invasion of microorganisms.[17] Therefore nosocomial infections that caused by *klebsiella pneumoniae* are the main causes of mortality and morbidity among patients.[18]

In the current study reported that *K.pneumoniae* was highly resistance to antibiotics especially to beta lactam and 3rd generation of cephalosporins (Table 4). This results are in agreement with many studies such as Coyle (2005).[19] and Iroha *et al*., (2011)[20] when they are reported that *klebsiella pneumoniae* was resistance to many antibiotics like : Amoxiclav with percentage 96%, Ceftazidime (98%), Cefotaxime (96.7%), Tobramycin (50%) and Gentamicin (74%). But in another study by Mariya and Sunil (2015)[21], *klebsiella pneumoniae* was resistance to amikacin with percentage (31.95%) , nitrfurantoin (52.78%) ,ceftriaxone (54.17%) ,cefotaxime (61.1%) and ceftazidime (62.5%.). *Klebsiella pneumoniae* strains have a high degree of resistance to third-generation cephalosporins (92%), cephalosporins are used as first-line therapy for burns infections and septicemia.[22, 23] *K. pneumoniae* is known for high resistance to various antibiotics, this bacterium has series of antibiotic resistance genes which can be transferred horizontally to other gram negative bacteria.[24] And associated with series of nosocomial infections in hospitals.[25, 26]

Multi drug resistance *K. pneumoniae* strains have caused many disease problems worldwide, the increasing prevalence of clinical MDR isolates has been associated with higher morbidity and mortality rates, posing a considerable threat to public health.[27]

In the current study some genes responsible for production of extended spectrum beta-lactams in *K. pneumoniae* have been detected, these genes are: *blaTEM* and *blaSHV* were detected by monoplex PCR using specific primer sequences which yielded product sizes of 643 bp and 714 bp, respectively. The result of the current study demonstrated that there was 12 isolates (100%) were positive for *blaTEM* gene and 11 isolates (91.66%) were positive for *blaSHV*. (Figures 1 and 2), respectively. The results of the present study are in agreement with Khosravi et *al.* (2013)[22] who they reported that the prevalence of *blaSHV* gene in *K.pneumoniae* isolates were 88%, on the other hand, in the same study, the prevalence of *blaTEM* gene was 34.61% of total *Klebsiella pneumoniae* isolates. About 80-90 % of *K.pneumoniae* strains are now considered to carry a *blaTEM* and *blaSHV* enzyme, transfer of plasmid between different bacterial species has been important way to transmission of drug resistance between bacterial species.

Plasmids resistance are the important source of extended spectrum beta lactamase transmission, transferable elements conferring resistance to antibiotics other than beta-lactams travel on or alongside the extended spectrum beta lactamase containing plasmids, lead to multidrug resistance bacteria. It is also that mechanism other than, addition to, plasmid mediated assist transfer of many kinds of resistance factors account for the phenomenon of co-resistance observed.[28] In Asia prevalence of extended spectrum beta-lactams producers among *K. pneumonia* a commonest nosocomial associated bacteria is reported to be more than 60-70% while for *Escherichia coli* it ranges from 40%-60%.[29] These pathogens account for an increased demands of beta-lactam drugs which lead to mutation of the bacteria resistance genes , this mutation causes production of the most feared beta-lactamase enzymes like *blaTEM* and *blaSHV* gens which have ability to hydrolyze all beta- lactam drugs including carbapenems and 3rd generation of cephalosporins antibiotics.[30]

CONCLUSION

There was strong relationship between highly prevalence of *blaTEM* and *blaSHV* gens and highly resistance to antibiotics especially to beta lactam and 3rd generation of cephalosporins in *Klebsiella pneumoniae* isolated from hospital's patients with burns infection in AL-Najaf Governorate – Iraq.

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