ISOLATION AND IN SILICOCHARACTERIZATION OF UGE GENE IN KLEBSIELLA PNEUMONIA ISOLATED FROM INDOOR DUST

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Abstract - Dust includes many micro-organisms such as bacteria named as airborne bacteria. Most types of these bacteria are harmful to humans and causing severe problems. Klebsiellapneumoniae consider a serious nosocomial pathogen that cause pneumonia, UTIs, wound and liver abscesses. One hundred fifty (150) samples isolated randomly from different indoor and outdoor areas during the period November 2014- February 2015. In the indoor isolates bacteriumKlebsiellapneumoniae was the commonest pathogen (36.70%) followed by Staphylococcus aureus (22.78%), Staphylococcus epidermidis (12.65%), Bacillus spp. (10.12%), Pseudomonas aeruginosa (5.06%), Enterobacter cloacae (5.06%), Streptococcus spp.(2.53%), Citrobacter spp.(1.26%) and Pantoea spp.(1.26%) respectively. While the pathogen Staphylococcus aureuscomprises the majority of outdoor bacteria as isolated in (41.66%) followed by Bacillus spp.(22.22%), Klebsiellapneumoniae(19.44%), Staphylococcus epidermidis (5.55%), Pseudomonas aeruginosa (5.55%), Aeromonas spp. (2.77%) and Chromobacteriumviolaceum (2.77%) respectively. All K. pneumoniaeisolates detected and confirmed by conventional PCR using Ugegene. The positive isolates were sequenced and search for homology. The multiple sequence alignment showed that our bacterial gene have differences in NAD-dependent epimerase and NAD(p)- binding domain sequence at different sites.These differences in the gene sequence may indicates special characterizations for theisolated strain which may give new approach for developing higher sensitive K. pneumoniae strains.

Keywords - In silico, Klebsiella pneumonia, Dust, Air borne, Phylogenetic

I. INTRODUCTION

According to APHA (American Public Health Association) ‘‘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 Klebsiellais caused mainly by K. pneumonia and K. oxytoca.They are opportunistic bacterial patho¬gens associated with nosocomial infections such as uri¬nary 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.

II. MATERIALS AND METHODS

2.1 Samples

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.

2.2 Isolation and identification of Klebsiellapneumoniae

Several different media were used for culture of Klebsiellapneumoniae from the isolates such as, Nutrient agar, NIH broth, EMB agar, Brain heart infusion broth, S.S. agar, MacConkey agar, MSA 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].

2.3 Molecular Detection of Klebsiellapneumoniae

2.3.1 Bacterial Genomic DNA Extraction

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

2.3.2 PCR Reaction

For identification and detection of Klebsiellapneumoniae, Ugegene was used. The gene primer (Table 1) was designed by Aher [8].

Table 1. The primer used in this study

Primer	Sequence		size	source
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-HClpH: 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; Annealing55ºC for 30 sec; Extention72 ºC for 5 min.

