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MOLECULAR TYPING OF *KLEBSIELLA PNEUMONIAE* ISOLATED FROM DIFFERENT CLINICAL SAMPLES

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Abstract

A total of (209) clinical specimens were collected from patients suffering from different infections such asburns, wounds, ear infections, diabetic foot ulcer, urinary tract infections, vaginal infections, and respiratory tract infection. All clinical specimens were cultured on different media. The results indicated that 30/209 (14%) of the isolates belonged to *Klebsiella pneumonie*. It was found that 13/30 (43.3%) isolates were isolated from urine, 3/30 (10%) from wound, 6/30 (20%) from diabetic foot, 5/30 (16.67%) isolates from burns and 1/30 (3.33%) from each sputum, ear and vaginal infections, In this study, used RAPD and ERIC-PCR for the detection of phylogenetic diversity of *K. pneumoniae* isolated from different samples, In general, 30 isolates characterized using the two molecular techniques had comparable number of bands with some degree of polymorphism. *K. pneumoniae* isolates from the same source were clustered in to different groups. The two molecular techniques generated 2 main clusters and the results of dendrogram of these techniques reveals that 20 polymorphic variants between *Klebsiella pneumoniae* clinical isolates detect by ERIC while the RAPD method show 24 polymorphic variants.

Introduction

Klebsiella pneumonia is an important opportunistic pathogen that causes a variety of infectious diseases in humans, including septicemia, liver abscesses, UTI, diarrhea, and pneumonia (Guo *et al*., 2017). It is a well-known hospital-acquired pathogen and associated with increased patient morbidity and mortality (Cabral *et al*., 2012). In addition to the clinical environment, *K. pneumoniae* is frequently found in foods including raw vegetables, powdered infant formula, meat, fish, and street foods, and has been considered as an important food-borne pathogen (Davis and Price, 2016). *Klebsiella* virulence factors differ according to the sites of infection because the host defense mechanisms differ from site to another site (Al-obadi, 2014). *K. pneumonia* strains possess a many of virulence factors that facilitate infection and survival in the host documented that the virulence factors of *K. pneumonia* consist of seven major bacterial virulence factors: capsule (for inhibition of phagocytosis), lipopolysaccharide (for avoidance of host serum complement factors), fimbriae (for adhesion), side rophores (for iron acquisition), bacteriocin (Al-obadi, 2014), serum resistance (Blackburn, 2010) and extended spectrum β-lactamases (ESBLs) (for protection from extended-spectrum cephalosporins). Random Amplified Polymorphic DNA (RAPD) -PCR technique as a useful tool for investigation of the genetic variation among *K. pneumonia* strains. RAPD-PCR has received considerable attention in recent years for epidemiological studies, due to its simplicity, rapidity, sensitivity, reproducibility, low cost (Nanvazadeh *et al*., 2013). Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR is a proper technique for DNA typing characterized as high type ability, stability and rapid reversal reproducibility with low complication and price, which can be used to screen, discriminate, and determine genetic relatedness among the strains with the same accuracy of pulsed-field gel electrophoresis PFGE. This method is a common technique in which the intergenic regions of target repetitive non-coding sequences in the genome of bacteria are represented (Fazeli *et al*., 2017).

Materials and Methods

Clinical Specimens

The specimens were obtained from different sites of infections (burns, wounds, ear, diabetic foot and urine,

vaginal, respiratory tract); each swab was taken carefully from the sites of infections and transfer to the laboratory of microbiology /college of medicine. Urine (mid-stream urine) was collected from patients suffering from UTIs in sterile screw-cap container. Swabs from burn, wound, ear were collected from patients before they take any antibiotics or cleaning and swab from diabetic foot patients who diagnosis depended on physician then the swab collect before cleaning. Each specimen was inoculated on selective media and identified by biochemical reaction according to the diagnostic procedures recommended in (Forbes *et al*., 2007).

Ethical approval

Agreement from the family and patients for sampling and carrying out this work was obtained.

DNA Extraction

This method was made according to the genomic DNA purification Kit supplemented by the manufacturing company (Genaid, UK).

Primer Sequences

The primer sequences and PCR conditions that were used are listed in table 1.

Table 1: The primer sequences and PCR conditions.

