

Some Oral Pathogenic Bacteria, Isolation and Diagnosis

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<http://dx.doi.org/10.22207/JPAM.12.3.54>

(Received: 15 May 2018; accepted: 20 July 2018)

The microbial community in oral cavity associated with oral infection and disease, variety of microbes have been recorded in oral cavity with different genetic properties, the present study aims to study some oral cavity isolates using classical methods and PCR for molecular detection O-antigen genes, results show that there were four isolates, three were *E. coli*; *Klebsilla pneumonia* and *Cardiobacterium hominis* while the fourth species was *Staphylococcus aureus*, it had different response to antibiotics *E. coli* and *Klebsilla pneumonia* were more resistance. The plasmid profile didn't show any plasmids as well as O- antigen genes it was negative for all isolates. The present study concluded that oral cavity have variety species with different genomic properties, it need more investigation about other genes and bioactivity.

Keywords: Oral cavity, O-antigen, plasmid profile.

The oral and dental infection cause by population of Microbial like periodontal diseases, pericoronitis, gingivitis, endodontitis, peri-implantitis, and post extraction infections each of these have distinct clinical and microbial features, The r microbial flora in the oral cavity contains about 1010 of bacterial species, more than 500 species yet to now recognized as normal inhabitants in oral cavity, However, only 150 microbial species have been isolated and cultured from root canals (Marina and Romana, 2007)

There are many species related with oral infections recoded by different studies like *Campylobacter rectus*, *Prevotella intermedia*, *Bacteroides forsythus*, *Actinobacillus actinomycetem-comitans*, *Porphyromonas gingivalis* *Eubacterium* species, *Fusobacterium nucleatum* also *Eikenella corrodens*, and *Peptostreptococcus micros*. *Treponema pallidum*-related spirochetes have been associated with

acute necrotizing ulcerative gingivitis, Also *Porphyromonas endodontalis* recorded to be specifically related to endodontic infections (Paster et al., 2000; Paste et al., 2001; Aas et al., 2005;; Dewhirs et al., 2010) it was responsible of two major oral diseases; caries (tooth decay) and periodontitis (gum diseases) Dahlén et al., 1993; Marsh et al., 1994; Nishihara et al., 2000).

The species Isolation which causing infection in oral cavity and teeth canal can be collected by strictly aerobic and anaerobic techniques, also Rapid selective culture and DNA probe have been used in identification of oral species microbes, however the detection by Molecular methods of oral microbial genera or by 16S rRNA genes sequencing improved that the mouth of human provides a habitat for about 700 species of bacteria, and that between 100 and 200 different species in the healthy mouth of any individual (Paster et al., 2006). other surveys suggested that the variety of bacteria in an individual oral cavity may be slightly higher by using pyrosequencing technologies, around 500 species (Zaura et al., 2009).

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Table 1. Antibiotic sensitivity of bacterial isolates

Bacterial isolates	Erythromycin 15 mcg	Cefazidime 30 mcg	Nitrofurantoin 300 mcg	Cefotaxime 30 mcg	Cefixime 5 mcg	Norfloxacin 10 mcg	Aztronam 30 mcg	Amikacin 30 mcg	Gentamicin 10 mcg	Tetracycline 30 mcg	Meropenem 10 mcg
<i>E. coli</i>	17	0	35	0	0	23	0	0	16	0	20
<i>Klebsilla pneumoniae</i>	0	0	0	23	0	30	0	14	13	33	0
<i>Staphylococcus aureus</i>	0	0	20	16	0	30	16	15	18	40	0
<i>Cardiobacterium hominis</i>	25	10	30	0	0	27	20	27	23	44	60

MATERIALS AND METHODS

Sample collection : samples collected using trans media swaps from dental canal after extraction , all patients were visited porsepa center for dentist clinic, swaps collected by specialist dr. Saadi shershap, then it transfer to lab for culture and analysis.

Culture and diagnosis: aerobic and anaerobic culture, then isolates were re-culture for purification isolates and diagnostic. Macroscopic and microscopic diagnostic : The features of colonies, culture in differential media and gam stain using to diagnostic isolates then antibiotic sensitivity was detected using disc methods to cefixime, erythromycin, gentamicin, amikacin, meropenem, Nitrofurantoin, ceftazidime, norfloxacin, tetracycline, cefotaxime, aztronam.