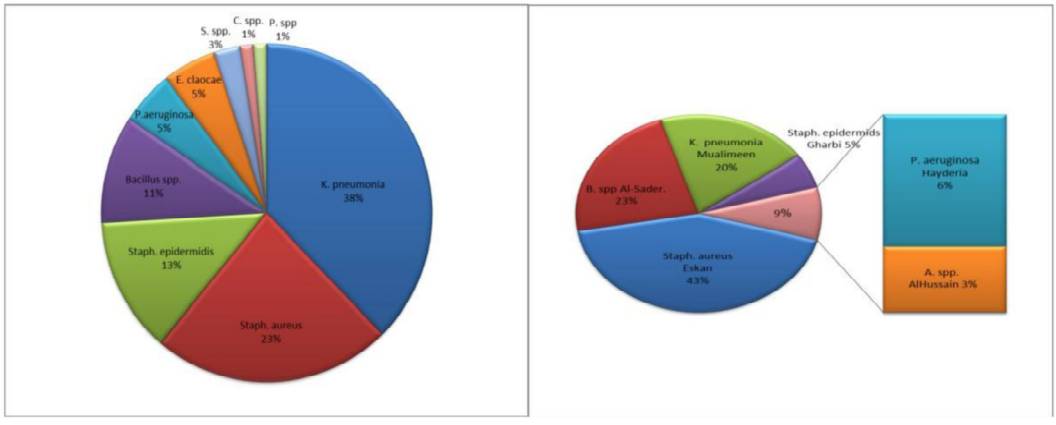
2.4 Statistics Analysis

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

III. RESULTS

3.1 Isolation and Identification of Bacteria

A total of (150) isolates were collected randomly from different areas in AL-Samawa City during the period 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 Klebsiellapneumoniae was the commonest pathogen (36.70%) followed by Staphylococcus aureus (22.78%), Staphylococcus epidermidis (12.65%), Bacillus spp. (10.12%), Pseudomonas aeruginosa (5.06%), Enterobacter cloacae (5.06%), Streptococcus spp.(2.53%), Citrobacter spp.(1.26%) and Pantoea spp.(1.26%) respectively (Figure 1a). While the pathogen Staphylococcus aureuscomprises the majority of outdoor bacteria as isolated in (41.66%) followed by Bacillus spp.(22.22%), Klebsiellapneumoniae(19.44%), Staphylococcus epidermidis (5.55%), Pseudomonas aeruginosa (5.55%), Aeromonas spp. (2.77%) and Chromobacteriumviolaceum (2.77%) respectively (Figure 1b).



A B

Figure 1.A: The Percentage of Indoor isolated bacterial species. B:The percentage of outdoor isolated bacterial species.

3.2 Diagnosis of Bacterial Isolates

The bacterial isolates were activated using Alternative Thioglycolate Medium (NIH ThioglycolateBroth) 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 35-37Cº for 24-48hr. Our results showedmucoid colonies with diffusing red pigment on MacConkey agar and were non-hemolytic on blood agar (Figure 2 a). bacterial swab stained by gram stain and examined under light microscope showed that K. pneumoniaeare gram-negative small rods.

3.3 Molecular Detection of K. pneumoniae

Three positive isolates were subjected to conventional PCR for the confirmation of K. pneumoniaeusing Ugegene as a diagnostic gene. It has been showed that all of them were positive for Ugegene (Figure3).

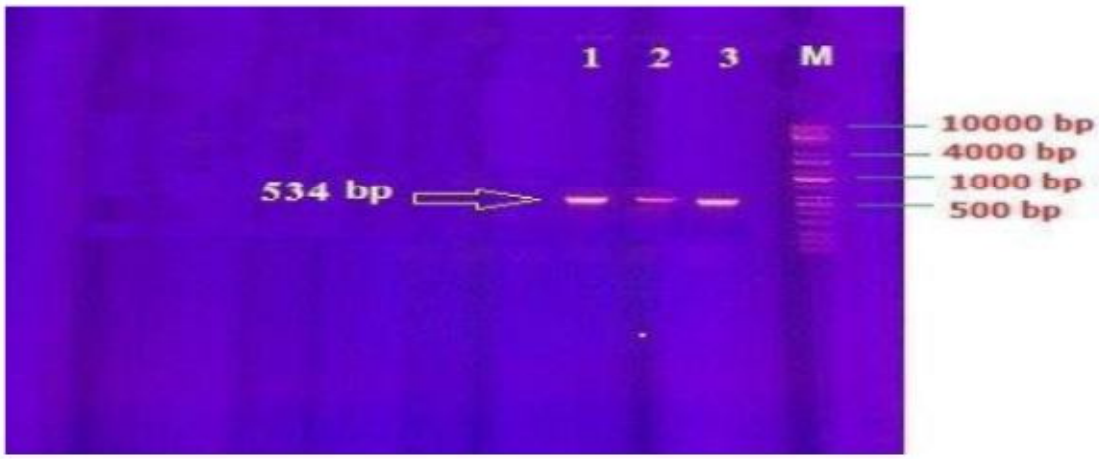


Figure 3. Agarose gel electrophoresis image that show the PCR product analysis of Uge gene (Virulence factor gene) in Klebsiellapneumoniae. Where M: marker (10000bp), lane (1-3) positive for virulence factor gene at (534bp) PCR product.

3.4 Bioinformatic Analysis

For sequence analysis, Uge gene was deposited at 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. pneumoniaestrain B-941 and K. pneumoniaestrain Mich. 61204 (94% of identity), and 93% for other studied species (Table 2). The ORF region that encode for Ugeprotein was detected using ORF Finder Program. The result showed that the actual part of Ugegene is 327bp in size encodes for Ugeprotein (108 amino acids).

Two structural domains in Uge protein; NAD-dependent epimerase and NAD(p)- binding domain (Figure 4) were analyzed further by multiple sequence alignment(Figure 5).

