

Antimicrobial susceptibility patterns of *Klebsiella pneumoniae* isolated from older smokers and non-smokers of inpatients in intensive care unit infected with chronic pneumonia in AL-Najaf hospital, Iraq

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Abstract

The main aim of this study is to detect the antimicrobial susceptibility patterns and the prevalence of two extended-spectrum beta-lactamase genes (*bla-SHV* and *bla-TEM*) in 80 *K. pneumoniae* isolates from older of inpatients (smokers and non-smokers) infected with chronic pneumonia in AL-Najaf hospital, Iraq. The antimicrobial susceptibility test of 80 isolates proved that *K. pneumoniae* were 100% resistance to amoxicillin and nitrofurantoin. While *K. pneumoniae* was resistance to Amoxiclav with percentage 88.75% , Cefotaxime 86.25% , Ceftriaxone 92.5% and Ceftazidime 85%. On the other hand, *K. pneumoniae* was resistance to Gentamicin, Amikacin , Tobramycin and Tetracycline with percentage 72.5%, 61.25%, 51.25% and 73.75% respectively. All *K. pneumoniae* isolates from smokers of inpatients were highly resistance to most antimicrobials as compare with those isolates from non-smokers. Also, the results proved that there were 73.75% isolates were multi-drug resistance, 15% isolates were extensive-drug resistance and 11.25% isolates were pan-drug resistance. The results of genotypic detection proved that there were 45 isolates (56.25%) were harbored *bla-TEM* , 31 isolates (38.75%) were harbored *bla-SHV* gene and 18 isolates (22.5%) were harbored both two genes. Most *K. pneumoniae* isolates from smokers of inpatients were harbored antimicrobials associated genes more that those isolates from non-smokers. In conclusions: *K. pneumoniae* isolates from older smokers of inpatients infected with chronic pneumonia were more virulent than those isolates from non-smokers and we must be careful because these bacteria may be transferred to other healthy persons.

Keywords: *Klebsiella pneumoniae*, older inpatients, chronic pneumonia, antimicrobials, multi-drug resistance, *bla-SHV*, *bla-TEM*.

INTRODUCTION

Smoking is a most risk factor to cause respiratory infections and pneumonia worldwide. One of the best important steps can take to lower the risk of respiratory infections and pneumonia caused by viruses and opportunistic bacteria such as *K. pneumoniae* is a quitting smoking (Campagna et al., 2016). *Klebsiella pneumoniae* is an opportunistic pathogen, cause many infections, such as pneumonia and blood infections in hospital patients, it accounts for a significant proportion of healthcare-associated, or nosocomial, *K. pneumoniae* is gram negative bacteria , mannitol fermenter, capsulated and non-motile (Zhong et al., 2013; Aljanaby and Alhasnawi (2017). Many reports have been published worldwide about outbreaks of pneumonia caused by *K. pneumoniae* in intensive care units (Liu et al ., 1992; Arpin et al., 2003) . The number of *K. pneumoniae* strains producing extended-spectrum beta-lactamase variants of the widespread plasmid-encoded beta-lactamases belonging to the enzyme families *TEM*, *SHV*, and *CTX-M* are constantly (Jacoby and Munoz-Price, 2005) . *Klebsiella pneumoniae* is naturally resistant to 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 (Benedić, 2000). *Klebsiella pneumoniae* is inherently resistant to penicillins and early cephalosporins due to constitutive production of a chromosomally encoded class a group 2b beta-lactamase Petit, (1992). The most common belong to the enzyme families *bla-TEM* , *bla-SHV* and *bla-CTX-M* (Patel et al., 2009). Bacterial resistance is a frequent and significant problem in the hospital environment. Increased resistance among members of the *Enterobacteriaceae* family have culminated in the ever more frequent appearance of multi-resistant species, which represent an important public health problem that is in expansion, requiring multidisciplinary efforts for prevention and control, besides efficient laboratorial detection (Giske et al., 2011). Therefore, the main goal of this study was to investigate of antimicrobial

resistance patterns and prevalence of *bla-TEM* and *bla-SHV* genes isolated from smokers and non-smokers older inpatients in intensive care units infected with chronic pneumonia in AL-Najaf hospital, Iraq.

