JOURNAL OF PURE AND APPLIED MICROBIOLOGY, August 2014. Vol. **8**(4), p. 2713-2720

Antimicrobial Susceptibility and Distribution of β-lactamases in Extended-Spectrum Cephalosporins Insensitive *Klebsiella pneumoniae* in Riyadh, Saudi Arabia

Muhannad Al-Jameel[1], Mohamed H. Al-Agamy[2,3]*, Ibrahim Aljufalli[2] and Atef M. Shibl[2]

[1]Saudi FDA
[2]Pharmaceutics department, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.
[3]Microbiology and Immunology Department, Faculty of Pharmacy, Cairo, Egypt.

(Received: 18 April 2014; accepted: 02 June 2014)

Two hundred fifty *Klebsiella pneumoniae* isolates were collected during October 2013 to February 2014 from two different tertiary hospitals in Riyadh, Saudi Arabia. Eighty out of 250 isolates *K. pneumoniae* were selected according to reduced susceptibility to extended-spectrum cephalosporins (ESC). Phenotypic tests for extended-spectrum β-lactamases (ESBLs), carbapenemases, and AmpC enzyme-producing isolates were performed to detect the resistance phenotype of the isolates. The antimicrobial susceptibility testing was done. The whole collection was found to be tigecycline, colistin and fosfomycin sensitive. The resistance rates to amoxicillin, amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, ceftazidime, cefotaxime, ceftriaxone, cefoxitin, imipenem, amikacin, ciprofloxacin, sulphamethoxazole/trimethoprim, tetracycline were 100%, 95%, 90%, 48.75%, 77.5%, 86.25%, 86.25%, 55%, 3.75%, 7.5%, 70%, 88.75%, and 83.60%, respectively. ESBLs, and carbapenemase, and AmpC production were seen in 80%, 25% and 13.75% of ESC insensitive *K. pneumoniae* isolates, respectively. The prevalence of metallo- and non-metallo carbapenemase producing *K. pneumoniae* isolates were 8.75% (n= 7/80) and 17.5% (n=14/80), respectively. All carbapenemase producers were also found to be ESBLs positive and/or AmpC positive. Our study documented the high rate of antibiotic resistances and high prevalences of ESBLs, AmpC and carbapenemases in *K. pneumoniae* isolates.

Key words: AmpC, ESBL, carbapenemases, *Klebsiella pneumoniae*, Saudi Arabia.

Klebsiella pneumoniae is Gram negative, non motile, encapsulated, facultative anaerobic, lactose fermenting, rod shaped bacterium. *K. pneumoniae* is common opportunistic pathogen of nosocomial infections that are associated with pneumonia, urinary tract infection (UTI), bacteremia, septicemia as well as bacterial meningitis and biliary tract infection (Grover *et al*., 2013; Struve and Krogfelt, 2004). Unsystematic antibiotic use is a major factor that often results in multi drug resistant strains. β-lactams are the most frequently used antimicrobials for empirical therapy (Grover *et al*., 2013). Increasing antimicrobial resistance, especially towards third and fourth generation cephalosporins, cephamycins, and carbapenems have been reported in the last decade and poses serious therapeutic problems when treating *K. pneumoniae* infections in humans (Pfeifer *et al*., 2010). Production of β-lactamases is one of the tactics adopted by bacteria to develop

* To whom all correspondence should be addressed.
Mobile: +966553227824
Tel: +96614677202; Fax: +96614676295
E-mail:elagamy71@yahoo.com malagamy@ksu.edu.sa

