Isolation and Molecular Characterization of *Klebsiella pneumonia* isolated from Dust

Lara Q. Hamzam[1,*], Laith Abdulhassan M. Jawad[1]

[1] *Department of Biology, Science College, AL-Muthanna University, Samawa, Iraq*

Received 27/12/2016, Accepted 26/2/2017, Published 9/3/2017

Abstract Dust includes many microorganisms such as airborne bacteria. Most types of these bacteria are harmful to humans and causing severe problems. *Klebsiella pneumoniae* considered as serious nosocomial pathogen that cause pneumonia, UTIs, wound and liver abscesses. One hundred fifty samples isolated randomly from different indoor and outdoor areas during the period November 2014- February 2015. In the indoor isolates bacterium *K. pneumoniae* was the commonest pathogen (36.70%) followed by *S. aureus* (22.78%), *S. epidermidis* (12.65%), *Bacillus* spp. (10.12%), *P. aeruginosa* (5.06%), *E. cloacae* (5.06%), *Streptococcus* spp.(2.53%), *Citrobacter* spp.(1.26%) and *Pantoea* spp.(1.26%). While *S. aureus* comprises the majority of outdoor bacteria in (41.66%). Using PCR method for detection of *Uge* gene and followed with direct DNA Sequencing. The multiple sequence alignment (MSA) showed highly conserved area in all studied strains. However, some variants were observed at different positions (24, 124, 152, 163, 165, 185, 199, 200, 207, 209, 248, 279, 284, 291). Also there were changes in a functional whole areas at the studied gene such as (GGCTGG) at the positions (212-217), (ATCCCG) at positions (226-231), (GCCC) at (233-236), (ACCG) at positions (242-245), (GATT) at (263-266), (TCAA) at (269-272). These differences in the gene sequence may indicates special characterizations for the isolated strain.

Keywords: klebsieua pneumonia, Molecular study, Dust Alignment,DNA sequencing.

Introduction

According to APHA (American Public Health Association) reported that airborne microorganisms can arise from many sources including air-conditioning systems, raw materials and specific food production systems [1]. These organisms include micro- and macro-fungi together with bacteria and their dormant spores [2]. These microorganisms are liable to spread infection.

A wide range of public health issues, such as infectious diseases, acute toxic effects and allergies can be arise from the exposure to the microorganisms [3]. People especially the patients are not only the sole source for the production of the airborne microorganisms. Other sources, however can produce the airborne microorganisms, such as different institutions where sick and injured people are treated and other indoor and outdoor conditions and surroundings [4]. In addition to their negative effects on the environment, airborne microbes are also metabolically active and well adapted for the harsh atmospheric conditions [5]. The infectious disease coming from *Klebsiella* is caused mainly by *K. pneumonia* and *K. oxytoca* .They are opportunistic bacterial pathogens associated with nosocomial infections such as urinary tract infection (UTI), pneumonia and septicemia [6]. In this study we aimed to identify types of indoor and outdoor bacteria, confirmed by molecular methods and study the evolutionary aspect of the detected species.

Materials and Methods

Sampling

A total of 150 indoor and outdoor isolates were collected randomly from AL-Samawa City during the period from November 2014 to February 2015. The isolates were

collected under sterilized conditions using sterilized cotton swabs from different indoor and outdoor places including: Air-Conditioning systems, School-desks, Furnish, Floors, Kitchens and streets edges.

Isolation and identification of *Klebsiella pneumoniae*

Several different media were used for culture of *Klebsiella pneumoniae* from the isolates such as, Nutrient agar, NIH broth, EMB agar, Brain heart infusion broth, Sallmonla-Shigella agar, MacConkey agar, Mannitole Salt agar and Blood agar [7]. The media were prepared under sterilized conditions at the laboratory of Biology department/ College of Science/ Al Muthanna University.

The identification of gram negative bacteria was performed by standard biochemical methods (Catalase test, Oxidase test, API 20E test) according to [7].

Bacterial Genomic DNA Extraction of *Klebsiella pneumoniae*

Bacterial genomic DNA of *Klebsiella pneumoniae* isolates were extracted by using (ZR Bacterial DNA MidiPrep™, Zymoresearch, USA), and done according to company instructions.

PCR Assay

For identification and detection of *Klebsiella pneumoniae, Uge* gene was used. The gene primer was designed by Aher et al. [8].

