WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 6, 322-328. Research Article ISSN 2277– 7105

DETECTION OF *KLEBSIELLA PNEUMONIAE* FROM CLINICAL AND MEDICAL WASTE SAMPLES USING EFFLUX GENES (*RAMA* AND *ACRA)*

Fatma Chalob Al Saadi* and Dr.Watheq Abas Al-Dreaghi

Genetic Engineering and Biotechnology Institute for Postgraduate Studies, Iraq.

Article Received on
29 March 2015,

Revised on 20 April 2015,
Accepted on 11 May 2015

***Correspondence for Author**
Fatma Chalob Al Saadi
Genetic Engineering and Biotechnology Institute for Postgraduate Studies, Iraq.

ABSTRACT

Infections with *K. pneumoniae* are usually hospital-acquired and occur mostly in individuals with a weakened immune system. The incidence of infections caused by multidrug-resistant *Klebsiella* strains has increased and be as serious problem. The present study is an attempt for molecular diagnosis of *K. pneumoniae* presence in samples patients and medical waste (Laboratory waste, Holocaust and outpatient clinics waste) by using of Polymerase chain reaction (PCR) technique using *ramA* and *acrA* genes. A total 160 samples were collected from 80 patients included (Urine, Blood, and Sputum) who attend to Baghdad city and 80 medical waste samples included (30 Laboratory waste, 40 the Holocaust and 10 outpatient clinics waste) to sum up, 115out of 160 specimens were detected for *K. pneumoniae*. Results obtained were 77% positive for the primer (*acrA*) and gave 100% positive results for the (*ramA*) which gave a fact that the *ramA* gene is ideal for detection of *K. pneumoniae* in different samples. This study presented high specificity and sensitivity for the diagnosis of Multidrug resistant *K. pneumoniae* strains using the PCR technology which is cheaper and faster than the classical methods currently used in the hospitals.

KEYWORDS: *K. pneumonia, Klebsiella, K. pneumonia.*

INTRODUCTION

Klebsiella is among the Gram negative pathogens most commonly encountered in hospital-acquired infections (Chan *et al*., 2009). As opportunistic pathogens, *Klebsiella spp.* primarily attack immunocompromised individuals who are hospitalized and suffer from sever underlying diseases such as diabetes mellitus or chronic pulmonary obstruction (Chien-Ko *et*

al., 2002). *Klebsiella* frequently cause human nosocomial infections, which is a common hospital-acquired pathogen causing severe respiratory infections such as pneumonia, other infections caused by this organism include urinary tract infection, wound infection, abscesses and diarrhea (Feizabadi *et al*., 2008). Although found in the normal flora of mouth, skin and intestines but can cause the destructive changes to human lungs if aspirated (Ryan and Ray, 2004). Efflux pumps are transport proteins that responsible for acquired resistance to various antibiotics based on the chromosomal or plasmids sources of the efflux genes (Van *et al*., 2000). One of the efflux systems involved in resistance phenotype is the AcrAB multidrug efflux system that in *K. pneumoniae* is encoded by the acrRAB operon. In this operon, *acrA* encode a periplasmic lipoprotein of 40 kDa, anchored to the inner membrane that bridges the outer and inner membranes and an integral membrane protein of 113.5 kDa with 12 membrane-spanning α-helices, located in the cytoplasmic membrane, respectively (Domenech-Sanchez *et al*., 2001). The *acrA* gene is coding the membrane fusion protein AcrA which is a membrane fusion protein build up an efflux pump whose overexpression leads to fluoroquinolone resistance (Mazzariol *et al*., 2002; Szabó *et al*., 2006).The AcrA efflux pump is play majore roles in multidruge resistance in *K.pneumoniae* by excreting antibiotics from different class including floroquinolones, It's associated in change the permeability of the bacterial membrane by antibiotics extrusion to external environment, thus the intracellular concentration of antibiotic reduces and the resistance to antibiotic will happen (Peleg *et al*., 2007).The *ramA* was originally discovered in *K. pneumoniae* by George *et al* (1995). It is thought to form an operon with a gene specifying the outer membrane protein RomA. The regulator RamA can also induce *acrA* expression and reduce antimicrobial susceptibility (Chollet *et al*., 2004; Ruzin *et al*., 2005; Keeney *et al*., 2007). This study has supported the PCR technique as a rapid and specific method for the identification of *K. pneumoniae* isolates by using primers specific (*ramA* and *acrA*) genes.

