Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(7):1102-1108

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Impact of antibiotic and heavy metals sensitivity on oral isolate: A case study

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ABSTRACT

Tooth decay are considered the most common in the world. The study aims to isolation and identification of important bacteria related to tooth decay, determining the sensitivity of bacteria of certain types of antimicrobial agents and studying the effect of heavy metals on bacterial isolates. A total (50) swabs were collected from mouths of patients from both gender and their ages range from 1-60 years which are referred to consult the dental clinics and specialized centers, in order to isolate and identify the causative agents that associated with oral diseases. The age stage group of infection rates showed groups (20-40) and (1-20) were the most infected compared to elder group (40-60) as was the incidence of 44and 32% respectively The antibiotic sensitivity test against the isolates showed the chloramphenicol up to 83.05% was the higher effect sensitivity of Gentamicin and Rifampicin up to 81.35% .While streptomycin 16.94% and penicillin G 64.40%. Also, these differences were found have lower effect for isolates against (7) heavy metals, where it showed resistance to Silver nitrate, Iron chloride, Zinc chloride, lead acetate to100%, while appeared sensitivity to mercury, cadmium and copper sulfate by 100,86.44and 1.69% respectively.

Keywords: Tooth decay; Bacteria; Antibiotic; Heavy metals

INTRODUCTION

Tooth decay is one of the most common infectious diseases affecting millions of people globally [1]. One of the occasional factors for the disease is dental biofilm, which is the bacterial charge that forms permanently on the tooth surfaces [2]. Hazard factors of the disease compose of unsuitable salivary flow, low quality of salivary buffer, incomplete fluoride exposure, and increase consumption of sugar [3]. Caries indicate to the centralize demolition of susceptible dental hard tissues by acidic by products from the bacterial fermentation of dietary carbohydrates [4]. It is a chronic disease that advances tardily in extreme people. It can be seen on smooth, hole and fissured surfaces of the crown and root of a tooth. According to the WHO) 60 – 90 % of school children worldwide have dental cavities [5].This decay is the result of the interaction of the oral micro flora plaque , the tooth surface, nourishment and the oral environment over time and results in a carious harm of the tooth enamel [6]. However in recent years overall happening of this disease has fall in industrialized nations, caries average are increasing in developing nations [7]. Moreover, caries spread is not evenly dole out across the population and communities with the highest happening are generally those in lower socioeconomic groups that have finite access to adequate oral health care [8]. In spite of the fact that studies display a decline in caries in the US , 10 billion are spent in this country each year on treatment of tooth decay [9]. In other industrialized nations such as the UK and China, caries prevalence in the past contract has been over 50 % in children. In developing countries, where oral health care is safely low available, caries average are increasing at an alarming rate. Studies done in the past contract in nation such as the Peru, Mexico, Philippines and Taiwan, detected caries in 75 - 90% of children [10].

Mutants streptococci a group of cariogenic bacteria, are implicated in the initiation of dental caries [11]. In addition to Mutants streptococci, Lactobacillus is another group of bacteria that is substantial in the development of caries. Lactobacillus does not avidly colonize on the tooth surface, but it may be transiently found in the oral cavity including the dorsum of the tongue [1]. Lactobacillus is not important in dental caries pointing .While, it is possible to play an important role in the caries advancement [12]. Due to the positive consortium between salivary levels of both of bacterial with caries and the relevance of this combination to carbohydrate exhaustion , counts of Streptococcus and Lactobacillus may, potentially, avail not only as a caries hazard foreteller but also as an indicator of carbohydrate consumption. [13] *Streptococcus mutans* is excessively accepted as one of the most substantial etiologic agents in caries evolution and has been shown to directly cause caries in germfree and specific pathogen free rat models. However, happening of caries have been found without *S. mutans* and high percentages of *S. mutans* have been recovered from non-carious persons, *S. mutans* remains the most combined species associated with caries. Also, in gnotobiotic and specific germfree rodent models, they have the paramount potential for generating caries [14]. Despite *S. mutans* has various properties that raise its cariogenicity, robust biofilm pointing in the presence of dietary sucrose is a stringent component in the development of caries.

Therefore, the aim of this study was to isolation and identification of important bacteria related to tooth decay and diseases of the mouth the other, determining the sensitivity bacteria of certain types of antimicrobial agents, studying the effect of some heavy metal for bacterial isolates .