Table 1. The primer used in this study

Primer	Sequence		size	Reference
Uge	F	TCTTCACGCCTTCCTTCACT	534 bp	Aher et al. (2012)
	R	GATCATCCGGTCTCCCTGTA		

PCR master mix reaction was prepared by using (2xHot start PCR Mastermix) and this master mix was done according to company instructions. The master mix including (1ng/µl) DNA template, 2xHot start PCR Mastermix (12.5 µl), each of forward and reverse gene primer (0.5 µl), Nuclease-free H2O (10.5 µl) for the total of (25µl). After that, these PCR master mix reaction components placed in standard PCR tubes containing the PCR PreMix as lyophilized materials containing all other components needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, MgCl2,stabilizer & tracking dye). Then the tube placed in Exispin vortex centrifuge for 3 minutes. Then transferred in Mygene PCR thermocycler. The PCR thermocycler conditions were set as 30-35 cycle of Denaturation 94ºC for 30 sec; Annealing 55ºC for 30 sec; Extention 72 ºC for 5 min.

Statistical analysis

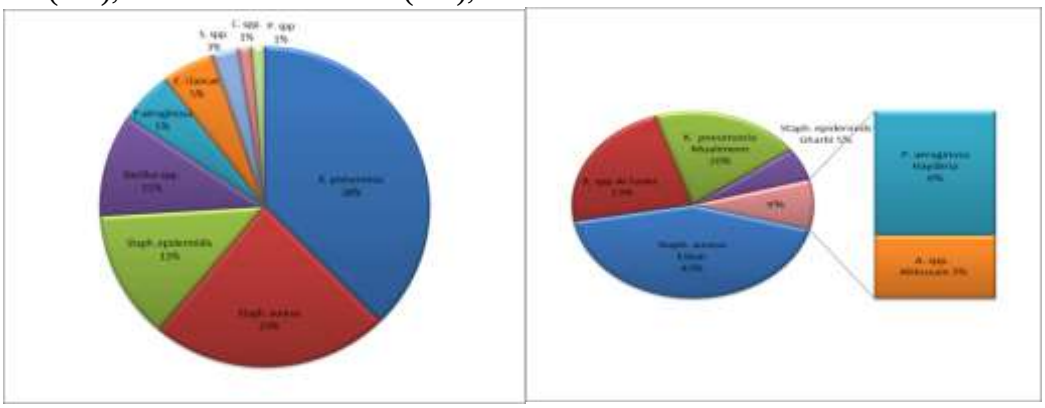
Statistical analysis was performed using SPSS computing program for the analysis of the obtained results (SSPS, 2008).

RESULTS

Isolation and identification of bacteria

A total of 150 isolates were collected randomly from different areas in Samawa City during the period from November 2014-February 2015. Our results include (100) isolates from indoor ,(79) of them were positive in the growth culture while (11) were negative (with percentage 79%).The outdoor isolates were (50), (14) were negative in the growth culture and (36) isolates were positive (with percentage 72%). In the indoor isolated bacterial species *K. pneumoniae* was the commonest pathogen (36.70%) followed by *S. aureus* (22.78%), *S. epidermidis* (12.65%), *Bacillus* spp. (10.12%), *P. aeruginosa* (5.06%), *E. cloacae* (5.06%), *Streptococcus* spp. (2.53%), *Citrobacter* spp. (1.26%) and *Pantoea* spp. (1.26%) respectively (Fig.1a). While the pathogen *S. aureus* comprises the majority of

outdoor bacteria as isolated in (41.66%) followed by *Bacillus* spp.(22.22%), *K. pneumoniae* (19.44%), *S. epidermidis* (5.55%), *P. aeruginosa* (5.55%), *Aeromonas* spp. (2.77%) and *C. violaceum* (2.77%) respectively (Fig.1b). The percentage of indoor species showed that *K. pneumoniae* comprises (38%) of all isolates followed by *S. aureus* (23%), *S. epidermidis* (13%), *Bacillus* spp.(11%), *P. aeruginosa* (5%), *E. cloacae* (5%), *Streptococcus.* spp. (3%), *Citrobacter* spp. (1%) and *Pantoea* spp. (1%), respectively. The percentage of outdoor species according to the place of collection showed that *staph. aureus* comprises (43%) in Al-Eskan, followed by *Bacillus* spp. (23%) in Al-Sader, *K. pneumoniae* (20%) in Al-Mualimeen, *S. epidermidis* (5%) in Al-Gharbi, *P. aeruginosa* (6%) in Al-Hayderia and (3%) for *Aeromonas* spp. in Al-Hussain.