MATERIALS AND METHODS

Isolation and identification of *K. pneumoniae*

A total of 80 *K. pneumoniae* strains were isolated from older inpatients in intensive care units (60-70 years old) 49 men and 31 women (41 smokers and 39 non-smokers) infected with chronic pneumonia (diagnosis by specialist physician) in AL-Najaf hospital, Iraq during period from January to July 2017. Sputum samples were collected by sterile cups (Hi-media, India). Immediately, Sputum incubated with brain hart infusion broth (Oxoid,UK) at 37°C overnight and streaked (by sterile swab, Bioanalyse, Turkey) on blood agar (Oxoid,UK) and MacConkey agar (Oxoid,UK) surface and incubated aerobically in 37°C for 24h. All *K. pneumoniae* isolates were identified according standard biochemical tests (MacFaddin, 2000) and by using the Vitek®2 system (bioMérieux, France).

Antimicrobial susceptibility testing

Susceptibility testing was done for ten antimicrobials were performed by using the disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute 2014 (CLSI, 2014). These antimicrobials are; Amoxicillin 25 µg , Amoxiclav 30 µg , Cefotaxime 30 µg , Ceftriaxone 30 µg , Ceftazidime 30 µg ,Gentamicin 15 µg , Amikacin 30 µg , Tobramycin 10 µg , Tetracycline 30 UI , Nitrofurantoin 30 µg. Any bacterial isolate resist to a minimum at least 3 different classes of antibiotics it is multi-drug resistance (MDR), any bacterial strain remain susceptible to only one or two class of antibiotics it is extensive-drug resistance (XDR) and any bacterial isolate resistance to all sub classes in all classes of antibiotics it is pan-drug resistance (PDR) (CLSI, 2014).

DNA extraction, PCR primers and thermo cycling conditions

Boiling method was used for total DNA extraction according to method by Aljanaby and Alhasnawi (2017). The primer sequences were as follows: *bla-SHV* forward, 5' GGCCGCGTAGGCATGATAGA 3'; *bla-SHV* reverse, 5' CCCGGCGATTGCTGATTTC 3' and *bla-TEM* forward, 5' CAGCGGTAAGATCCTTGAGA3'; *bla-TEM* reverse, 5' ACTCCCGTCGTGTAGATAA 3' with product size 714 and 643 bp respectively (Ensor et al., 2009). The PCR program consisted of an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of DNA denaturation at 52 °C for 45s, primer annealing at 52 °C for 45s (for *bla-TEM* gene) and 55 C for 60s (for *bla-SHV* gene) extension 72°C, 45s . After the last cycle, a final extension step at 72°C for 7 min (Ensor et al., 2009). Fifteen-microliter aliquots of PCR product were analyzed by gel electrophoresis with 1.5% agarose. Gels were stained with ethidium bromide at 1.5 mg/ml and visualized by UV transillumination. A 100-bp DNA ladder.

Statistical analysis

Percentages were used for the comparison between samples of the study by using GraphPad Prism version 6 computer software.

RESULTS

Antimicrobial susceptibility testing

The results demonstrated that all 80 *K. pneumoniae* isolates (100%) were resistance to Amoxicillin and Nitrofurantoin. On the other hand, *K. pneumoniae* isolates were resist to Amoxiclav 88.75% , Cefotaxime 86.25%, Ceftriaxone 92.5%, Ceftazidime 85% and Tetracycline 73.75%. Also, *K. pneumoniae* showed moderate resistance to Gentamicin 72.5, Amikacin 61.25% And Tobramycin 51.25% (Table 1) and (Figure 1). All *K. pneumoniae* isolates from smokers patients were high resistance to

antimicrobials other than those isolates from nonsmokers patients (Table 2) and (Figure 2). The results proved that there were 59 isolates (73.75%) were multi-drug resistance (33 isolates from smokers patients and 26 isolates from nonsmokers patients), 12 isolates (15%) were extensive-drug resistance (8 isolates from smokers patients and 4 isolates from nonsmokers patients) and 9 isolates were (11.25%) pan-drug resistance (7 isolates from smokers patients and 7 isolates from nonsmokers patients) (Figure 3) and (Table 3).