resistance to β-lactams. Broad-spectrum β-lactamases are known to hydrolyze amino- and uriedopenicillins and first generation cephalosporins. ESBLs are known to hydrolyze all penicillins, extended-spectrum cephalosporins (ESC) and monobactams, but they lack hydrolytic activity on cephamycins and carbapenems. ESBLs are inhibited by β lactamase inhibitors (clavulanic acid, tazobactam, sulbactam). AmpC β-lactamases confer resistance to penicillins, cephalosporins, cephamycins and monobactams. The organisms develop resistance to β-lactam/β-lactamase inhibitor combinations but are usually sensitive to carbapenems. This lack of inhibition by cephamycins and β-lactamase inhibitors differentiates AmpC producers from the ESBL producers. Mechanism of resistance in AmpC can be chromosomal or plasmid mediated (Philippon *et al*., 2002). Carbapenems have become the drug of choice for serious infections by gram negative hospital associated pathogens including ESBL and AmpC producing *Enterobacteriaceae* (Pitout, 2008; Philippon *et al*., 2002). Carbapenem resistance in *Enterobacteriaceae* has been largely due to acquisition of carbapenemase genes belonging to Ambler classes A, B and D β-lactamases (Queenan and Bush, 2007). There are some limited studies which characterize ESBL and carbapenemases and there is no study is undertaken to characterize AmpC in Riyadh, Saudi Arabia. Therefore this study was devoted to update the resistance profile of *K. pneumoniae* isolates in Riyadh, Saudi Arabia during October 2013 to February 2014and to characterize the phenotypic resistance mechanisms to β-lactam antibiotics.

MATERIALS AND METHODS

Bacterial isolates

K. pneumoniae isolates (n=250) were isolated from clinical specimens submitted for bacteriological testing from in-patients admitted to hospital A and hospital B, Riyadh, Saudi Arabia**.** *K. pneumoniae* isolates were collected over a period of 5 months from October 2013 to February 2014. Eighty out of 250 isolates were selected according to reduced susceptibility (zone diameter of ≤ 22 mm and/or MIC ≤ 1μg/mL) to any ESC (ceftazidime, cefotaxime, or aztreonam). With regard to the specimen site, *K. pneumoniae* were obtained from stool specimens (n = 23), wound swab (n = 14), urine (n = 12), endotracheal tube specimens (n = 9), sputum (n = 7), blood (n = 4), bed sore (n = 2), tissue culture (n = 2), high vaginal swab (n = 1), urethral swab (n = 1), umbilical (n = 1), cerebrospinal fluid (n = 1), aseitic fluid (n = 1), gastroectomy swab (n = 1), and catheter tip specimen (n = 1).

Bacterial Identification

Identification of *K. pneumoniae* was done on the basis of Gram staining, colony morphologies on MacConkey's agar, oxidase reaction, and the biochemical tests included in the API 20E identification kit (Biomerieux, Marcyl'´Etoile, France). The identified strains were stored in glycerol broth cultures at-70^{0}C.

Preparation of inocula

Three to five well isolated colonies from an overnight tryptone soy agar plate (Merck®, Darmstadt, Germany,) were suspended in 5 mL saline (0.9% NaCl) using Vortex mixer (Daigger®, NY, USA) to obtain an inoculum equivalent to 0.5 McFarland standard. The suspension was measured spectrophotometry using Spectrophotometer (LKB® Ultrospec) at 560 nm to give absorbance 0.1-0.14. The obtained suspension was diluted 1:100 in 0.9% saline to obtain~ 5x10^5 CFU/mL. This suspension was swabbed on a Mueller-Hinton agar plate (Scharlau®, Sentmenat, Spain) and allowed to dry completely. Those plates were ready for the susceptibility testing and β-lactamase screening.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the disc diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2013). The antibiotics tested were amoxicillin (AMX 20mg), amoxicillin/clavulanate (AMC 20/10mg), piperacillin (PIP 100mg), piperacillin+tazobactam (TZP 100/10mg), cefotaxime (CTX 30mg), cefuroxime (CRO 30mg), ceftazidime (CAZ 30mg), imipenem (IMP 10mg), aztreonam (ATM), cefoxitin (FOX 30mg), cefotetan (CTT 30mg), amikacin (AN 30mg), ciprofloxacin (CIP 5mg), sulphamethoxazole/trimethoprim (SXT 23.75/ 1.25 mg), and tetracycline (TE 30 mg) (Becton, Dickinson and company, MD, USA).

The minimum inhibitory concentration (MIC) was determined by an E-strip test (Liofilchem s.r.l, Roseto, Italy) as described by the manufacturer. A laboratory control strain,

Escherichia coli ATCC 25922, was used in the sensitivity test and in the MIC determination. MIC to imipenem (IMP), doripenem (DOR), ertapenem (ERT), cefotetan (CTT), cefoxitin (FOX), ceftazidime (CAZ), cefepime (FEP), colistin (COL), fosfomycin (FOS), and tigecycline (TGC) was determined.