MATERIAL AND METHODS

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by disk diffusion method according to National Committee for Clinical Laboratory standards (NCCLs) guideline. Each *Klebsiella pneumoniae* isolate was tested for susceptibility to twenty (20) different antibiotics including Ampicillin, Carbenicillin, Pipracillin, Amoxicillin / Clavulania acid, Cefotaxime, Ciprofloxacin, tetracycline, Nalidixic acid, Norfloxacin, Aztreonam, Kanamycin,

levofloxacin, Ceftriaxone, Ceftazidime, Streptomycin, Gentamicin, tobramycin, Amikacin, Imipenem, Meropenem.

Genomic DNA extraction

This procedure was done by using commercially available DNA extraction and purification kit (Geneaid Genomic DNA extraction Kit) .The procedure was explained in details in user's manual.

Polymerase chain reaction (PCR)

Amplification of *ramA* and *acrA* fragments were done by using polymerase chain reaction method. A 499bp (*ramA* gene), as well as 495 bp (*acrA* gene), were amplified by PCR using forward and reverse primers according to Schneiders *et al*., (2003) and Pakzad *et al*., (2013). Specific primers for *ramA* gene (*ramA*-F, 5´- GGGTCGCCGATAAGACGC- 3´ and *ramA*-R, 5´-GCTGGGCGCCATTGAGTAT-3´).

Specific primers for *acrA* gene(*acrA*-F,5´-ATGAACAAAAACAGAGG-3´and *acrA*-R, 5´-TTTCAACGGCAGTTTTCG- 3´).

PCR reaction was performed in mixtures (by BioNeer, Korea) containing 1.5 µM MgCl2, 250 µM dNTPs, 1 U Tag DNA polymerase, 30 µM KCl, 10 µM Tric-HCl (pH 9.0), 10 pmoll^{-1} of each primer, 5µl DNA template, AccuPower® PCR Premix and distilled water up to final mixture volume of 20µl and *ramA* primer was done as follow: initial denaturation step at 95°C for 5min followed by 30 cycles consisting of denaturation (94°C for 50 sec.), annealing(56°C for50 sec.) and extension (72°C for 1min), followed by afinal extension step at 72°C for 5min. PCR products were analyzed by electrophoresis in a 2% vw^{-1}agarose gel with ethidium bromide at constant voltage (10v/cm) for 30 minutes to test the amplification success (Othman *et al*., 2012). While *acrA* peimer was done as follow: initial denaturation step at 94°C for 5min followed by 30 cycles consisting of denaturation (94°C for 1min), annealing(42°C for 1min) and extension (72°C for 1min), followed by afinal extension step at 72°C for 5min. PCR products were analyzed by electrophoresis in a 2% vw^{-1} agarose gel with ethidium bromide at constant voltage (10v/cm) for 30 minutes to test the amplification success (Othman *et al*., 2012). at an optimized annealing temperature that was determined for each primer.

RESULT AND DISCUSSION

Results declared that all isolates *K.pneumoniae* have been multidrug resistant. The resistance of medical waste isolates to ciprofloxacin and tetracycline were higher than resistance of clinical isolates due to heavy antibiotic use hospital waste contains larger numbers of ciprofloxacin and tetracycline antibiotics and this leads to the development of resistant strains to antibiotics as well as the possibility of acquiring genetic factors.