DNA extraction and PCR experiment: DNA was extracted and PCR performed according to PCR colony methods then the following primers were used to amplify game negative isolates O45 wzx1 (F) 3'-CCG GGT TTC GAT TTG TGAAGG TTG-5' ,(R) 3'-CAC AAC AGC CAC TAC TAG GCA GAA-5' , and O45 wzy1 (F) 3'-GAA ATT ATG CCA TCT TGG CGA GCG-5' , (R) 3'-CAT GTGAAG CCTGAAGGC AA ACTC-5' (DebRoy et al., 2004). PCR conditions and size products: PCR experiments performed as a following; pre-denaturation for 5 min at 94°C, then 35 cycles (30 s at 94°C, 30 s at 59°C, 30 s at 72°C, and finally 10 min at 72°C). Electrophoresis was implemented using 1% agarose 0.5 X for 60 min , 70 V and 20 mA all testes had control represented by *E coli* HB101 [pomega]

RESULTS AND DISCUSSION

The results of bacterial culture show four species of bacteria three of its was gram GV- included *E. coli* ; *Klebsilla pneumonia* and *Cardiobacterium hominis* , the fourth species was *Staphylococcus aureaus*.

Antibiotic resistance the antibiotic sensitivity of isolates were show in table (1) *E coli* was more resistance than other isolates it was resistance to Ceftazidime, Cefotaxime, Cefixime, Aztronam, Amikacin and Tetracycline while it more sensitive to Nitrofurantoin than other isolates. *Klebsilla pneumonia* isolates was resistance to

seven types of antibiotics and it more sensitive to Tetracycline as well as the other two isolates, *Staphylococcus aureus* was resistance to 4 types while *Cardiobacterium hominis* was resistance to two types only. The resistance to antibiotics types dependent on the genetic of isolates and its predisposition to acquire a new antibiotic resistance gene by conjugation, transformation and transduction way. The oral cavity was complex ecosystem that consist of hundred different taxa of microorganisms may be detected founded, some studies suggested that the incoming bacteria to oral cavity like *E. coli* that don't considered as a part of the normal oral thus can able to attach to extracellular matrix or resident members, and more importantly, overcome the invasion resistance in order to integrate into the community [14], He et al., (2012) concluded that the some bacterial species in oral cavity can be integrated in this

community by strategy of evasion to development oral microbial community; which prevents detection by antagonistic oral bacteria and allows integration.

The results of DNA profile didn't show any plasmids in all isolates (figure 1), also all isolates didn't had o-antigen gene; the PCR product show negative results (figure2).

The review of literature about plasmid profile in oral cavity bacterial were little, study performed by Marcia et al., (2003) about plasmid profile in oral *Fusobacterium nucleatum*, they found three groups of plasmid were classified according to its size. Although of absence plasmids in present isolates it were had antibiotics resistance, this can be explanation by plasmid curing in oral cavity that resulted from construction microbial biofilm initial from adherence to the oral tissues and teeth, cooperation between bacterial species, signaling between the bacteria and its role in pathogenesis, and the transfer of DNA between bacteria by different genetic transfer processes (Kolenbrander et al., 2010).

The Biofilms consider good environment to exchange DNA because of a close proximity of cells that able DNA to trapped in extracellular matrix. Roberts et al., (2001) and Warburton et al., (2007) improved horizontal gene transfer between oral streptococci in biofilm communities. Mira (2008) clarified that the DNA transfer between some oral bacteria genera was happen conjugative transposons also t Analysis of the genomes of some oral isolates improved that past horizontal gene transfer happened in about 5% and 45% of genes in different species. DNA transferring between

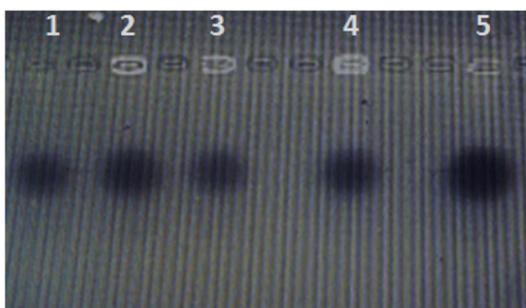


Fig. 1. Plasmid profile of oral bacterial isolates lane 1 positive control *E. coli* Hb101, lane 2-5 oral bacterial isolates *E. coli*, *Klebsilla pneumonia*, *Staphylococcus aureus*, *Cardiobacterium hominis* respectively



Fig. 2. Negative Multiplex PCRs targeting the *Owzx* and *Owzy* genes Lane 1 M, molecular weight, lane2-8 bacterial isolates lane 9, *E. coli* HB101. The Positive multiplex PCR showing the amplified *wzx* 255 bp and *wzy* 451 bp

different strains *P. gingivalis* and *Escherichia coli*, events by conjugation; its suggested by Koehler et al., (2003). In some genera DNA can be transmitted without direct cells contact as in oral streptococci (Wang et al., 2002).

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