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Detection the Genetic Diversity among Oral Cavity Klebsiella spp Isolates

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ABSTRACT

The present study was carried out to Detection The genetic diversity among oral cavity Klebsiella spp isolates for different proofs, oral cavity swaps were collected from dental clinic attendances after classical microbial investigations Klebsiella spp was more frequent isolates, RAPD-PCR used for genetic diversity of isolates that show primers recorded 18, 21, 27, and 35 bands for A, B, C, and D respectively the most similar isolates were (7 vs 3,4 and 6) for primers A, primer B (7 vs 5) , primer C (4 vs1, 2, 3) primer D (6 vs 3) , and the mean of similarity index for all primers shows that the more similarity between (4 vs 3) and low similarity with E coli HB101. The overall diversity shows no strong similarity among isolates which habitat in oral cavity The genetic similarity pointed to possibility of diverted in genetic component in Klebsiella spp which pointed that no strong genetic variation among isolates and it may be belong on the some oral health problems in Iraqi populations.

Keywords: Klebsiella spp, genetic similarity, oral health problems, RAPD-PCR.
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INTRODUCTION

The oral infection can be defined as un-equilibrium in microorganism community inhabiting the oral cavity, it has main role in etiology of periodontitis, destruction of tissue and tooth loos (1,2) the products of these microorganism can be effect in this infection, it may be trigger host cells to released degradation enzymes and stimulated immune response by immune cells macrophage and lymphocytes, the cytokines which products by immune response activated more than one pathways of degradation , the metalloproteinase of matrix, plasminogen-dependent, phagocytic and polymorphonuclear-serine proteinase pathways and ultimately osteoclastic bone re sorption, the dental processing like extraction tooth, root scaling, treatment of endodontic and periodontal surgery may contribution in microorganisms entrance to blood stream or lymphatic system which may causes other infectious (3,4).

There are several genus and species still recorded in oral microorganisms community included bacteria, viral, fungus and parasites some of them founded normal flora of oral cavity while other consider as pathogenesis, researchers classified it's into different classing's like Strict anaerobic bacteria and Facultative anaerobic bacteria in addition of it gram negative or positive to each class (5).

Oral microbial contributed in some disease and oral health problem like Caries which one of the chronic infectious disease, the bacteria is major pathogen caused chronic and destruction in dental hard tissue, and can be occur in any age. Caries in early childhood is the most harmful and big public health problem among preschool children globally (6,7). The Mucosal diseases included oral lichen planus , Oral leukoplakia and systemic lupus erythematosus characterized by specific manifestation of systematic diseases in oral mucosa, (8). also the Periodontal diseases occur in human mouth that divided to gingival diseases and periodontitis (9, 10) .

MATERIALS AND METHODS

Study subjects: samples collected from oral cavity of individuals suffering from dental caries by trans media swaps, patient's attendance to dental clinic in college of dentist according to ethical approval of ministry of higher education and scientific research in Iraq. Swaps collected by specialist Dr. Maytham Ali, next it transferred to lab studying.

Macroscopic and microscopic analysis: aerobic and anaerobic culture were implemented on suitable media; then more frequent isolates were collected and were re-culture for diagnostic. The features of colonies, culture in differential media and gram stain using to diagnostic isolates**,** antibiotic sensitivity was implemented by disc diffusion method using different antibiotics**.**

DNA extraction

Bacterial DNA extraction according to colony PCR method in briefly a single colony of isolates were re-culture on Luria agar for 18 hours. Then pick from colonies was transferred to PCR- tube contain 30 µl of dH2O with mixing , the Mixtures incubated at 95°C for 7 minutes in water bath, after incubation Mixtures centrifuged at 10.000 rpm for 15 min, then transferred the supernatant to a new tubes and stored at -20 C till it used for PCR.

PCR amplifications: Amplification of RAPD implemented using four primers represented in table 1. Amplification mixtures consisted of 1 µl of DNA tamplet, 10 pm primer and then it completed to 20 µl by dH2O (intron). amplifications conditions were 95 C for 5 min, 40 cycles (45 sec at 95 C , 45 sec at 44-52 C then 1 min at 72), final extension 72C for 10 min. products were show by the electrophoresis 1.5 % agaros for 60 min at 70V, 20mA.

Table 1 Primers used in RAPD-PCR

Primer	Sequences(5 to 3)	Annealing TM C ◦	References
A	GAGGCCCTTC	44.3	(11)

B	CGCAGACCTC	44.2	(12)
C	AACGCGCAAC	42	(13)
D	GTTTCCGCCC	52.1	

Phylogenetic analysis

The phylogenetic tree constructed by (UPGMA) algorithm by DendroUPGMA: Adendrogram construction utility, similarity index were calculated according to Jaccard index.