The primers *ramA* and *acrA* were amplified with DNA fragment, which is used as a template for PCR reaction. The PCR amplification was confirmed by running 10µl of PCR product along with 100bp DNA marker in 2 agarose gel. The amplified PCR products (*ramA* and *acrA*) were visualized as a single band of expected size under the UV with the marker, which were 499bp for *ramA* and and 495bp for *acrA* (Figure 1 and 2).

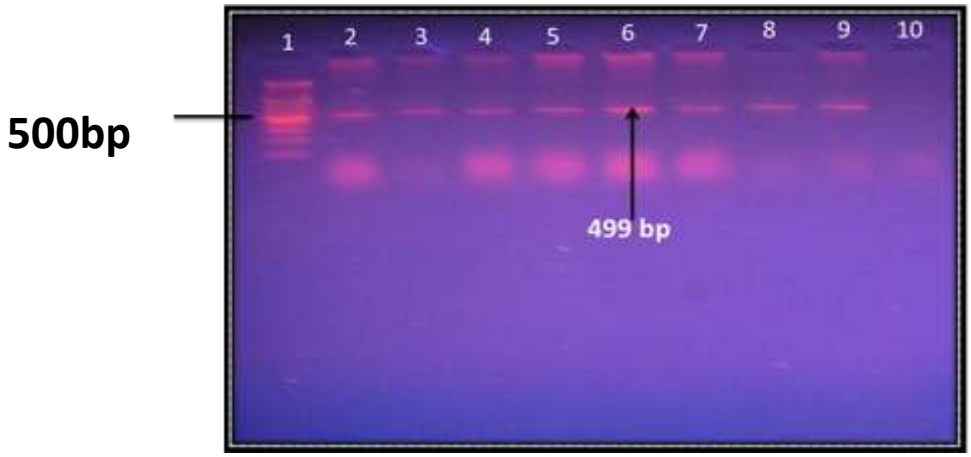

Figure (1): Gel electrophoresis of PCR product of *ramA* gene for *K. pneumoniae* using 2% agarose gel at 70Volt for 45min. Lane 1: 100bp DNA ladder; Lane (2-9): positive results for ramA gene. Lane 10: Negative control.

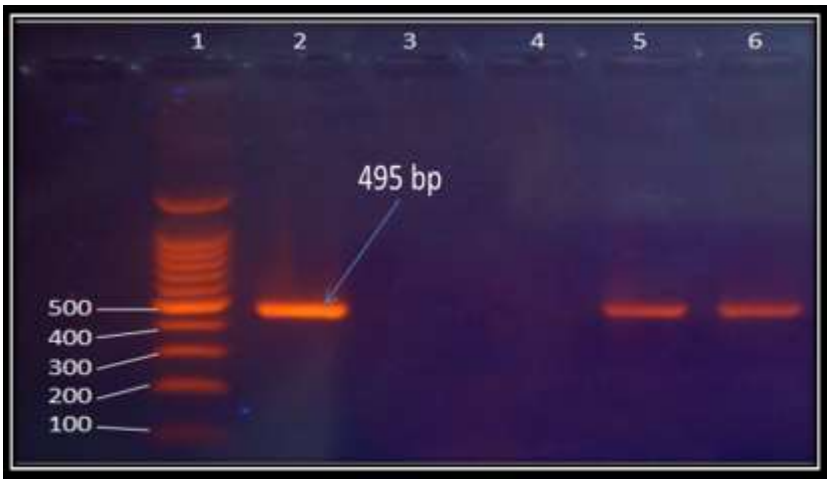

Figure (2): Gel electrophoresis of PCR product of *acrA* gene for *K. pneumoniae* using 2% agarose gel at 70Volt for 1hour. Lane 1: 100-bp DNA ladder; Lane (2, 5, And 6): positive result for *acrA* gene; Lane 3, 4: Negative control.