A **B**

Fig.(1): A. The Percentage of Indoor isolated bacterial species. B. The percentage of outdoor isolated bacterial species.

Table 2: significant differences between indoor and outdoor sources:

Bacterial species	Indoor source	Outdoor source
Staphylococcus aureus	**22.78**	**41.66****
Bacillus spp.	**10.12**	**22.22****
Klebsiella pneumoniae	**36.7****	**19.44**
Staphylococcus epidermidis	**12.65***	**5.55**
Pseudomonas aeruginosa	**5.06**	**5.55**

*Significant differences at ($p \leq 0.05$).
** Significant differences at ($p \leq 0.01$).

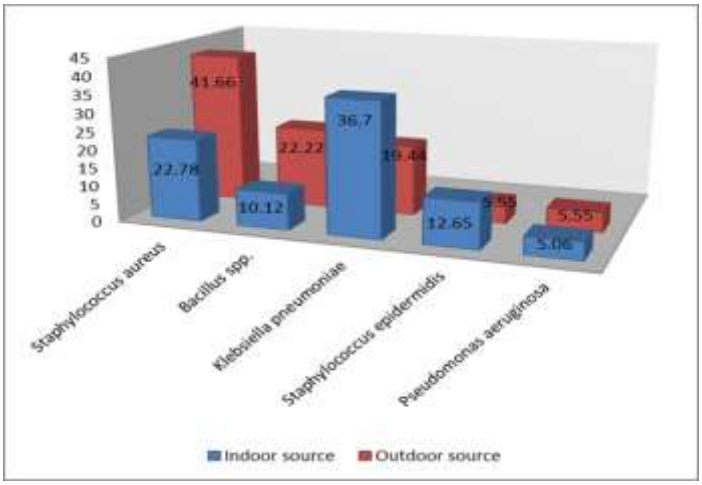


Fig. (2): significant differences between indoor and outdoor sources.

The bacterial isolates were activated using Alternative Thioglycolate Medium (NIH Thioglycolate Broth) for sterility purpose, also in order to isolate and growth the largest possible number of bacterial species [9] which cultured by streaking on different media at temperature between 35-37C^{o} for 24-48hr. Our results showed mucoid colonies with diffusing red pigment on MacConkey agar and were non-hemolytic on blood agar. bacterial swab stained by gram stain and examined under light microscope showed that *K. pneumoniae* are gram-negative small rods.

a **b**

Fig.(3): A. The positive result of catalase test. B. The API 20E test result.

Three positive isolates were subjected to conventional PCR for the confirmation of *K. pneumoniae* using *Uge* gene as a diagnostic gene. It has been showed that all of them were positive for *Uge* gene (Fig.4).

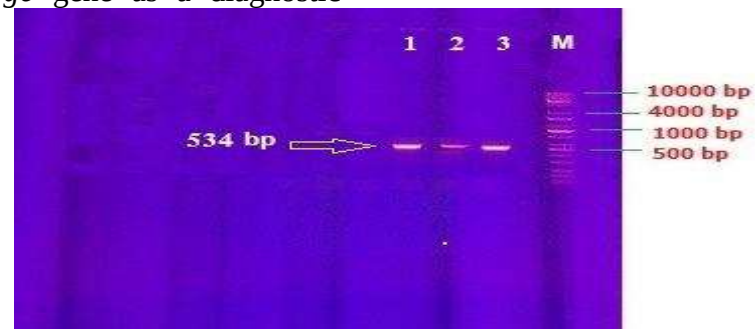


Fig.(4): Agarose gel electrophoresis image that show PCR product analysis of *Uge* gene in *K. pneumoniae*. Where M: marker (10000bp), lane (1-3) positive gene at (534bp) PCR product.

For sequence analysis, *Uge* gene was deposited to the NCBI database under the GeneBank accession number KX668629. The sequenced PCR products were submitted for the sequence of similarity using BLASTnt of NCBI. The results showed that our gene sequence was showed a high degree of homology with *K. pneumoniae* strain B-941 and *K. pneumoniae* strain Mich. 61204 (94% of identity), and 93% for other studied species. The ORF region that encode for *Uge* protein was detected using ORF Finder Program. The result showed that the actual part of *Uge* gene is 327bp in size encodes for *Uge* protein (108 amino acids).