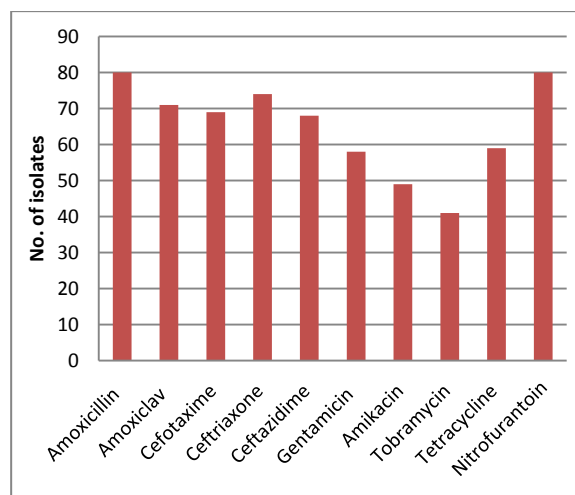


Figure 1: Total numbers of *K. pneumoniae* isolates from older inpatients infected with chronic pneumonia that were resistance to 10 antimicrobials. (N= 80 isolates).

Table 1: Antimicrobial susceptibility test of 80 *K. pneumoniae* isolates from older inpatients infected with chronic pneumonia.

Antimicrobials	Resistance (100%)	Intermediate (100%)	Sensitive (100%)
Amoxicillin 25 µg	80(100)	0(0.0)	0(0.0)
Amoxiclav 30 µg	71(88.75)	0(0.0)	9(11.25)
Cefotaxime 30 µg	69(86.25)	2(2.5)	9(11.25)
Ceftriaxone 30 µg	74(92.5)	0(0.0)	6(7.5)
Ceftazidime 30 µg	68(85)	2(2.5)	10(12.5)
Gentamicin 15 µg	58(72.5)	3(3.75)	19(23.75)
Amikacin 30 µg	49(61.25)	1(1.25)	30(37.5)
Tobramycin 10 µg	41(51.25)	9(11.25)	30(37.5)
Tetracycline 30 UI	59(73.75)	3(3.75)	18(22.5)
Nitrofurantoin 30 µg	80(100)	0(0.0)	0(0.0)

Table 2: Total numbers and percentages of *K. pneumoniae* isolates that were resistance to 10 antimicrobials isolated from older inpatients (smokers and non-smokers) infected with chronic pneumonia according to gender and. (N= 80 isolates).

Antimicrobials	Men (49)		Women (31)		Total resistance 80 (100%)
	Smokers 29 (100%)	Non-smokers 20 (100%)	Smokers 12(100%)	Non-smokers 19 (100%)	
Amoxicillin 25µg	29(100)	20(100)	12(100)	19(100)	80(100)
Amoxiclav 30µg	29(100)	20(100)	12(100)	10(52.6)	71(88.75)
Cefotaxime 30µg	29(100)	20(100)	12(100)	8(42.1)	69(86.25)
Ceftriaxone 30µg	29(100)	20(100)	12(100)	13(68.4)	74(92.5)
Ceftazidime 30µg	29(100)	20(100)	12(100)	7(36.8)	68(85)
Gentamicin 15µg	27(93.1)	15(75)	10(83.3)	6(31.5)	58(72.5)
Amikacin 30µg	20(68.9)	12(60)	10(83.3)	7(36.8)	49(61.25)
Tobramycin 10µg	14(48.2)	11(55)	10(83.3)	6(31.5)	41(51.25)
Tetracycline 30UI	24(82.7)	15(75)	8(66.6)	12(63.1)	59(73.75)
Nitrofurantoin 30µg	29(100)	20(100)	12(100)	19(100)	80(100)