ESBL screening

The isolates which showed reduced susceptibility to CAZ, CTX, or ATM (zone diameter of less than or equal 22 mm and/or MIC more than or equal 1 µg/mL) were selected to screen ESBL production (EUCAST, 2013).

ESBL confirmatory testing

Combination disc test and gradient test using E tests ESBL strips were performed to seek ESBL production (EUCAST, 2013).

Combination disc test

Two disks of CAZ (30µg) were used, each with and without clavulanate (10µg). The isolate is ESBL producer if the zone expansion diameter is ≥ 5 mm larger with clavulanate than without.

Gradient test method

E-test ESBL strips were in accordance with the manufacturer's directions to seek ESBL production. The ceftazidime/ceftazidime-clavulanate (CAZ/CAL) ESBL Etest strip and cefepime/cefepime-clavulanate (FEP/FEL) Etest ESBL strip were used. The test is positive if ≥ 8 fold reduction is observed in the MIC of CAZ/CAL and/or FEP/FEL compared with the MIC of CAZ and/or FEP alone or if a phantom zone or deformed ellipse is present.

AmpC screening

Bacterial susceptibility to FOX was tested according to the standard disk diffusion method. The isolates which showing <19 mm zone size and/or MIC >8µg/mL, were selected to screen AmpC production (EUCAST, 2013).

AmpC confirmatory testing

E-test ESBL strip, cefotetan/cefotetan+cloxacillin (CTT/CXT), was used to seek AmpC production. The test was in accordance with the manufacturer's directions. MIC ratio of ≥ 8 and/or the presence of a phantom zone or deformation of the ellipse were considered positive for AmpC.

Carbapenemase screening

Bacterial susceptibility to IMP was tested according to the standard disk diffusion method, and isolates showing <27 mm zone size and/or MIC > 0.12 µg/mL were selected to screen carbapenemase production (EUCAST, 2013).

Modified Hodge test (MHT)

MHT was used to detect carbapenemase production. This test was conducted according to the CLSI 2013 guidelines for search of carbapenemase production (CLSI, 2013). A 0.5 McFarland's suspension of ATCC *Escherichia coli* 25922 was diluted 1:10 in sterile saline. This was inoculated on a Mueller Hinton agar plate. IMP disc was placed in the centre of the agar plate. 3-5 colonies of the test organism were picked and inoculated in a straight line, from the edge of the disc, up to a distance of at least 20 mm. The plates were incubated at 37^0C overnight and they were examined next day. Isolates with cloverleaf images of inhibition zone were considered as a carbapenemase-producing *K. pneumoniae*.

MBL confirmatory test

Combination disc test and gradient test using E tests MBL strip were performed to seek MBL production (Pitout *et al*., 2005; EUCAST, 2013).

Combination disc test

Two disks of IMP (30µg) were used, each with and without ethylenediaminetetraacetic acid (EDTA) (930µg). In brief, overnight cultures at a 0.5McFarland standard were inoculated on Mueller Hinton agar. Meropenem or IMP disks were supplemented with 5 µl of 0.5M EDTA (930 µg EDTA) and the differences in zone diameters with and without EDTA were measured. The isolate is MBL producer if the zone expansion diameter is ≥ 5 mm larger with EDTA than without.

Gradient test method

Production of MBL was evaluated using E-test MBL strip (IMP/IMP + EDTA). A ratio of the MICs of IMP to IMP+EDTA of ≥ 8 or the presence of a phantom zone was interpreted as being positive for MBL production.

RESULTS

Antimicrobial susceptibility

The antimicrobial susceptibility testing was carried out by disc diffusion method to 80 ESC insensitive clinical isolates of *K. pneumoniae*. The resistant rates of antimicrobial agents are illustrated in Table 1 and Fig. 1. The whole collection of *K. pneumoniae* isolates was resistant

to AMX (100%). Four (5%) out of 80 isolates of *K. pneumoniae* showed intermediate resistance to IMP. The resistance rates for all isolates to AMC, PIP, PIP/TAZ, CAZ, CTX, CRO, FOX, IMP, AN, CIP, SXT and TE were 87.5%, 88.75%, 48.75%, 75%, 86.25%, 86.25%, 73.75%, 4.54%, 7.5%, 70%, 88.75%, and 78.75% respectively.