Table 3: The Homology between *K. pneumoniae Uge* gene and other studied strains:

Species strain	Sequence ID	Identity
Klebsiella pneumoniae	**KX668629**	**-**
***Klebsiella pneumonia* strain B-941/14**	**KP760052.1**	**94%**
***Klebsiella pneumonia* strain Mich. 61 O4 lipopolysaccharide biosynthesis gene**	**KU310493.1**	**94%**

***Klebsiella pneumonia* strain F-35**	**KP760055.1**	**93%**
***Klebsiella pneumonia* strain I-6208**	**KP760056.1**	**93%**
***Klebsiella pneumonia* strain B-690/14**	**KP760057.1**	**93%**
***Klebsiella pneumonia* strain B-958/14**	**KP760053.1**	**93%**

The MSA showed highly conserved area in all studied strains. However, there are differences in the type of nucleotides at some places. Clearly changes were observed in our sequence at some regions (C to G) at positions (24, 124, 152, 163, 165, 185, 199, 200, 207, 209, 248, 279, 284, 291) respectively, while (A to G) at positions (151, 194, 251) also changes in the nucleotide (T to G) in positions (191, 254, 256, 258, 281, 321), where C

changed to A in the position (188, 293, 307) and (T to A) in position (286, 306) while (C to T) were in positions (249) and (T to C) in position (288) of nucleotides sequence. Also there were changes as a whole codons such as (GGCTGG) were changed to (CAGGAT) in the positions (212-217) and (ATCCCG) to (GCAGGC) in positions (226-231), also (GCCC) instead of (AATG) at (233-236), (ACCG) to (TGGA).
in position (242-245), (GATT) to (CCCG) at (263-266), (TCAA) to (CACC) at (269-272), (TTGTA) to (AACAT) at (297-301) and (TTTTTC) to (CACCAG) in the positions (313-318) respectively, while other sequences were highly conserved with C,T,A and G (Fig. 5).