RESULTS AND DISCUSSION

The results of present study shows numerous bacterial species in aerobic and anaerobic cultures, the more frequent species was *Klebsiella spp* (8 isolates) in aerobic culture, antibiotic sensitivity show that all isolates high sensitive to amikacin, three isolates low sensitive to penciling and one isolate highly sensitive and other isolate low sensitive to clarithromycin, one isolates was low sensitive to carbenicillin. The antibiotic resistance belong to genes encoded to these resistance, these genes carried on chromosome or on plasmid which acquired by some mechanisms like genetic transfer, transduction and conjugations, also bad uses of antibiotics led to development a new mechanisms to resistance of antibiotics in bacterial isolates that introduced a new generations have completely resistance to antibiotics and high virulence factors (14) .

The similarity index was used in present study to detection relation among the *Klebsiella* spp. Which caused dental decay in Iraqi individuals which lastly suffered from dental carries and oral medicine problems, in previous study deal with microorganisms in oral community founded that four bacterial spp. Isolated from dental carries one of these was *klipsella spp* (15) .

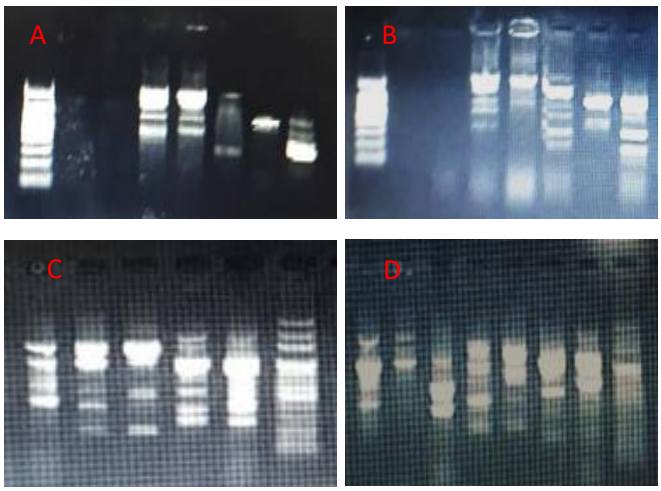

Figure (1) Electrophoresis pattern of PCR-RAPD for isolates M DNA marker, 1-11 e coli isolates (1.5% agarose, 70V, 20mA, for 60 min). A Primer A, B Primer B, C primer C, D primer D.

Table (2) similarity matrix of isolates according to primers

Primer A	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	1.000	1.000	0.000	0.000	0.000	0.000	0.000
2		1	0.000	0.000	0.000	0.000	0.000
3			1	1.000	0.333	0.200	0.333
4				1	0.333	0.200	0.333
5					1	0.000	0.200
6						1	0.333
7							1
Primer B	1	2	3	4	5	6	7
1	1.000	1.000	0.000	0.000	0.000	0.000	0.000
2		1	0.000	0.000	0.000	0.000	0.000
3			1	0.200	0.333	0.200	0.143
4				1	0.000	0.000	0.000
5					1	0.000	0.500
6						1	0.000
a7							1
Primer C	1	2	3	4	5	6	7
1	1.000	1.000	1.000	1.000	0.000	0.000	0.000
2		1	1.000	1.000	0.000	0.000	0.000
3			1	1.000	0.000	0.000	0.000
4				1	0.000	0.000	0.000
5					1	0.333	0.222
6						1	0.300
7							1

Primer D	1	2	3	4	5	6	7
1	1.000	1.000	0.000	0.000	0.000	0.000	0.000
2		1	0.000	0.000	0.000	0.000	0.000
3			1	0.125	0.667	0.714	0.500
4				1	0.167	0.286	0.500
5					1	0.429	0.429
6						1	0.500
7							1
Mean of primers E	1	2	3	4	5	6	7
1	1.000	1.000	0.000	0.250	0.000	0.000	0.000
2		1	0.000	0.250	0.000	0.000	0.000
3			1	0.581	0,333	0.278	0.243
4				1	0.125	0.121	0.207
5					1	0.189	0.337
6						1	0.283
7							1

Table (3) the Cophenetic Correlation Coefficient (CP) among bacterial isolates

Primer no.	Number of bands	Cophenetic Correlation Coefficient (CP)
A	18	0.979098415100737
B	21	0.968129864192401
C	27	0.999622800359871
D	35	0.954004117872539
E	Mean	0.932286840029516