The resistance rates at Hospital A to AMC, PIP, PIP/TAZ, CAZ, CTX, CRO, FOX, IMP, AN, CIP, SXT and TE were 93.93%, 92.42%, 51.51%, 72.72%, 87.87%, 87.87%, 83.33%, 3.03%, 9.09%, 74.24%, 92.42% and 77.27%, respectively (Table 1 and Fig. 2). On the other hand, the resistance rates at Hospital B to AMC, PIP, PIP/TAZ, CAZ, CTX, CRO, FOX, IMP, AN, CIP, SXT and TE were 100%, 71.42%, 35.71%, 85.71%, 78.57%, 78.57%, 28.57%, 0%, 0%, 50%, 71.42% and 85.71% respectively (Table 1 and Fig. 3).

Prevalence of ESBL

Eighty isolates which showed reduced susceptibility to CAZ, CTX, or ATM (zone diameter of less than or equal 22 mm and/or MIC more than or equal 1 µg/mL) were selected to screen ESBL by combination disc test and E test ESBL strips. The results are shown in Table 2. The prevalence of ESBL in insensitive ESC *K. pneumoniae* isolates was 91.25% (n=73/80). The prevalence of ESBL in the whole *K. pneumoniae* isolates (n=250) was 29.2% (n= 73/250).

The prevalence of ESBL in insensitive ESC *K. pneumoniae* isolates in Hospital and Hospital B is shown in Table 2. The prevalence of ESBL producing *K. pneumoniae* isolates in Hospital B were 92.85% (n= 13/14) while the prevalence of ESBL in Hospital A *K. pneumoniae* isolates were 90.90% (n= 60/66).

Table 1. Antimicrobial resistance patterns of 80 *K. pneumoniae* isolated from two hospitals, Riyadh

Antibiotics	Number (%) of resistant isolates					
	Total isolates (n= 80)		Hospital A (n =66)		Hospital B (n=14)	
	No	%	No	%	No	%
AMX	80	100	80	100	80	100
AMC	70	87.5	62	93.93	8	100
PIP	71	88.75	61	92.42	10	71.42
TZP	39	48.75	34	51.51	5	35.71
CAZ	60	75	48	72.72	12	85.71
CTX	69	86.25	58	87.87	11	78.57
CRO	69	86.25	58	87.87	11	78.57
FOX	59	73.75	55	83.33	4	28.57
IMP	3	3.75	3	4.54	0	0
AN	6	7.5	6	9.09	0	0
CIP	56	70	49	74.24	7	50
SXT	71	88.75	61	92.42	10	71.42
TE	63	78.75	51	77.27	12	85.71

Table 2. Prevalence of ESBL, AmpC, and carbapenemases in *K. pneumoniae* isolates

Antibiotics	Number (%) of resistant isolates					
	Total isolates (n= 80)		Hospital A (n =66)		Hospital B (n=14)	
	No	%	No	%	No	%
ESBL	73	91.25	60	90.90	13	92.85
AmpC	11	13.75	10	15.15	1	7.14
Carbapenmases	21	26.25	18	27.27	3	21.4
Metallo carbapenamses	7	8.75	7	10.60	0	0
Non-metallocarbapenmase	14	17.5	11	16.66	3	21.42

Prevalence of AmpC

Forty three (53.75%) out of 80 insensitive ESC *K. pneumoniae* isolates showing reduced susceptibility to FOX, (zone diameter of <19 mm and/or MIC >8µg/mL) were selected to screen AmpC by CTT/CXT E test strips. Eleven (25.6%) out of 43 isolates, which were positive in an initial screening, were AmpC positive. The prevalence of AmpC is illustrated in Table 2. The prevalence of AmpC producing *K. pneumoniae* isolates was 13.75% (n= 11/80).