Genes Name	Primer sequence (5´- 3´)	Size of product bp	PCR condition	Ref.
*RAPD*4	AAG ACG CCG T		94C°5min.45 Cycle94C°1min., 36C°1min.,72C°1min.72C°9min.	(Venieri*et al*., 2015)
*ERIC*1	SenseATG TAA CCT GGG GAT TCA C AntisenseAAG TAA GTG ACT GGG GTG AGC G		94C°5min.35 Cycle94C°1min., 49C°1min.,72C°3min.72C°10min.	(Zhang *et al*., 2018)

Results

Isolation of *K. pneumonia* from Clinical Samples

A total of 30 strains of *K. pneumoniae*, were originally isolated from a variety of clinical specimens: urine (13), wound swab (3), ear swab (1), swab from burn (5) and from diabetic foot (6), sputum (1) and HVS (1). The strains were identified as *K. pneumoniae* on the basis of typical morphology by gram negative staining, a negative oxidase and positive catalase reaction and then diagnosis by Vitek 2 compact system.

Detection of phylogenetic diversity of *Klebsiella pneumonia* isolated from different samples by RAPD-PCR:

A total of 30 amplified DNA fragments ranging in size from 300-1500 bp detected by using one random primer .The primer reveals (0-10) fragment with size ranging from (300-1500 bp) among the isolates, it was found that three isolates formed no band as shown in Fig. 1, 2.

The results of RAPD analysis revealed that the amplified products of most isolates vary in the molecular size patterns even with the equal total fragment. Dendrogram analysis was constructed on the basis of

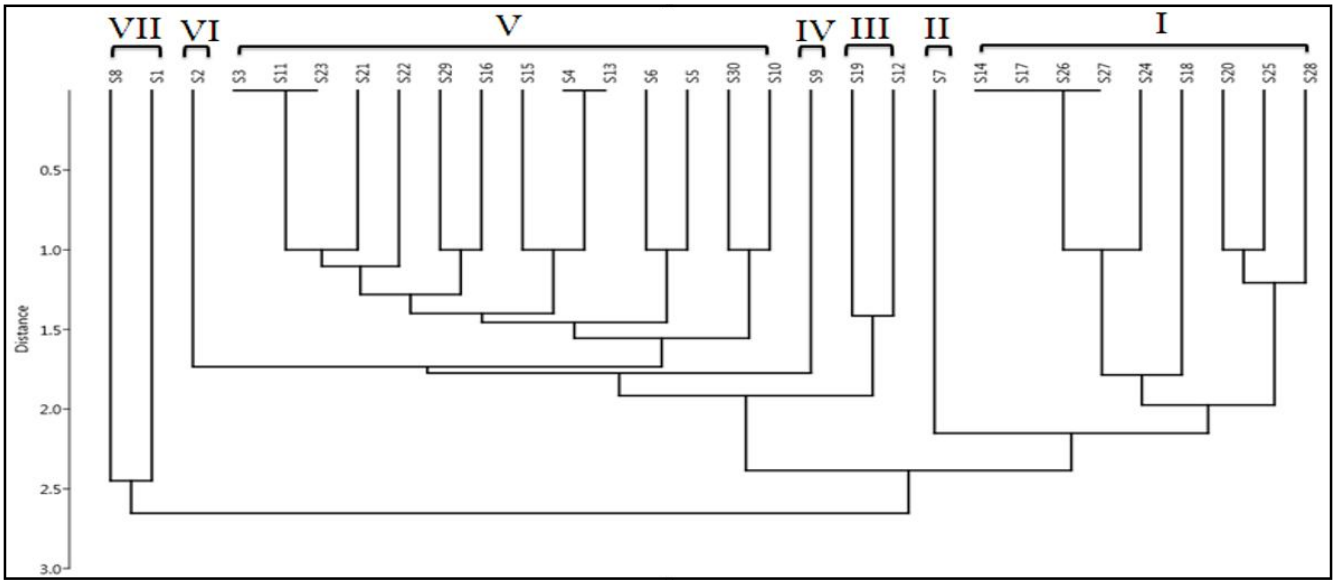

Fig. 1: RAPD-PCR dendrogram tree analysis of *Klebsiella pneumonia* isolates by using (Paleontological Statistics version 4.0). The Cluster analysis using (algorithm Ward's method) were showed 7 cluster variants and 24 polymorphic variants between *Klebsiella pneumonia* clinical isolates.