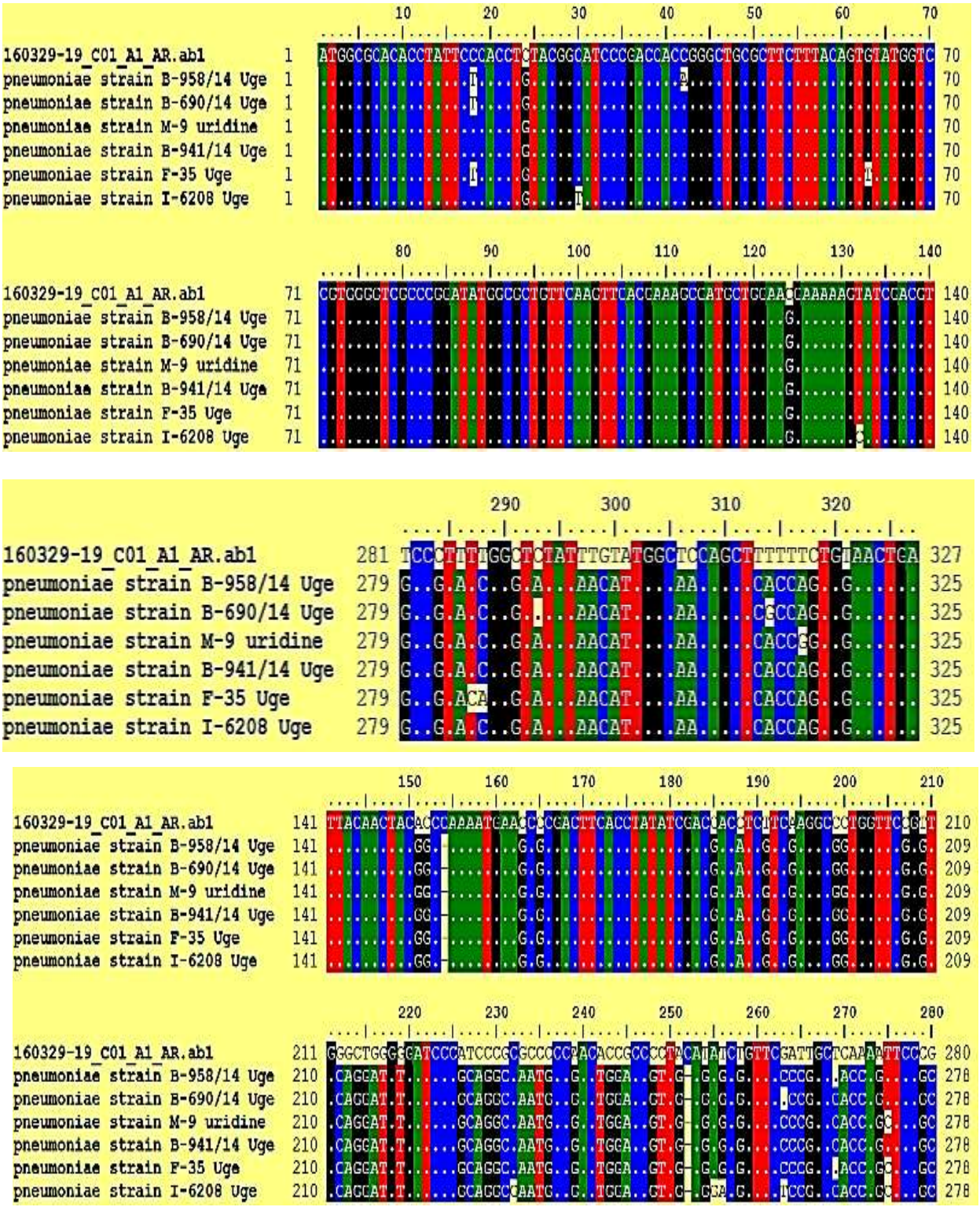


Fig.(5): The multiple sequence alignment between our isolate and other selected strains

In the indoor isolated bacterial species *K. pneumoniae* was the commonest pathogen followed by *S. aureus*, *S. epidermidis*, *Bacillus* spp., *P. aeruginosa*, *E. cloacae*, *Streptococcus*

spp., *Citrobacter* spp. and *Pantoea* spp. respectively.

As described above, *K. pneumoniae* was the most common pathogen of indoor air in this study, as isolated in (36.70%) of indoor samples. A previous study by Jones et al. [10] showed that *K. pneumoniae* was the third most commonly isolated Gram-Negative microorganism behind *P. aeruginosa and Escherichia coli*. Also, *Klebsiella spp*. are reported to be responsible for (7-10%) of bloodstream infections in Europe, North America and South America according to data collected by the SENTRY Antimicrobial Surveillance Program [11].

The indoor results according to the percentage showed significant differences at ($P \leq 0.05$). On the other hand, the pathogen *Staphylococcus aureus* comprises the majority of outdoor bacteria followed by *Bacillus* spp., *K. pneumoniae*, *S. epidermidis*, *P. aeruginosa*, *Aeromonas* spp. and *C. violaceum*, respectively.

As shown above the bacteria *S. aureus* comprises the majority of outdoor bacteria, as isolated in (41.66%) of outdoor samples in this study. These results bring into line with a study by Abdel Hameed and Awad (2002) suggested that the higher counts of airborne staphylococci and total viable bacteria are due to insufficiency of the air conditioning system and ventilation rate, passenger activities [12]. The outdoor results between percentages showed significant differences at ($P \leq 0.05$).

According to a study by Rudan et al. [13] reported that *K. pneumoniae* and *Staphylococcus aureus* were the most responsible cause of severe cases of pneumonia among children aged under 5 years, and Since the individuals spend most of their time in the indoor environments compared to the outdoors as mentioned by Lebowitz [14], So we focused on *K. pneumoniae* in this study for this reason since it comprises the majority of indoor pathogens.

A study by Soleimani et al. [15] showed that *K. pneumoniae* in the normal weather conditions comprises (3.4%) of total 88 samples in the Universities while in Dusty weather it encompasses (9.1%) of total 22 samples.

The bacterial isolates were collected under sterilized conditions using sterilized cotton swabs. Samples had been activated using Alternative Thioglycolate Medium (NIH Thioglycolate Broth) for sterility purpose and also in order to isolate and growth the largest possible number of bacterial species [9] which cultured by streaking on different media at temperature between (35-37C^{o}) for (24-48hr).In the primary culture, It was found that culturing on media without using the anti-fungal (Nystatin) and doing subculture on media containing Nystatin was better to get bacterial growth, as reported in a study by Haidaris and Brownlow showing that airborne fungi are the most commonly environmental contaminants that grow rapidly as fuzzy colonies on a bacterial plate. They found out two prominent sources of contamination which are the introduction of microbes from the environment and the mix-up of two or more closely related strains of bacteria [16], So the best way to get a pure culture of bacteria in this study was by using antifungal to prevent contamination.