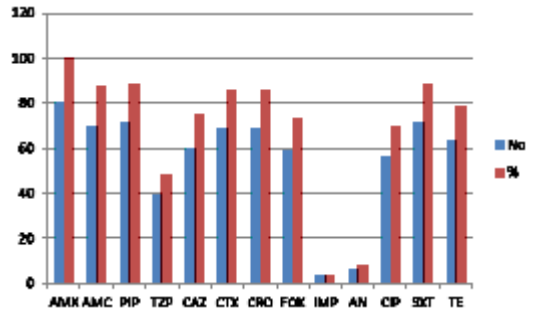

Fig. 1. Resiatance rates for 80 *K. pneumoniae* isolated from two hospitals, Riyadh

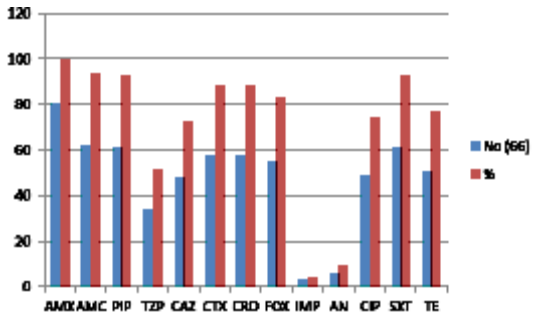

Fig. 2. Resiatance rates for 66 *K. pneumoniae* strains isolated from Hospital A

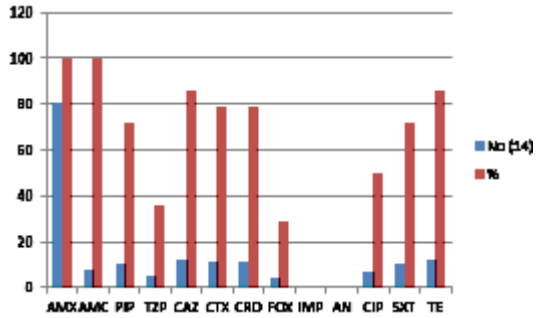

Fig. 3. Resiatance rates for *K. pneumoniae* strains isolated from Hospital B

The prevalence of AmpC (Table 2) producing *K. pneumoniae* isolates in Hospital B were 7.14% (n= 1/14). On the other hand, the prevalence of AmpC (Table 2) positive isolates in Hospital A were 15.15% (n = 10/66).

Prevalence of carbapenemase

Twenty five (31.25%) out of insensitive ESC 80 isolates showing reduced susceptibility to IMP (zone diameter of <30 mm and/or MIC > 0.12 µg/mL) were selected to screen carbapenemase by MHT. Twenty one (84%) out of 25 isolates, which were positive in an initial screening, were carbapenemase positive. The prevalence of carbapenemase (Table 2) producing *K. pneumoniae* isolates were 26.25% (n= 21/80). The prevalence of metallo- and non- metallo carbapenemase (Table 2) producing *K. pneumoniae* were 33.33% (n=7/21) and 66.66% (n=14/21) respectively. The prevalence of metallo- and non- metallo carbapenemase (Table 2) producing *K. pneumoniae* isolates among whole collection were 8.75% (n= 7/80) and 17.5% (n=14/80) respectively. The prevalence of carbapenemase positive isolates in Hospital B were 21.4% (n= 3/14), while, the prevalence of carbapenemase positive isolates in Hospital A were 27.27% (n = 18/66) (Table 2).

DISCUSSION

K. pneumoniae is mainly accountable for causing diseases such as nosocomial pneumonia, septicaemia, wound infections, UTI, and neonatal septicaemia (Rossolini *et al*., 2007). Resistance to ESC was first found in *K. pneumoniae* in 1983 (Knothe *et al*., 1983). The emergence of antibacterial resistance and the production of β-lactamases are responsible for the frequently observed empirical therapy failures in many bacterial infections. The alarming rate of resistance noted among these isolates in the present study, is of concern. Resistance of ESC insensitive isolates was found to coexist with resistance to two or more antibiotics such as piperacillin, ciprofloxacin, sulfamethoxazole/trimethoprim and tetracycline. Carbapenems, tigecycline and colistin have gained recent attention for their broad-spectrum antimicrobial activity. In the current study, 3.75% of the isolates were found to be resistant to imipenem.