EXPERIMENTAL SECTION

Isolation of microbial isolates from patients

Collection of samples: specimens have been collected in this study from the dental unit in the health center and dental clinics in Gambang, Malaysia with the assistance of the dentist and by using sterile swabs into the middle nutritious carrier for age 1 to 60 years both of genders. Collected samples were transferred to the laboratory of Universiti Malaysia Pahang.

Microbial culture**:** Samples from the mouth of patients were cultured on nutrient agar plates and were incubated at 37º for 24 hours then purified and cultured on to agar slants and kept in the chiller until use.

Antimicrobial Activity test Using Disc Diffusion Method

Antibiotic sensitivity test

All antibiotics used in this study from Mast disc^{tm} , Mast Diagnostics , mast group Ltd., mersey side. UK. Except penicillin G from Oxoid Ltd. Basingstoke. Hampshire England and streptomycin prepared in laboratory. Antibiotic disc(Amoxicillin 10µg, Neomycin10µg, Ampicillin 10µg, Tetracycline 10 µg, Gentamicin 10 µg, Chloramphenicol 10 µg, Penicillin G 10 µg, streptomycin10 µg, Rifampicin 5 µg.

Muller- Hinton agar from Hardy Diagnostics was prepared according to the manufacturers recommendations, autoclaved at 121 ^{o}C for 15 minute, then the medium was cooled to 45-50 °C and poured into the plates, allowed to set on a level surface to a depth of approximately 4mm. Inoculums from primary culture plates were prepared by touching 3-5 colonies with a swap and transferred into plate and mixed with two drops of sterile distal water, then spread in three plates.

The nine antibiotic discs were placed on to the inoculated plates. Then putted into the chiller for 15 min and then incubated at 37 °C, after overnight incubation ,the diameter of each inhibition zone was measured and recorded in mm [15].

Heavy metals activity test

Prepare concentration**:** Prepared concentration 10 µg / ml for (7) heavy metals (Silver nitrate, Iron chloride, Zinc chloride, lead acetate, copper sulfate, cadmium and mercury) that used in experimental. The stock solution was prepared for concentration and the filter paper disc was prepared and uploaded with 25 µl of heavy metal and continue as previous mentioned [16].

RESULTS AND DISCUSSION

Patients Isolates

In this study, has been obtained from the mouths of 50 patients in different ages and both of genders with percentage of 54% males and 46% females , as shown bacteria and yeast (59) isolates in the table and Figure1. It has been shown the primary isolation of samples.

Table 1- Primary isolation of samples and percent ages with gender

Patient No.	Gender	Age	Isolates No.	Patient No.	Gender	Age	Isolates No.
1	M	30	1	26	F	29	1
2	M	5	1	27	M	30	1
3	M	28	1	28	M	37	2
4	F	48	1	29	M	60	NG
5	M	19	2	30	M	19	1
6	F	23	2	31	F	12	1
7	F	17	1	32	M	5	1
8	F	10	1	33	F	24	2
9	M	52	1	34	M	19	2
10	F	29	1	35	F	51	1
11	F	53	NG	36	M	44	1
12	M	46	3	37	M	3	1
13	F	58	3	38	M	3	2
14	F	45	2	39	F	35	2
15	F	29	NG	40	M	32	1
16	M	26	1	41	F	7	NG
17	F	26	1	42	F	30	NG
18	F	20	1	43	F	26	1
19	F	6	1	44	F	31	1
20	M	22	1	45	M	18	2
21	F	30	2	46	M	28	1
22	F	47	1	47	M	33	1
23	M	5	1	48	M	60	1
24	M	24	1	49	M	49	1
25	M	18	1	50	M	24	1

M: male, F: female, NG: not growth

The impact of age stage group on the infection rates of the tooth caries showed the age stage groups of (20-40 years) and (1-20 years) were the most of the infected compared to elder group (40-60 years) , as was the incidence of 44% and 32% respectively.

