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Detection O Antigens Genes in Antibiotics Resistant Oral *Klebsiella*Spp Isolates Through Using Multiplex PCR Technique

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Abstract

The present study was suggested to estimated two of the O-antigen in *klebsiellaspp* that resistance to some antibiotics isolated from oral samples, these isolates were choosing were more frequent than others, O*wzx*and O*wzy*were detection using multiplex PCR, the present results show that isolates 25% of isolates have *wzx*, while 62.55% have *wzy* gene, the present of both genes in isolates are represented by present *wzy* and *wzx* together was 25% , absent both genes was 37.5% of isolates, present *wzx* absents *wzy* didn't appeared in genetic detection while absence *wzx* and present *wzy* was shown in 37.5% of isolates , the present study concluded that the *wzy*may beassociated with the teeth health problems, its need more investigations about other types of O antigen.

Key words: O antigen, *wzy* and *wzx*, multiplexPCR, *klebsiella spp*.

Introduction

Oral bacterial community compose of different surface every surface covered by divers of bacterial species several of oral species contributed in oral problems like tooth decay and periodontitis, the review of literature show large number of isolates species collected from oral cavity for different age categories and different patients suffering from diseases or form healthy individuals (1) investigations show that some types of oral bacterial have major role in pathogenicity of other disease like preterm low birth weight (2,3) osteomyelitis in children (4)bacterial endocarditis (5). In addition of oral disease, these disease resulted from collaboration factors one of these is unequilibrium in oral bacterial community especially oral disease, to yet bacterial isolates which responsible on oral infection and tooth decay are better known but its genetic components are still diverse(6) , thus the this study aims to detection some virulence gene in oral bacterial isolates .Dental caries causes by large numerous of mutants streptococci like*Strmutans*or*Strsobrinus*in addition to actinomycetes and

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lactobacilli which have been detected in several studies(7) the chronic inflammatory of Periodontal in adults causes tooth loss when appropriate treatment was absent, the diseases of periodontitis are divided into two types; the infections affectedin the gingiva only (gingivitis) which affected in the underlying and tooth-supporting tissues of the periodontium(periodontitis) comprised ofthe alveolar bone and the periodontalligament and (8).There are numerous types of the O-antigen forms of the most clinically relevant Shiga toxin-producing *E. coli* (STEC) serotypes, more than 200 different STEC O:H serotypes have been associated with human disease (9) the O*wzx*and O*wzy*were studied in present study.

Materials and methods

Study samples: samples were collected using trans media swaps from oral cavity from individuals attendance to dental clinic in dentist college according to ethical approval of ministry of higher education and scientific research in Iraq. Sampleswere taken by specialist dentist in clinic, next it transferredto labstudying.

The analysis by Macroscopic and microscopic : aerobic and anaerobic culture were implemented on suitable media, then more frequent isolates were collected and were re-culture for further analysis,classical lab work used for isolates detection.

Extraction of DNA and PCR Conditions: DNA was extracted according to leaflet of DNA extraction kit and PCR implemented using multiplex PCR by following primer setsprevious descripted by (10). The thermo cyclers were pre-denaturation (5 min / 94˚C), next 35 cycles consist of (30 s 94˚C, 30 s 59˚C, 30 s 72˚C) after that(10 min / 72˚C)PCR products were Electrophoresed using 1% agarose 0.5 X TBE buffer for 60 min , 70 V all isolates had control represented by *E coli* HB101 [pomega](11).

Results and discussion

the present study which implemented by culture each samples on aerobic and an aerobic condition show different microorganism species of gram positive and negative bacteria, these diversity included staphylococcusspp and streptococcusspp, E coli, bacillus, lactobacillus, and *klebsiella*spp which it more frequented than other genus in samples culture in addition of candida and Neisseria for one time, these genus were re-culture in selective media for accurate diagnostics with microscopic and macroscopic diagnostics , then *klebsiella*spp isolates were chosen to antibiotics sensitivity and genetic study in present study because it more genus causes dental

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decay and caries in patients, in previous study carried out on patients attended to dental clinic suffered from caris and decay we found that *klebsiella* spp. Is one of the most bacterial causes dental decay (12). The antibiotic resistance of present isolates shows that there was high percentage to resistance to antibiotics except amikacine 10 mg, all isolated were sensitive in different inhibition zones. (table1) the resistance to antibiotics considered that it is a big problems contributed by multifactorial in last decade, numerous studies deal with this problem were performed to overcome the factor contributed in resistance to antibiotics.

Figure (1) gel electrophoresis of Multiplex PCRs targeting the O*wzx*and O*wzy*genesM, DNA marker, lane 1, negative control; lane 2-8 bacterial isolates lane 9, *E coli* HB101. amplifiedproducts are *wzx*255 bp and *wzy*451 bp.