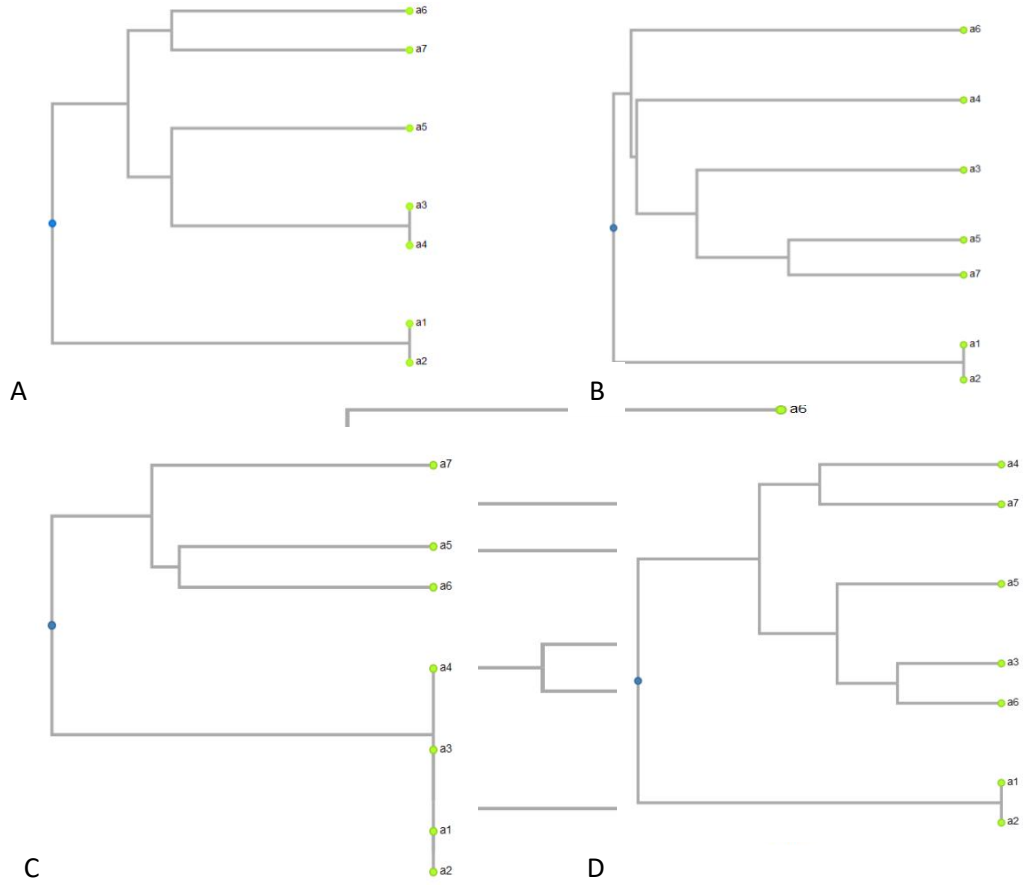

Figure (2) the phylogenic trees of bacterial isolates a1-a6 *klebsiella* spp a7 *E coli* HB101, A,B, C and D RAPD primers, E mean of overall similarity index

Oral disease like dental carries, gum inflammations and others become one of the most problems in Iraqi populations in last decades, thus the present study was suggested to evaluation the genetic background of more bacterial spp isolates which more frequents in oral cavity and dental carries. The similarity index was depended in present study according to Jaccard index that implemented using UPGM dndrogram software, the similarity index was divers according to primers sequences and its loci, primers recorded 18, 21, 27, and

35 bands for A, B, C, and D respectively with Cophenetic Correlation Coefficient ranged (0.95400- 0.99962) (figure 1and table 3), the most similar isolates were (7 vs 3,4 and 6) for primers A, primer B (7 vs 5) , primer C (4 vs1, 2, 3) primer D (6 vs 3) (table 2, figure 1). The mean of similarity index for all primers shows that the more similarity between (4 vs 3) and low similarity with 7 (*E coli* HB101).

The phylogenic trees show that all trees consist of more than one clusters and groups and the genetic distance variance according to primers sequence, overall the diversity in bacterial isolated resulted from different factors included spontaneous mutation like point mutation and DNA re-arrangement in addition of horizontal genetic transfer as well as transformation, transduction , conjugation and recombination, all these processes causes genetic diversity in bacterial populations which are lived at the same environments, although of the present isolates were members of Enterobacteriaceae it can be survived in saliva and these phenomena have been observed in some oral clinical investigations (15,16).

The ways of bacterial transfer to mouth cavity can be contributed in dental health disorder which will acquire genetic adaptation later, via contaminated eating, some mouth tools and dental clinic causes transfer multidrug resistance bacteria like *Klebsiella* spp. (17- 19). The present study aims to detection the genetic diversity of more frequents oral bacterial isolates to investigate the sources of oral infections, controlled on the oral infections which high incidence recorded in last years and for possibility to control on the side infections Accompanying with other diseases like oral ulcer , from these results we determined that the source of oral infection is entrobacterecea, and the bacterial isolates that detected in present study can be contributed in other oral infections like dental decay and Periodontal diseases (20). in addition of oral ulcer correlated with other disease like autoimmune disease and immune suppression drug consumptions. The genetic similarity pointed to possibility of diverted in genetic component in *Klebsiella spp* as in figure (2 E) which pointed that no strong genetic variation among isolates and it may be belong on the some oral health problems in Iraqi populations , moreover its need more investigated to improve the genetic similarity among oral cavity microorganisms which causes some problems in tooth health in Iraqi population, thus we need more researches deal with other types of oral microorganisms species and the types of communications among them to detect the source of these health problems.

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