Resistance to ESC is increasing and may be mediated by upregulated chromosomal (AmpC) β-lactamases, efflux or porin loss or by acquired β-lactamases, included ESBLs, AmpC plasmid mediated and carbapenemases (Pfeifer *et al*., 2010). Several studies mainly focus on a variety of function enzymes produced in *K. pneumoniae*, including ESBLs, plasmid-mediated AmpCs, and carbapenemases (Marchaim *et al*., 2008). In the present study, the proportion of the ESBL positive cases was highest, followed by carbapenemases-producing stains and, AmpC-producing stains.

A few studies were undertaken to determine the prevalence of ESBL and carbapenemases in Saudi Arabia and there is not any study to characterize AmpC β-lactamases. Therefore, this study was devoted to determine the prevalence of ESBLs, AmpC and carbapenemases either metallo- or non-metallo-carbapenemases in ESC insensitive *K. pneumoniae* isolates. ESBLs are the major cause of resistance to ESC and monobactams while being inhibited by β-lactamase inhibitors. The prevalence of ESBLs among *K. pneumoniae* clinical isolates has increased significantly over the past two decades (Peirano and Pitout, 2010). The prevalence of ESBLs among *K. pneumoniae* clinical isolates varies worldwide, and these patterns rapidly change over time. The prevalence of ESBLs among *K. pneumoniae* clinical isolates from Saudi Arabia is also variable. There is a rarity of local reports on the prevalence of ESBL positive *K. pneumoniae*. Previous studies examining *K. pneumoniae* isolates in Saudi Arabia have reported prevalences of ESBL-producing *K. pneumoniae* ranging from 10.4 to 63% (Al-Qahtani *et al*., 2014; Marie *et al*., 2013; Tawfik *et al*., 2011; Al Johani *et al*., 2010; Ahmad *et al*., 2009; Al-agamy *et al*., 2009). In the present study, the prevalence of ESBL in insensitive ESC *K. pneumoniae* isolates was 91.25% (n=73/80) however, the prevalence of ESBL in the whole *K. pneumoniae* isolates (n=250) was 29.2% (n= 73/250). The differences in ESBL prevalence are difficult to clarify and may owe to dissimilarities in the types and amounts of third-generation cephalosporins used and variations in isolate collection dates, time periods, and geographic areas.

Unlike other β-lactamases, AmpC β-lactamases are not inhibited by β-lactamase inhibitors. AmpC-producing organisms are generally resistant to broad-spectrum penicillins, cephamycins, oxyimino- and 7-α-methoxy-cephalosporins, and aztreonam, but are susceptible to cefepime, cefpirome, and carbapenems (Pitout and Laupland, 2008; Philippon *et al*., 2002). Phenotypic characterization of 80 isolates of ESC-insensitive *K. pneumoniae* was done. Among 80 ESC insensitive *K. pneumoniae* isolates, 92.25%, 13.75%, and 26.25% were found to have ESBL, AmpC, and carbapenemases, respectively. Co-production of AmpC β-lactamase and ESBL among the isolates in this study has been detected in four out of 80 *K. pneumoniae* isolates however AmpC was detected solely in 7 isolates.

Production of carbapenemases is an important mechanism for *K. pneumoniae* resistance to carbapenems (Nordmann *et al*., 2011; Queenan and Bush, 2007). These enzymes can hydrolyze not only carbapenems but also most ²-lactams except monobactam. So far, there have been about 70 kinds of carbapenemases reported in the world. Among Enterobactericaeae, clinically significant carbapenemase are metallo carbapenemase which include Ambler class B (VIM, IMP and NDM) and serine carbapenemase which include Ambler class A (KPC), and Ambler class D (OXA-48) (Nordmann *et al*., 2011). Metallo carbapenemases are inhibited by EDTA while non-metallo carbapenemase are not inhibited by EDTA. In the present study, 25 (31.25%) out of 80 ESC insensitive *K. pneumoniae* isolates showed reduced susceptibility to carbapenem. These isolates screened for carbapenemase phenotypically by Modified Hodge Test. The prevalence of carbapenemase was 26.25% (21/80). Four isolates did not produce carbapenemase. In the present study, carbapenemase always coexist with ESBL-positive strains or AmpC-positive strains. To differentiate phenotypically between metallo carbapenemase and serine carbapenemase, MBL E test strip (IMP/ IMP+ EDTA) was used. The prevalence of metallo carbapenemase and non-metallo carbapenemase producing *K. pneumoniae* were 33.33% (n=7/21) and 66.66% (n=14/21) respectively. The present study was in agreement with the previous study in Saudi Arabia (Shibl *et al*., 2013). They reported that, the prevalence of metallo carbapenemase and non-metallo carbapenemase was 22% and 78%, respectively