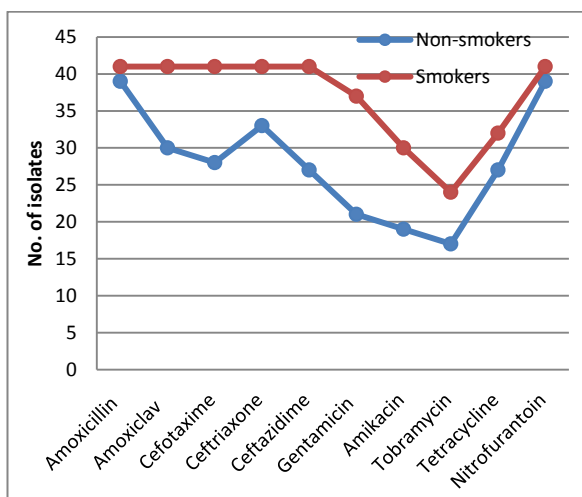


Figure 2: Total numbers of *K. pneumoniae* isolates from older smokers and non-smokers of inpatients infected with chronic pneumonia. (N= 80 isolates).

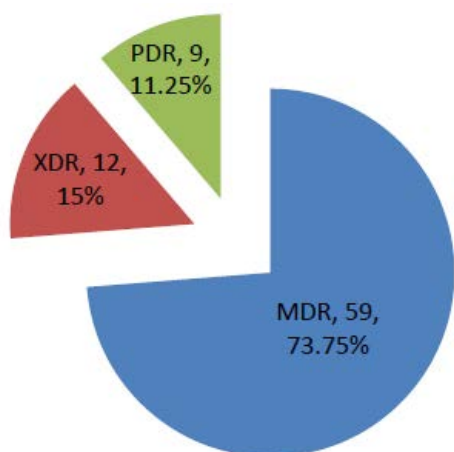


Figure 3: Numbers and percentages of multi-drug resistance, extensive-drug resistance and pan-drug resistance *K. pneumoniae* isolates from older inpatients infected with chronic pneumonia. MDR: multi-drug resistance, XDR: extensive-drug resistance, PDR: pan-drug resistance.

Table 3: Numbers and percentages of multi-drug resistance, extensive-drug resistance and pan-drug resistance *K. pneumoniae* isolates from older inpatients (smokers and non-smokers) infected with chronic pneumonia.

Older patients	MDR (100%)	XDR (100%)	PDR (100%)
Nonsmokers	26(32.5)	4(5)	2(2.5)
Smokers	33(41.25)	8(10)	7(8.75)
Total	59(73.75)	12(15)	9(11.25)

MDR: multi-drug resistance, XDR: extensive-drug resistance, PDR: pan-drug resistance.

Genotype detection of antimicrobials associated genes

The result of the current study proved that out of 80 *K. pneumoniae* isolates there were 45 isolates (56.25%) were harbored *blaTEM* (Figure 4) 35 isolates from smokers patients and 10 isolates from nonsmokers patients , 31 isolates (38.75%) were harbored *blaSHV* gene (Figure 5) 24 isolates from smokers patients and 7 isolates from nonsmokers patients , 18 isolates (22.5%) were harbored both genes (Figure 6) 12 isolates from smokers patients and 6 isolates from nonsmokers patients (Table 4).