Table 2. The Homology between K. pneumoniaeUge gene and other studied strains

Species strain	Sequence ID	Identity
Klebsiellapneumoniae	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%

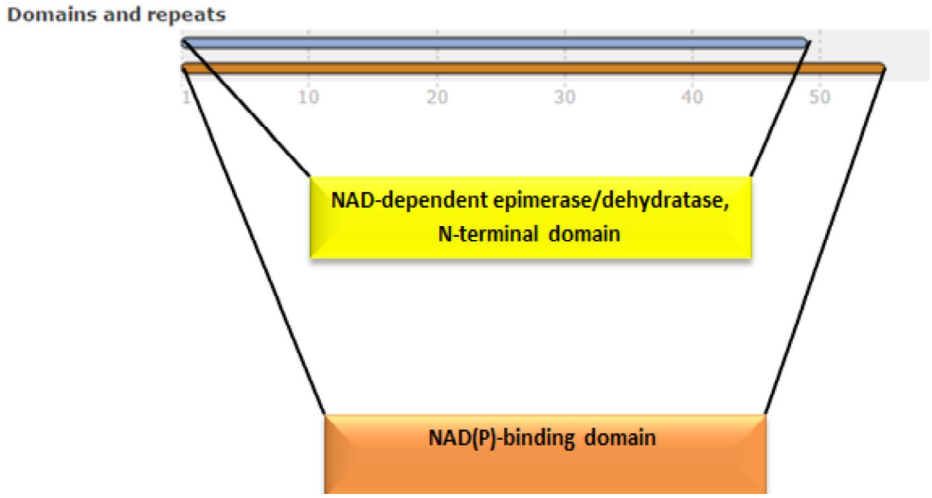


Figure 4. The main domains in open reading frame encoding for Uge gene in Klebsiella pneumonia

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 the positions (C:18,C:24,C:42,G:63,C:151,T:163) respectively where nucleotides C,G and T only located in our strain while other sequences were highly conserved with C,T,A and G(Figure 6).

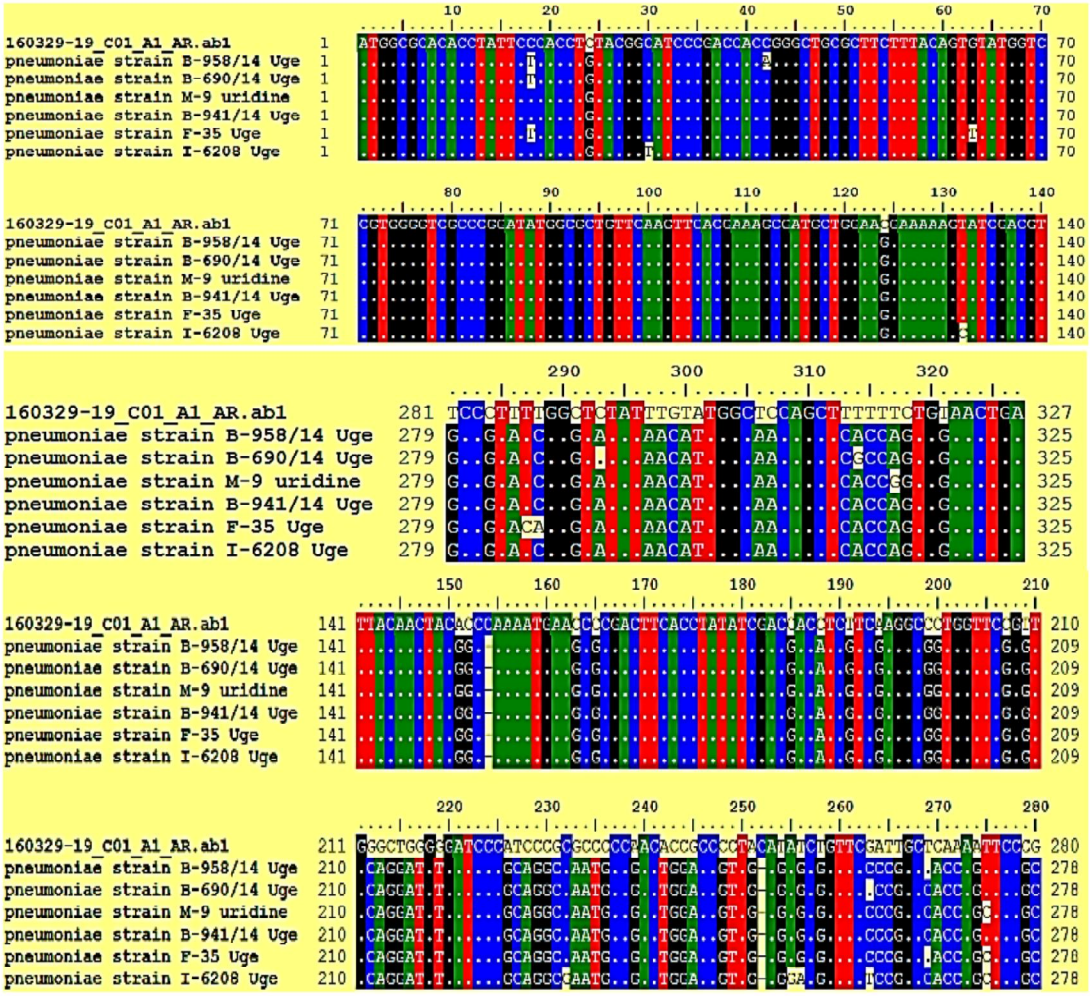


Figure 6.The multiple sequence alignment between our isolate and other selected strains.