(Shibl *et al*., 2013). In most of studies in Saudi Arabia the metallo carbapenemase and non-metallo carbapenemase were mainly NDM and OXA-48, respectively (Zowawi *et al*., 2014; Al-agamy *et al*., 2013; Shibl *et al*., 2013). However from previous studies we can expect that OXA-48 and NDM are dominant carbapenemases in Saudi *K. pneumoniae*.

One of the limitations of this study is no genotypic identification of the resistance determinants of ESBL, AmpC and carbapenemases. PCRs for ESBL genes (TEM, SHV, CTX-M), for carbapenemase genes (KPC, OXA-48, VIM, IMP, NDM), and for AmpC (CMY, MOX, FOX, ACT, LAT, MIR, DHA, CIT) must been done in the future work.

CONCLUSION

In conclusion, this work provides the evidence of heterogeneity of determinants conferring ESC resistance in clinical *K. pneumoniae* isolates from Riyadh, Saudi Arabia. Our study revealed that ESBLs positive *K. pneumoniae* were resistant to the majority of ESC and other non ²-lactams, and some strains also carry AmpC and carbapenemase together, which lead to multidrug resistance. Our results also revealed that high prevalence of ESBLs, carbapenemase, and AmpC in ESC insensitive *K. pneumoniae* isolates. Empirical use of carbapenems for the treatment of infection caused by ESC insensitive *K. pneumoniae* isolates should be carefully monitored in Saudi hospitals**.** Tigecycline and colistin are the most active drugs against ESBL, AmpC and/or carbapenemase positive *K. pneumoniae* isolates.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group Project no. RGP-VPP-038.

REFERENCES

1. Ahmad S, Al-Juaid NF, Alenzi FQ, Mattar EH, Bakheet Oel-S. Prevalence, Antibiotic Susceptibility pattern and production of extended-spectrum beta-lactamases amongst clinical isolates of Klebsiella pneumoniae at Armed Forces Hospital in Saudi Arabia. *J Coll Physicians Surg Pak.* 2009; **9**(4): 264–5.
2. Al Johani SM, Akhter J, Balkhy H, El-Saed A, Younan M, Memish Z. Prevalence of antimicrobial resistance among gram negative isolates in an adult intensive care unit at a tertiary care center in Saudi Arabia. *Ann Saudi Med.* 2010; **30**(5): 364–9
3. Al-Agamy MH, Shibl AM, Elkhizzi NA, Meunier D, Turton JF, Livermore DM. Persistence of Klebsiella pneumoniae clones with OXA-48 or NDM carbapenemases causing bacteraemias in a Riyadh hospital. *Diagn Microbiol Infect Dis.* 2013; **76**(2): 214-6.
4. Al-Agamy MH, Shibl AM, Tawfik AF. Prevalence and molecular characterization of extended-spectrum b-lactamaseproducing Klebsiella pneumoniae in Riyadh, Saudi Arabia. *Ann Saudi Med*. 2009; **29**(4): 253–7.
5. Al-Qahtani AA, Al-Agamy MH, Ali MS, Al-Ahdal MN, Aljohi MA, Shibl AM. Characterization of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* from Riyadh, Saudi Arabia. *J Chemother.* 2014; **26**(3): 139-45.
6. Clinical and Laboratory Standards Institute (CLSI) Performance standards for antimicrobial susceptibility testing. Twenty-first informational supplement. M100-S23. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.
7. Grover N, Sahni AK, Bhattacharya S.Therapeutic challenges of ESBLS and AmpC beta-lactamase producers in a tertiary care center. *Med J Armed Forces India.Med J Armed Forces India*. 2013; **69**(1): 4-10.
8. Knothe, H., Shah, P., Krcmery, V., Antal, M., Mitsuhashi, S.Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. *Infection*, 1983; **11**: 315–317.
9. Marchaim D, Navon-Venezia S, Schwaber MJ, Carmeli Y., Isolation of imipenem- resistant Enterobacter species: emergence of KPC-2 carbapenemase, molecular characterization, epidemiology, and outcomes. *Antimicrob Agents Chemother* 2008; **52**: 1413–1418
10. Marie M.A., John J., Krishnappa L.G., Gopalkrishnan S., Molecular characterization of the β-lactamases in *Escherichia coli* and *Klebsiella pneumoniae* from a tertiary care hospital in Riyadh, Saudi Arabia. *Microbiol. Immunol.* 2013; **57**(12):805-810.

11. Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis.* 2011;**17**(10):1791-8.
12. Peirano G., Pitout J.D.D., Molecular epidemiology of *Escherichia coli* producing CTX-M β-lactamases: the worldwide emergence of clone ST131 O25:H4. *Int. J. Antimicrob. Agents.* 2010; **35**: 316-321.
13. Pfeifer, Y., Cullik, A., Witte, W. Resistance to cephalosporins and carbapenems in Gram-negative bacterial pathogens. *Int. J. Med. Microbiol*., 2010; **300**: 371–9.
14. Philippon A., Arlet G., Jacoby G.A. Plasmid-determined AmpC type beta lactamases. *Antimicrob. Agents Chemother*., 2002; **46**(1):1–11.
15. Pitout JD, Gregson DB, Poirel L, McClure JA, Le P, Church DL., Detection of Pseudomonas aeruginosa producing metallobeta-lactamases in a large centralized laboratory. *J Clin Microbiol* 2005; **43**:3129–3135
16. Pitout JD, Laupland KB., Extended-spectrum beta-lactamase producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect. Dis*. 2008; **8**: 159-166.
17. Pitout JD. Multiresistant Enterobacteriaceae: new threat of an old problem. *Expert Rev Anti Infect Ther* 2008; **6**: 657-69
18. Queenan AM, Bush K., Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev* 2007; **20**: 440-458.
19. Rossolini GM, Mantengoli E, Docquier JD, Musmanno RA, Coratza G., Epidemiology of infections caused by multiresistant Gram-negatives: ESBLs, MBLs, panresistant strains. *New Microbiol* 2007; **30**: 332–339.
20. Shibl A, Al-Agamy M, Memish Z, Senok A, Khader SA, Assiri A. The emergence of OXA-48- and NDM-1-positive *Klebsiella pneumoniae* in Riyadh, Saudi Arabia. *Int J Infect Dis*. 2013; **17**(12): e1130-3.
21. Struve C, Krogfelt KA., Pathogenic potential of environmental *Klebsiella pneumoniae* isolates. *Environ Microbiol* 2004; **6**: 584–590.
22. Tawfik AF, Alswailem AM, Shibl AM, Al-Agamy MH. Prevalence and genetic characteristics of TEM, SHV, and CTX-M in clinical Klebsiella pneumoniae isolates from Saudi Arabia. *Microb Drug Resist.* 2011;**17**(3):383–8.
23. The European Committee on Antimicrobial Susceptibility Testing., Antimicrobial Susceptibility Testing. Available at http://www.eucast.orgwww.eucast.org, 2013.
24. Zowawi HM, Sartor AL, Balkhy HH, Walsh TR, Al Johani SM, AlJindan RY, Alfaresi M, Ibrahim E, Al-Jardani A, Al-Abri S, Al Salman J, Dashti AA, Kutbi AH, Schlebusch S, Sidjabat HE, Paterson DL. Molecular Characterization of Carbapenemase-Producing *Escherichia coli* and *Klebsiella pneumoniae* in the Countries of the Gulf Cooperation Council: Dominance of OXA-48 and NDM Producers. *Antimicrob Agents Chemother*. 2014; **58**(6):3085-3090.