K. pneumoniae and *S. aureus* significant differences between indoor and outdoor sources were at ($p \leq 0.01$), *S. epidermidis* were at ($p \leq 0.05$). While *P. aeruginosa* has no significant differences between indoor and outdoor sources. An outdoor study conducted by Kennedy et al. [17] showed that *S. aureus* can survive and grow under conditions of higher temperatures from 7^{o}C – 47.8^{o}C in the presence of UV light. This result bring into line with our study where *S. aureus* showed high percentage in the outdoor environment more than the indoor where *K. pneumoniae* comprised the higher percentage. The bacteria capable of transmission through aerosols such as, *Klebsiella* species can survive in high RH and in low temperature [18],[19].

A previous study by [20] demonstrated that the survival of *K. pneumoniae* was to some extent dependent upon relative humidity 60%.

Molecular study

In our study we used Conventional PCR which is commonly used technique to determine the presence of virulence gene *Uge* (encoding for Uridine diphosphate galactourinate 4-epimerase) that associated with the virulence in *K. pneumoniae*. A study by Regué [21] proved that the mutant strains of *K. pneumoniae* (without *Uge* gene) were non-virulent in laboratory animals; this fact proves the important role of *Uge* gene in pathogenicity of *K. pneumoniae*.

All *K. pneumoniae* isolates tested by Conventional PCR in this study were positive for *Uge* gene. The positive isolates of *Uge* gene were sequenced for homology using BLASTnt. The sequenced PCR product results showed that *uge* gene sequence was showed a high degree of homology with *K. pneumoniae* strain B-941 and *K. pneumoniae* strain Mich. 61 O4 (94% of identity), and 93% for other studied species. The *K. pneumoniae* strain B-941/14 was isolated in Russia from endotracheal aspirate of *K. pneumoniae sub.pneumoniae wabG* gene (620bp) which encodes for glucosyltransferase [22] which showed a high similarity with our gene 94% of identity, Also the *K. pneumoniae* strain Mich. 61 O4 lipopolysaccharide biosynthesis gene was isolated by Fang e [23] that encodes for phosphogluconate dehydrogenase in *K. pneumoniae*.

According to Cortes [24], capsule associated gene (*Uge*) promote infection by resistance to phagocytosis. This gene was commonly found in *K. pneumoniae* isolates, it seem to be at the basis of pathogenicity of *K. pneumoniae* [24]. A previous research conducted by Ho [25] on mutant ugd based on both anti K1 serum test by double immunodiffusion assay and string test, all mutants obtained lost the K1 serotype and mucoviscosity while remaining O1 serotype positive, suggesting that these mutants produce little or no CPS. Moreover, "deletion of *Uge* in the proposed *lps* locus led to loss of O1 serotype, confirming the proposed boundary between cps and *lps* loci. Since the deletion mutants make little or no CPS, they are expected to lose pathogenicity also" [25].

The multiple sequence alignment (MSA) showed highly conserved area in all studied strains. However, there are differences in the type of nucleotides at some places. Clearly changes were observed in our sequence at some regions (C to G) at positions (24, 124, 152, 163, 165, 185, 199, 200, 207, 209, 248, 279, 284, 291) respectively, while (A to G) at positions (151, 194, 251) also changes in the nucleotide (T to G) in positions (191, 254, 256, 258, 281, 321), where C changed to A in the position (188, 293, 307) and (T to A) in position (286, 306) while (C to T) were in positions (249) and (T to C) in position (288) of nucleotides sequence. Also there were changes as a whole codons such as (GGCTGG) were changed to (CAGGAT) in the positions (212-217) and (ATCCCG) to (GCAGGC) in positions (226-231), also (GCCC) instead of (AATG) at (233-236), (ACCG) to (TGGA) in position (242-245), (GATT) to (CCCG) at (263-266), (TCAA) to (CACC) at (269-272), (TTGTA) to (AACAT) at (297-301) and (TTTTTC) to (CACCAG) in the positions (313-318) respectively, while other sequences were highly conserved with C,T,A and G. These differences in our gene sequence may indicates special characterizations for this strain.

Also in fungi the uge gene is predicted to encode a putative UDP glucose-4-epimerase gene (*Uge* A), required for the biosynthesis of Galf as well as for Galp metabolism in *Aspergillus niger*. The mutation in the *Uge* A gene of mutant (A to G) caused the change of a codon from AAC to GAC which consequently resulted in the change of Asn to Asp at position 191 in the *Uge* A protein [26].