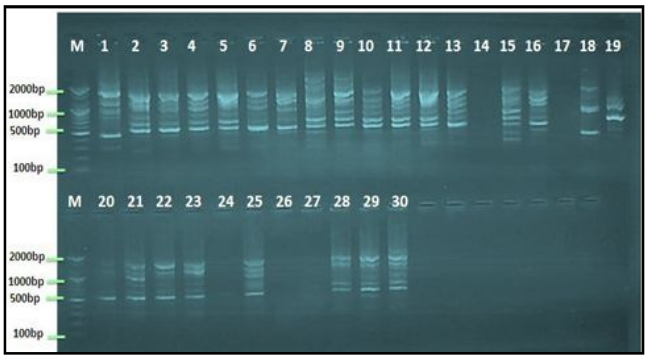

Fig. 2: Agarose gel electrophoresis at 70 volts for 50 min. for *RAPD* gene in *K. pneumoniae*. PCR product visualized under U.V light at 280nm. After staining with ethidium bromide. The primer reveals (0-10) fragment with size ranging from (300-1500bp).

the averaged similarity with the use of (UPGMA) has been used to show the phylogeny occurrence of strains.

According to the dendrogram, the (30) strains of *K. pneumonia* were distributed into two clusters as (A and B), where cluster A included two sub-clusters and contain (2) isolates which divided into two branches, the first branch contains (10) isolates in which isolates (14,17,27 and 26) show 100% similarity.

The second branch contains one isolate, while the cluster (B) contains 16 isolates, isolates number (4,13) and isolates (11, 3 and 23) show 100% similarity, as shown in Fig. 2.

In this study, the RAPD primer showed DNA polymorphism among *K. pneumonia* isolates from different clinical sample, either in the occurrence of amplified fragment or in the variable genetic similarities of each isolates with the other and the results revealed 24 polymorphic variants.

The diversity could be due to the fact that they all were obtained from different sources, or due to the genetic instability of *K. pneumoniae*. Mahmudi *et al*., 2016 show that RAPD-PCR markers are powerful and effective techniques in identity and initial screening of samples, examining the differences among species and identify at the level of strain as well as compared with biochemical methods need to spend less time and lower cost (Mahmmudi *et al*., 2016).

RAPD finger printing method could be used to rapidly identify major clones of *Klebsiella* spp. including multi drug resistant, pathogenic *K. pneumoniae* population are highly heterogeneous. The numerous serotypes in *Klebsiella* spp. could explain the relevant degree of genetic diversity by RAPD analysis (Venieri *et al*., 2015).

Enterobacterial Repetitive Intergenic Consensus (ERIC) DNA fingerprint Analysis:

ERIC-PCR fingerprinting revealed (18) genetic

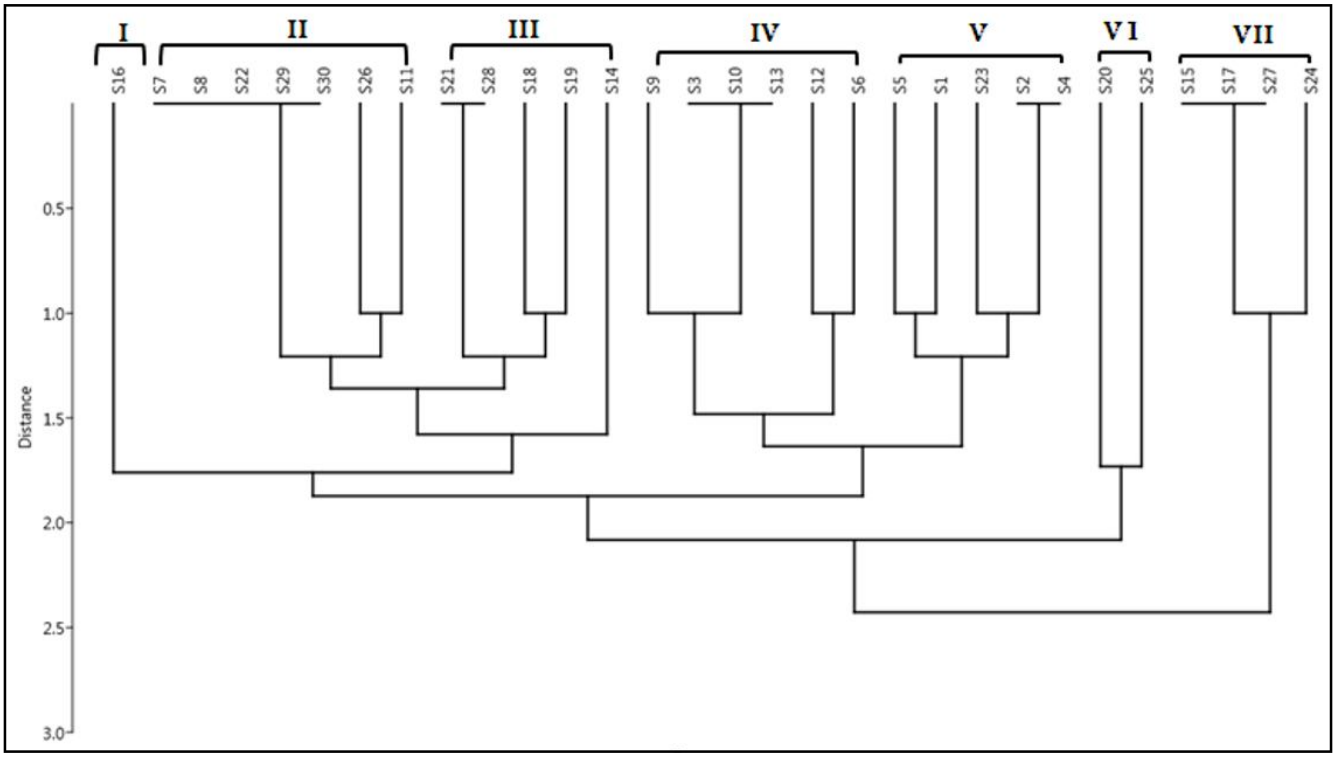

Fig. 3: ERIC-PCR dendrogram tree analysis of *Klebsiella pneumonia* isolates by using (Paleontological Statistics version 4.0). The Cluster analysis using (algorithm Ward's method) were showed 7 cluster variants and 20 polymorphic variants between *Klebsiella pneumoniae* clinical isolates.

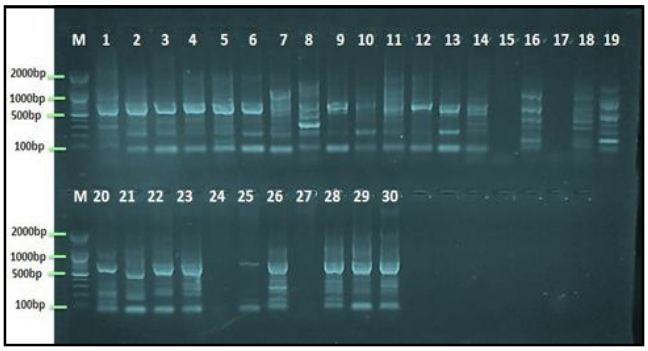

Fig. 4: Agarose gel electrophoresis at 70 volts for 50 min. for *ERIC* gene in *K.pneumoniae*. PCR product visualized under U.V light at 280nm. After staining with ethidium bromide. The primer reveals (0-9) fragment with size ranging from (200-1500bp).

patterns Fig. 3, 4. The cluster was shown in (0-9) band between (200-1500) bp.

ERIC-PCR typing revealed 20 main genotypes. The dendrogram results show to main clusters (A and B), were cluster A contains three isolates which are all identical 100% isolates as shown in Fig. 3 while cluster B contains two sub-cluster, one sub-cluster contain 6 group which are given similarity 100% in their isolates. It was also observed that the same genetic types of bacteria were isolated from different sites of infection, so this was possible to postulate that the same clone was present in the environment which probably epidemiologically are related (Wolska and Szweda, 2009), also, ERIC-PCR. Can provide more discriminative DNA patterns of bacterial source and was able to show the species-specific comparison for other type methods.

The results revealed that some isolates from the same site of infection have different patterns, this may be attributed to the presence of the mutation (insertion or deletion) in that locus. These markers were able to distinguish bacterial isolates into different genotype groups showing discriminative power to differentiate closely related bacteria strains, isolates from the different source were found to cluster to gather, the isolates still show some genetic similarities and unchanged throughout the evolutionary pathway (Ramazanzadeh *et al*., 2013).

Conclusions

Molecular typing is a potent tool for the study of nosocomial infections. RAPD is a widely used genotyping tool for *K. pneumoniae* strains, It was observed that a high degree of heterogeneity existed among *K. pneumoniae* isolates.

Acknowledgment

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