All samples show positive results for presence of *ramA* gene. Our results obtained from the use of *ramA* gene were not compatible with (Schneiders *et al.*, 2003) since there were 30% positive of *ramA* gene because our strain *K. pneumoniae* was multidrug resistant (MDR) strains. Studies that concerned in transposon insertion mapping classified the *ramA* gene as a related to MDR, Transcription of *ramA* was detected in fluoroquinolone-resistant clinical isolates of *K. pneumoniae* (Ruzin *et al*., 2005; Emma Padilla *et al*., 2010). Genes encoding MDR pumps are normal constituents of bacterial chromosomes and thus provide to bacteria the intrinsic potential to develop the MDR phenotype without acquisition of antibiotic resistance genes, the activation of multidrug efflux pump genes by mutations or induction caused by stress of exposure to xenobiotics results in overexpression of pumps (George, 1996; Grkovic *et al*., 2001). The expression of *ramA* plays a role in conferring resistance to various classes of antibiotics including chloramphenicol, fluroquinolones and the tetracyclines (Elkins and Nikaido, 2002). While results obtained from the use of *acrA* gene were (77%) which compatible with Pakzad *et al.*, (2013). But the study of Schneiders *et al.*, (2003) was incompatible with our results were shown 5 positive results out of 10 which reached to(50%) because in our study all samples showed positive results for presence of *ramA* gene. The overexpression of *acrA* is linked to the increased level of transcription of *ramA.* Increased AcrAB efflux pump expression in fluoroquinolone-resistant *K.pneumoniae* strains was caused by overexpression of the transcriptional regulator *ramA* (Schneiders *et al.*, 2003). Ruzin *et al* (2005) observed that *ramA* over-expression correlated with acrAB overexpression, leading to the premise that acrAB may be under the transcriptional control of *ramA.* AcrAB efflux system belongs to the resistance-nodulation-division (RND) family of transporters and utilizes the outer membrane protein TolC.Substrates for this efflux system has included fluoroquinolones, chloramphenicol and tetracycline (Nikaido, 1996). As showed in our study clinical isolates were resemble the medical waste isolates in genome content, this result were expected because of most of medical waste resources is completely came from material that used in detection, diagnosis and treatment for patient infected with clinical isolates.

REFERENCES

1. Chan, Y.; Liu, J.; Pociask, D.; Zheng, M.; Mietzner, T. and Berger, T. Lipocalin 2 is required for pulmonary host defense against Klebsiella infection. J Immunol. 2009; 182(8): 4947-56.