A study by Seifert [27] on RHD1gene in *Arabidopsis thaliana* concluded that the flux of galactose from UDP-D-Gal into different downstream products is compartmentalized at the level of cytosolic *Uge* iso-forms. This suggests that substrate channeling plays a role in the regulation of plant cell wall biosynthesis and the single UGE isoforms from *Homo sapiens* and *Escherichia coli* and the amino-terminal moiety of Gal10 from *Saccharomyces cerevisiae* are 53%, 49%, and 48% identical to RHD1, respectively [27].

A previously detection of uge gene by Aher & Kumar [8] in isolated strains of *K.*

pneumoniae from goats revealed that this gene was the second most prevalent virulence gene.

A NAD-dependent epimerase/ dehydratase (N-terminal) domain is predicted to occupy amino acids 1 to 50 in *Uge* sequence where the NAD (p) binding domain occupy amino acids 1 to 55 in the gene (Figure 4-12). The *Uge* catalyzes the conversion of UDP-D-glucuronate to UDP-D-galacturonate, which is also present in both bacterial capsules and LPS.

The effects of a *Uge* null mutation on colonization and virulence were studied in *K. pneumoniae* 52145, which is a highly virulent strain able to colonize different surfaces. A *Uge* deletion reduced colonization and rendered the strain completely a virulent in an experimental model of pneumonia. This suggests that the *Uge* mutation could have important, measurable effects on colonization and virulence [21].

The ORF results of the region encodes for *Uge* protein showed that the actual part of *Uge* gene is (327bp) in size encodes for *Uge* protein (108 amino acids).

The proteins encoded by this operon are also involved in the degradation of urea in ammonia and carbamate in carbonic acid, causing PH decreasing and allow survival to acid environment, like metabolic acidosis in hosts with sepsis [28].

Conclusion

The dusty air consider a suitable environment for aerosolized pathogenic bacteria. *S. aureus* was the most rate bacteria in the outdoor environment that cause a serious diseases. *K. pneumoniae* comprises the high incidence rate of the nosocomial pathogenics as airborne bacteria. *Uge* gene sequence in this study was different in specific regions from other studied strains.

References

[1]Speck, M. L. (1984). Compendium of methods for the microbiological examination of foods (No. 576.163 AMEc). American Public Health Association.

[2]Medrela-Kuder, E. (2003). Seasonal variations in the occurrence of culturable airborne fungi in outdoor and indoor air in Cracow. International Biodeterioration & Biodegradation, 52(4), 203-205.

[3]Douwes, J., Thorne, P., Pearce, N.& Heederik, D. (2003). Bioaerosol health effects and exposure assessment: progress and prospects. Annals of Occupational Hygiene, 47(3), 187-200.

[4]Scaltriti, S., Cencetti, S., Rovesti, S., Marchesi, I., Bargellini, A.,& Borella, P. (2007). Risk factors for particulate and microbial contamination of air in operating theatres. Journal of Hospital Infection, 66(4), 320-326.

[5]Vaïtilingom, M., Attard, E., Gaiani, N., Sancelme, M., Deguillaume, L., Flossmann, A. I., ...& Delort, A. M. (2012). Long-term features of cloud microbiology at the puy de Dôme (France). Atmospheric environment, 56, 88-100.

[6]Podschun, R., & Ullmann, U. (1998). Klebsiella spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clinical microbiology reviews, 11(4), 589-603.

[7]MacFaddin, J.E. (2000). Individual biochemical tests. In: Biochemical tests for identification of medical bacteria. pp.1-456, 3rd ed., Lippincott Williams and Wilkins, Philadelphia.

[8]Aher, T., Roy, A., & Kumar, P. (2012). Molecular detection of virulence genes associated with pathogenicity of Klebsiella spp. isolated from the respiratory tract of apparently healthy as well as sick goats. Israel Journal of Veterinary Medicine, 67(4), 249-252.

[9]Nungester, W. J., Mary N. Hood, and Mary K. Warren (1943). "Use of Thioglycollate Media for Testing Disinfectants."Experimental Biology and Medicine 52.4: 287-289.

[10] Jones, M. E., Draghi, D. C., Thornsberry, C., Karlowsky, J. A., Sahm, D. F., & Wenzel, R. P. (2004). Emerging resistance among bacterial pathogens in the intensive care unit–a European and North American Surveillance study (2000–2002). Annals of Clinical Microbiology and Antimicrobials, 3(1), 14.