IV. DISCUSSION

4.1 Clinical and Epidemiological Features

In the indoor isolated bacterial species Klebsiellapneumoniae was the commonest pathogen (36.70%) followed by Staphylococcus aureus (22.78%), Staphylococcus epidermidis (12.65%), Bacillus spp. (10.12%), Pseudomonas aeruginosa (5.06%), Enterobacter cloacae (5.06%), Streptococcus spp.(2.53%), Citrobacter spp.(1.26%) and Pantoea spp.(1.26%) respectively. As described above, Klebsiellapneumoniae 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 Klebsiellapneumoniae was the third most commonly isolated Gram-Negative microorganism behind Pseudomonas 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 percentage of indoor species showed that K. pneumoniae comprises (38%) of all isolates followed by Staph. aureus (23%), Staph. epidermidis (13%), Bacillus spp.(11%), P.aeruginosa(5%), E.cloacae(5%), Streptococcus. spp. (3%), Citrobacter spp. (1%) and Pantoea spp. (1%), respectively.All at significant differences at ($P \leq 0.05$).

While the pathogen Staphylococcus aureuscomprises the majority of outdoor bacteria as isolated in (41.66%) followed by Bacillus spp.(22.22%), Klebsiellapneumoniae(19.44%), Staphylococcus epidermidis (5.55%), Pseudomonas aeruginosa (5.55%), Aeromonas spp. (2.77%) and Chromobacteriumviolaceum (2.77%), respectively.

As shown above the bacteria Staphylococcus 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 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, Staph. epidermidis (5%) in Al-Gharbi, P. aeruginosa(6%) in Al-Hayderia and (3%) for Aeromonas spp. in Al-Hussain. All at significant differences at ($P \leq 0.05$).

According to a study by Rudan et al. [13] reported that Klebsiellapneumoniae and Staphylococcus aureuswere 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 the pathogen Klebsiellapneumoniaein this study for this reason since it comprises the majority of indoor pathogens. A study by Soleimani et al. [15] showed that K. pneumoniaein 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.

4.2 Diagnosis of Bacterial Isolates

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 (35-37Cº) 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.

4.3 Significant Differences Between Indoor and Outdoor Sources

The Significant differences between the outdoor and indoor Staphylococcus aureusand Bacillus spp. were at ($p \leq 0.01$), While Klebsiellapneumoniaesignificant difference between indoor and outdoor sources was at ($p \leq 0.01$). Staphylococcus epidermidis significant difference in the indoor to outdoor was at ($p \leq 0.05$), While Pseudomonas aeruginosa has no significant differences between indoor and outdoor sources.

An outdoor study conducted by Kennedy et al. [17] showed that S. aureuscan survive and grow under conditions of higher temperatures from 7ºC – 47.8ºCin 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. pneumoniaecomprised the higher percentage. The bacteria capable of transmission through aerosols such as, Klebsiellaspecies can survive in high RH and in low temperature [18],[19]. A previous study by [20] demonstrated that the survival of K. pneumoniaewas to some extent dependent upon relative humidity 60%.

4.4 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 Klebsiellapneumoniae. 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. pneumoniaeisolates tested by Conventional PCR in this study were positive for Ugegene. The positive isolates ofUgegene 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. pneumoniaestrain B-941 and K. pneumoniaestrain Mich. 61 O4 (94% of identity), and 93% for other studied species.TheK. pneumoniaestrain B-941/14 was isolated in Russia from endotracheal aspirate of K. pneumoniaesub.pneumoniaewabG gene (620bp) which encodes for glucosyltransferase [22] which showed a high similarity with our gene 94% of identity, Also the K. pneumoniaestrain 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. pneumo¬niaeisolates, it seem to be at the basis of pathogenic¬ity 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 that there are some differences between our sequence and other studied

strains. In our sequence, the differences showed changes at regions: (C:18), (C:24), (C:42), (G:63),(C:151), (T:163). 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 Aspergillusniger. The mutation in the ugeA 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 UgeA 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 isoforms. 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 ofuge 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 Ugegene is (327bp) in size encodes for Ugeprotein (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. Staphylococcus aureuswas the most rate bacteria in the outdoor environment that cause a serious diseases. Klebsiellapneumoniaecomprises the high incidence rate of the nosocomial pathogenics as airborne bacteria. Ugegene is responsible of Klebsiellapneumoniaevirulence which contributes in the biosynthesis of lipopolysaccharides. Ugegene sequence in this study was different in specific regions from other studied strains.

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