About 25% of isolates have wzx, while 62.55% have *wzy* gene, the present of both genes in isolates are represented by present wzy and wzx together was 25% , absent both genes was 37.5% of isolates, present wzx absents wzy didn't appeared in genetic detection while absence wzx and present wzy was shown in 37.5% of isolates (figure 1,2)

Table (1) antibiotic sensitivity of ***klebsiellas*pp** isolates from oral cavity.

Samples	amikacin 10	penciling 10 mg	Augmentin	clarithromycin	carbenicillin.
12	20 mm	12 mm	-	-	-
1	20	-	-	20mm	8mm
5	22	-	-	-	-
6	12	-	-	-	-
13	13	-	-	-	-
22	23	9mm	-	-	-
2	20	-	-	-	-
15	19	-	-	-	-
- **Resistance**					

Antibiotics sensitivity shows that all isolates resistance to Au and sensitive to AK other results show that isolates sensitive to one antibiotic at least.

The information's about plasmids in oral cavity bacteria were poor, investigateimplemented byMarcia et al., (8) found in oral

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*Fusobacteriumnucleatum*three different size groups of plasmid. In spite of didn't found any types of plasmids in present study it resists to some antibiotics this may be because the oral cavity causes plasmid curing during creation microbial biofilm whichbegan inoral tissues and teeth adherence, collaboration among bacterial species, the signaling among bacteria and its role in pathogenesis, alsogenetic molecules transfer among bacteria. (13).

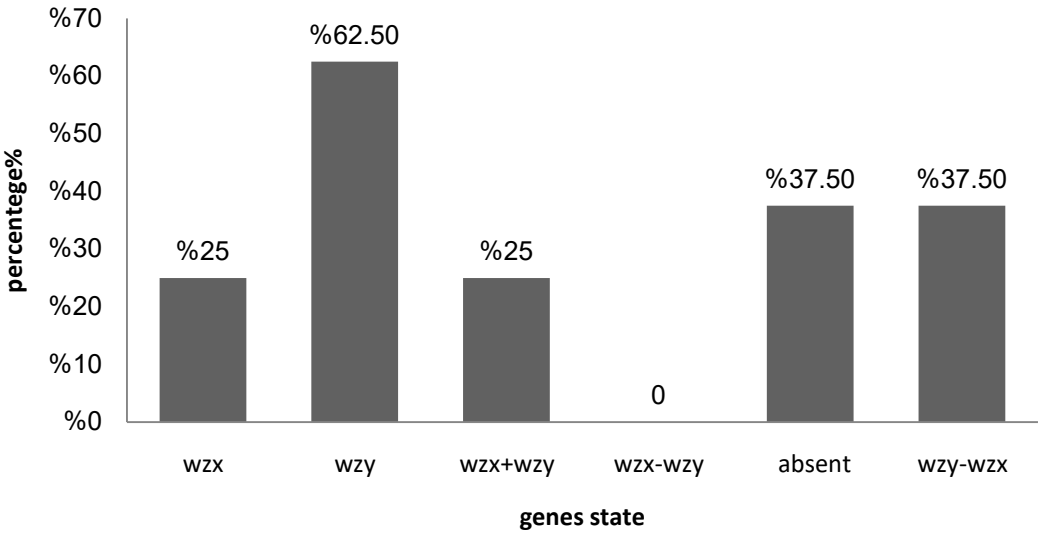

Figure (2) the percentage of genes wzx and wzy in *klebsiella*spp isolates

Researchers considered that the biofilm is a good environment to DNA transfer and exchange due to the closely proximity of cells capable DNA to be uptakewithin the extracellular matrix, Roberts et al., (14) and Warburton et al., (15) proved of the horizontal gene transfer capability that happened among oral streptococci in biofilm communities. DNA transferring between different strains *P. gingivalis*and *Escherichia coli*, events by conjugation; it's suggested by Koehler et al., (16). In some genera DNA can be transmitted without direct cells contact as in oral streptococci (17.18).

The results of this study show that the high percentages of isolates have *wzy* than wzx, also low percentages have been found for present booth genes together , and thabsent of both genes was 37.5% of isolates, present wzx absents wzy didn't appeared in genetic detection while absence wzx and present wzy was shown in 37.5% of isolates . the studies in Iraq about this types of gene were poor , we started to study this genes in different environment enterobacteriaceae have role in disease

incidence , a study deal with this genes in environmental *E coli* which found that 100% of isolates had O45 wzx gene and 25% had O45 wzy gene and about 25% of isolates had both genes using multiplex PCR (10).

Conclusions

The *klebsiellaspp* have two of the O-antigen genes that resistance to antibiotics, which include O*wzx*and O*wzy*, thisstudy show that 25% of this bacterial species have *wzx*, while 62.55% have *wzy* gene, the present study explined that the *wzy*may beassociated with the teeth health problems.

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