[11]Alves, M. S., da Silva Dias, R. C., de Castro, A. C. D., Riley, L. W., & Moreira, B. M. (2006). Identification of clinical isolates of indole-positive and indole-negative Klebsiella

spp. Journal of clinical microbiology, 44(10), 3640-3646.
[12]Awad, A. H. A. (2002). Environmental study in subway metro stations in Cairo, Egypt. Journal of Occupational Health, 44(2), 112-118.
[13]Rudan, I., Boschi-Pinto, C., Biloglav, Z., Mulholland, K.& Campbell, H. (2008). Epidemiology and etiology of childhood pneumonia. Bulletin of the World Health Organization, 86(5), 408-416B.
[14]Lebowitz, M. D., & Walkinshaw, D. S. (1992). Indoor Air'90: Health effects associated with indoor air contaminants. Archives of Environmental Health: An International Journal, 47(1), 6-7.
[15]Soleimani, Z., Parhizgari, N., Rad, H. D., Akhoond, M. R., Kermani, M., Marzouni, M. B., ...& Goudarzi, G. (2015). Normal and dusty days comparison of culturable indoor airborne bacteria in Ahvaz, Iran. Aerobiologia, 31(2), 127-141.
[16]Haidaris, C. G., & Brownlow, E. J. (2001). Working safely with biological samples. Molecular Biology Problem Solver, a laboratory guide, 136-138.
[17]Kennedy, J., Blair, I. S., McDowell, D. A.,& Bolton, D. J. (2005). An investigation of the thermal inactivation of Staphylococcus aureus and the potential for increased thermotolerance as a result of chilled storage. Journal of applied microbiology, 99(5), 1229-1235.
[18]Marthi, B., Fieland, V. P., Walter, M., & Seidler, R. J. (1990). Survival of bacteria during aerosolization. Applied and environmental microbiology, 56(11), 3463-3467.
[19]Walter, M. V., Marthi, B., Fieland, V. P., & Ganio, L. M. (1990). Effect of aerosolization on subsequent bacterial survival. Applied and environmental microbiology, 56(11), 3468-3472.
[20]Bolister, N. J., Johnson, H. E., & Wathes, C. M. (1992). The ability of airborne Klebsiella pneumoniae to colonize mouse lungs. Epidemiology and infection, 109(1), 121.
[21]Regué, M., Hita, B., Piqué, N., Izquierdo, L., Merino, S., Fresno, S., ... & Tomás, J. M. (2004). A gene, uge, is essential for Klebsiella pneumoniae virulence. Infection and immunity, 72(1), 54-61.
[22]Astashkin, S. V. (2015). Disjointly homogeneous rearrangement invariant spaces via interpolation. Journal of Mathematical Analysis and Applications, 421(1), 338-361.
[23]Fang, C. T., Shih, Y. J., Cheong, C. M., & Yi, W. C. (2015). Rapid and accurate determination of lipopolysaccharide O-antigen types in Klebsiella pneumoniae with a novel PCR-based O-genotyping method. Journal of clinical microbiology, JCM-02494.
[24]Cortés, G., Borrell, N., de Astorza, B., Gómez, C., Sauleda, J., & Albertí, S. (2002). Molecular analysis of the contribution of the capsular polysaccharide and the lipopolysaccharide O side chain to the virulence of Klebsiella pneumoniae in a murine model of pneumonia. Infection and immunity, 70 (5), 2583-2590.
[25]Ho, J. Y., Lin, T. L., Li, C. Y., Lee, A., Cheng, A. N., Chen, M. C., ... & Tsai, M. D. (2011). Functions of some capsular polysaccharide biosynthetic genes in Klebsiella pneumoniae NTUH K-2044. PLoS One, 6(7), e21664.
[26]Park, J., Tefsen, B., Arentshorst, M., Lagendijk, E., van den Hondel, C. A., van Die, I., & Ram, A. F. (2014). Identification of the UDP-glucose-4-epimerase required for galactofuranose biosynthesis and galactose metabolism in A. niger. Fungal Biology and Biotechnology, 1(1), 6.
[27]Seifert, G. J., Barber, C., Wells, B., Dolan, L., & Roberts, K. (2002). Galactose biosynthesis in Arabidopsis: genetic evidence for substrate channeling from UDP-D-galactose into cell wall polymers. Current Biology, 12(21), 1840-1845.
[28]Kakinuma, Y., Iida, H., Sekizuka, T., Usui, K., Murayama, O., Takamiya, S., ...& Matsuda, M. (2007). Cloning, sequencing and characterization of a urease gene operon from urease-positive thermophilic Campylobacter (UPTC). Journal of applied microbiology, 103(1), 252-260.