DISCUSSION

There are many factors effect on lung and heart and lead to different complications. Smoking injures lung and alveolar epithelial cells resulting in diffuse infiltrates and parenchymal fibrosis (Caminati et al., 2012; Kumar et al., 2017). *Klebsiella pneumoniae* is one of the most important gram negative bacteria cause several diseases such as, pneumonia , wounds , blood infections and liver abscess (Aljanaby and Alhasnawi, 2017). *Klebsiella pneumoniae* harbored *bla-TEM bla-SHV and bla-CTX-M* genes, therefore remain the most important extended spectrum beta-lactamase producing bacteria isolated from different clinical courses from hospital's patients worldwide (Chang et al., 2015). In the present study proved that *K. pneumoniae* was high resistance to different antimicrobials such as beta lactam and 3rd generation cephalosporins (Table 1 and Figure 1). All *K. pneumoniae* isolates from smokers older of inpatients were highly resistance to most antimicrobials as a compare with those isolates from non-smokers (Table 2 and Figure 2). Also, the results of current study proved that there were 73.75% isolates were multi-drug resistance, 15% isolates were extensive-drug resistance and 11.25% isolates were pan-drug resistance (Figure 3 and Table 3) and most *K. pneumoniae* isolates from smokers older of inpatients were multi-drug resistance as a compare with those isolates from non-smokers.

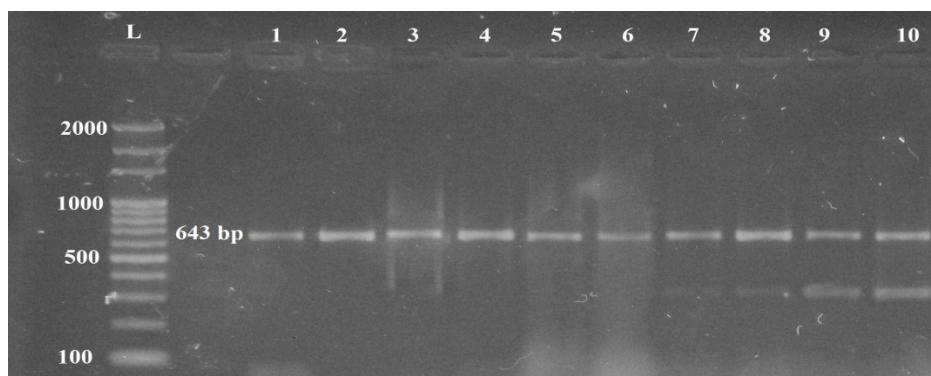


Figure 4: Ethidium bromide-stained agarose gel electrophoresis of PCR amplified products from extracted total DNA of *k. pneumoniae*. Lane: (1 to 10 isolates) amplified with diagnostic *blaTEM* gene, show positive results at 643 bp. The electrophoresis was performed at 70 volt for 80 Minutes. (L): DNA molecular size marker (100bp ladder).

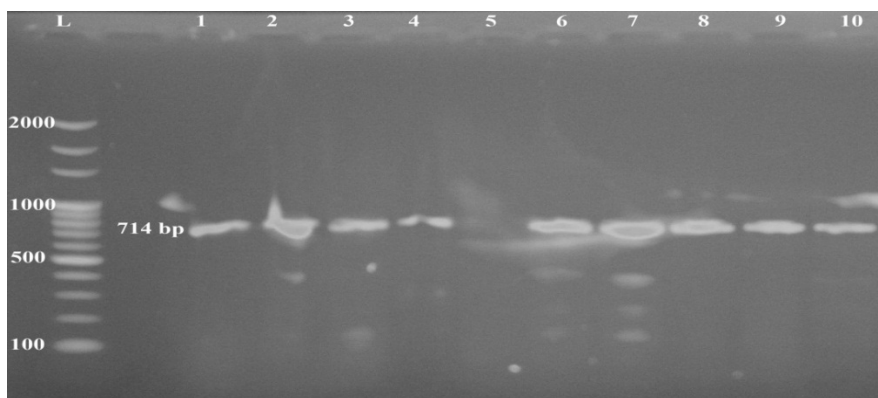


Figure 5: Ethidium bromide-stained agarose gel electrophoresis of amplified products from extracted total DNA of *K. pneumoniae*. Lane: (1 to 10 isolates) amplified with diagnostic *blaSHV* gene, show positive results at 714 bp . The electrophoresis was performed at 70 volt for 80 Minutes. (L): DNA molecular size marker (100bp ladder).