2. Chien Ko- Wen; Patersotr, D. L; Sagnimeni, A. J.; Hansen, D.S.; Von Gottberg, A.; Mohapatra, S.; Casellas, J. M.; Goossens, H.; Mulazimoglu, L.; Trenholme, G.; Klugman, K. p.; McCormack, J. G. and Yu, V. L. Community-Acquired Klebsiella pneumoniae Bacteremia: Global Differences in Clinical Patterns. Emerging Infectious Diseases. 2002; 8: 160-166.
3. Chollet, R.; Chevalier, J.; and Bollet,CRamA is an alternate of the multidrug resistance cascade in Enterobacter aerogenes. Antimicrob Agents Chemother. 2004; 48: 2518–2523.
4. Domenech-Sanchez, A.; S. Alberti, L. Martinez-Martinez, A. Pascual, I. Garcia, and V.J. Benedi. (2001). Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. C1-2018.
5. Elkins, CA. and Nikaido, H. Substrate specificity of the RND-type multi-drug efflux pumps AcrB and AcrD of Escherichia coli is determined predominately by two large periplasmic loops. Journal of Bacteriology. 2002; 184: 6490-6498.
6. Emma Padilla, Enrique Llobet, Antonio Doménech-Sánchez, Luis Martínez-Martínez, José Antonio Bengoechea and Sebastián Albertí. Klebsiella pneumoniae AcrAB Efflux Pump Contributes to Antimicrobial Resistance and Virulence. Antimicrob Agents Chemother. 2010; 54(1): 177.
7. Feizabadi, M.M.; Farahani, A.S.; Rahmati, M. and Asadi, S. Phenotypic characterization and plasmid analysis of Klebsiella pneumoniae strains from Iranian patients. R. Ci. Méd. Boil., Salvador. 2008; 7(3): 273-279.
8. George, AM.; Hall, RM.; Stokes, HW. Multi-drug resistance in Klebsiella pneumoniae: a novel gene, ramA, confers a multi-drug resistance phenotype in Escherichia coli. Microbiology. 1995; 141: 1909-1920.
9. George, A. M. Multidrug resistance in enteric and other Gram-negative bacteria. FEMS Microbiol. 1996; Lett. 139: 1–10.
10. Grkovic, S., M. H. Brown, and R. A. SkurrayTranscriptional regulation of multidrug efflux pumps in bacteria. Semin. Cell Dev. Biol. 2001; 12: 225– 237.
11. Keeney, D.; Ruzin, A.; and Bradford, PA. RamA, a transcriptional regulator, and AcrAB, an RND-type efflux pump, are associated with decreased susceptibility to tigecycline in Enterobacter cloacae. Microb Drug Resist. 2007; 13: 1–6.
12. Mazzariol, A.; Zuliani, J.; Cornaglia, G.; Rossolini, GM.; Fontana, R.. AcrAB Efflux System: Expression and Contribution to Fluoroquinolone Resistance in Klebsiella spp. Antimicrob Agents Chemother. 2002 Dec; 46(12): 3984-3986.

13. Nikaido, H. Multidrug efflux pumps of Gram-negative bacteria. J Bacteriol, 1996; 178: 5853–5859.
14. Othman EO, Abdel-Samad MF, Abo El Maaty NA, Sewify KM. Evaluation of DNA polymorphism in Egyption buffalo growth hormone and its receptor genes. J. Appl.Biol.Sci. 2012; 6: 37-42.
15. Pakzad, I.; Zayyen Karin, M.;and Taherikalani, M.; Boustanshenas, M.; Lari, AR. .Contribution of AcrAB efflux pump to ciprofloxacin resistance in Klebsiella pneumoniae isolated from burn patients. GMS Hyg Infect Control. 2013; 8(2).
16. Peleg, AY.; Adams, J.; and Paterson DL. Tigecycline Efflux as a Mechanism for Nonsusceptibility in Acinetobacter baumannii. Antimicrob Agents Chemother. 2007; 51(6): 2065-2069.
17. Ruzin, A.;Visalli, MA.; Keeney, D.; and Bradford, PA.. Influence of transcriptional activator RamA on expression of multidrug efflux pump AcrAB and tigecycline susceptibility in Klebsiell pneumoniae. Antimicrob Agents Chemother. 2005; 49(3): 1017-1022.
18. Ryan,KJ. and Ray,CG. Sherris Medical Microbiology.McGra Hill. ISBN. 2004; 8385-8529.
19. Schneiders, T.;Amyes, SG.; and Levy, SB. (Role of AcrR and RamA in fluoroquinolone resistance in clinical Klebsiella pneumoniae isolates from Singapore. Antimicrob Agents Chemother. 2003; 47(9): 2831-2837.
20. Szabó, D.; Silveira, F.; Hujer, AM.; Bonomo, RA.; Hujer, KM.; Marsh, JW.; Bethel, CR.; Doi, Y.; Deeley, K. and Paterson, DL. (Outer membrane protein changes and efflux pump expression together may confer resistance to ertapenem in Enterobacter cloacae Antimicrob Agents Chemother; 2006; 50: 2833-2835.
21. Van Bambeke, F.; Balzi, E. and Tulkens, PM. Antibiotic efflux pumps. Biochem Pharmacol. 2000; 60(4): 457-470.