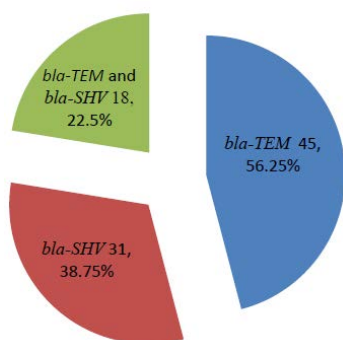


Figure 6: Prevalence of *bla-TEM* and *bla-SHV* genes in 80 *K. pneumoniae* isolates from older inpatients infected with chronic pneumonia.

Table 4: Numbers and percentages of prevalence of *bla-TEM* and *bla-SHV* genes in *K. pneumoniae* isolates from older inpatients (smokers and non-smokers) infected with chronic pneumonia. (N= 80 isolates).

Older patients	<i>bla-TEM</i> (100%)	<i>bla-SHV</i> (100%)	<i>Bla-TEM</i> and <i>bla-SHV</i> (100%)
Nonsmokers	10 (12.5)	7 (8.75)	6 (7.5)
Smokers	35 (43.75)	24 (30)	12 (15)
Totals	45 (56.25)	31 (38.75)	18 (22.5)

This results are in agreement with many reporters such as Coyle (2005) , Abdulsalaam *et al.*, (2013) and Aljanaby and Alhasnawi (2017) when they are reported that *k. pneumoniae* was resistance to Amoxiclav , Cefotaxime and Cefotaxime with high percentage. But in another study by Mariya and Sunil (2015) *k. pneumoniae* was resistance to amikacin , nitrofurantoin ,ceftriaxone, cefotaxime and ceftazidime with moderate percentage. *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 (Khosravi *et al.*, 2013; Cruz-Córdova *et al.*, 2014). *K. pneumoniae* is capable of resistance to different antibiotics , this bacterium has series of antibiotic resistance genes which can be transferred horizontally to other gram negative bacteria (Piddock, 2006) and associated with series of nosocomial infections in hospitals (Lewis *et al.*, 2007; Chikere *et al.*, 2008). Multi drug resistance *K. pneumoniae* caused many problems worldwide; the increasing prevalence of clinical MDR isolates has been associated with higher morbidity and mortality rates (Cao *et al.*, 2014).

In the present study some genes responsible for production of extended spectrum beta-lactams (*blaTEM* and *blaSHV*) in *K. pneumoniae* have been detected by PCR using specific primer sequences which yielded product sizes of 643 bp and 714 bp, respectively. The result of the 38.75% isolates were harbored

blaSHV gene (Figures 5) and 22.5% isolates were harbored both genes (Figure 6) and most *K. pneumoniae* isolates from smokers older of inpatients were harbored *blaTEM* gene , *blaSHV* gene and both genes as a compare with those isolates from non-smokers (Table 4).

The results of the present study are in agreement with Khosravi *et al.*, (2013) who they reported that the prevalence of *bla-SHV* gene in *K.pneumoniae* isolates were 88%, on the other hand, in the same study, the prevalence of *bla-TEM* gene was 34.61% of total *K. 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.

In middle east, the prevalence of extended spectrum beta-lactams producers among *K. pneumoniae* a commonest nosocomial associated bacteria is reported to be more than 65 % while for *Escherichia coli* it ranges from 45%-65% (Newire *et al.*, 2013; (Aljanaby and Alhasnawi, 2017; Aljanaby and Medhat , 2017).

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 (Vaidya, 2011). 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* genes which have ability to hydrolyze all beta- lactam drugs including carbapenems and 3rd generation of cephalosporins antibiotics (Aljanaby and Medhat , 2017).

CONCLUSION

K. pneumoniae isolates from older smokers of inpatients infected with chronic pneumonia were more virulent than those isolates from non-smokers and we must be careful because these bacteria may be transferred to other healthy persons.

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