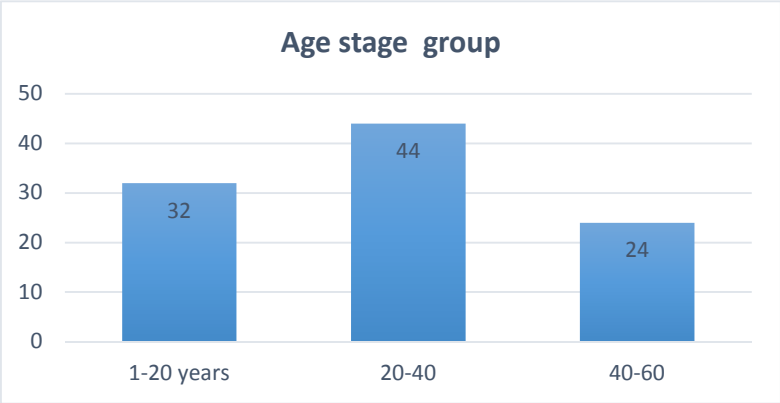

Fig 1- Percentage of isolates according to age group

The study confirmed that children and the younger are more susceptible to mouth infection. This may be due largely to reasons related to immune shortages of the infected people in these age groups as well as consciousness of health or other factors related to nutrition and public health that increase the rates of infection in patients of the children and the younger [17] stated that children are more susceptible to the bacteria that cause decay. That necrosis of the infected children appear to have teeth mutans with its different kinds and with high rates. Also, the frequent consumption of sugar play an important role in the infection with emphasis on the role of the mother as a source of transmission of disease from her infected teeth to her baby, where the levels of these bacteria with mothers are similar to those found at their children.

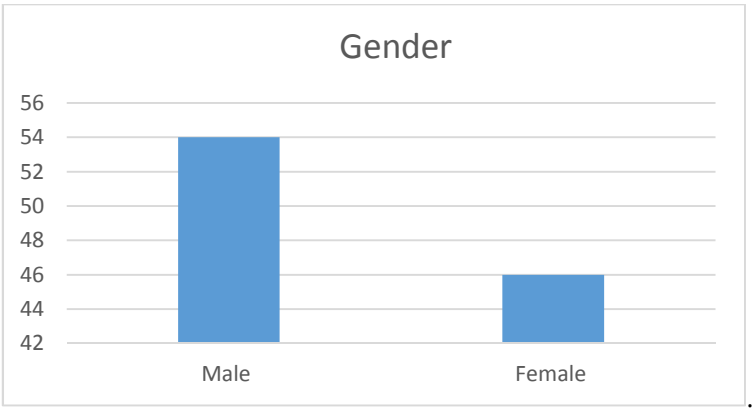

Fig 2- Percentage of isolates according to gender

Age stage group relate with Gender

The impact of age stage group on the infection relate with gender rates of the tooth caries showed the age stage groups of (20-40 years) and (40-60 years) were the similar of the infected with gender compared to first age stage group (1-20 years) , as was the incidence of 20% male and 12% female .

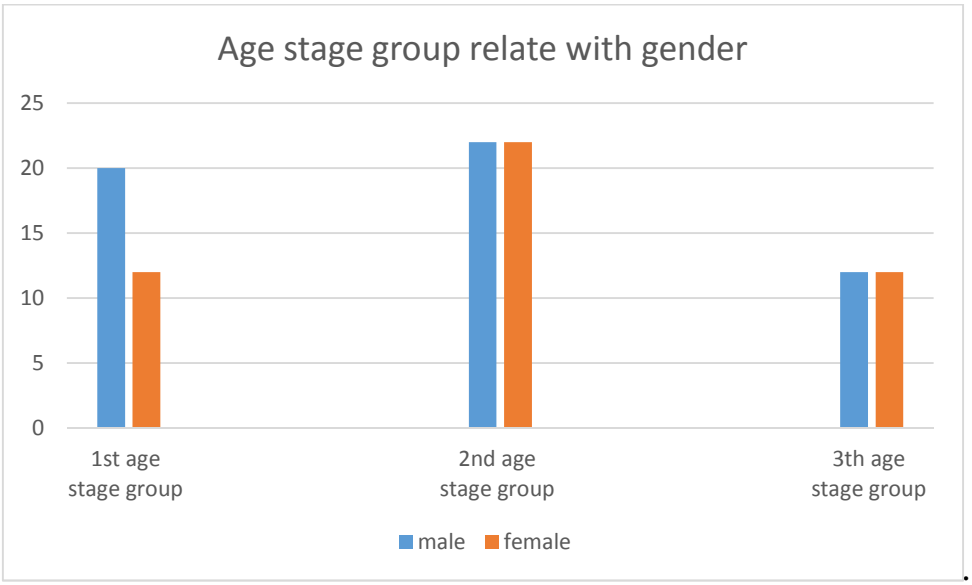

Fig 3-Age stage group relate with Gender

The Sensitivity of Bacteria to Antibiotics.

Data represented in Figure 4 show the percentage sensitivity of bacterial isolates against nine antibiotics, where it showed they were streptomycin and penicillin G 16.94%, 64.40% respectively. While noted the highest sensitivity to antibiotics was chloramphenicol (83.05%), similarity sensitive for Gentamicin and Rifampicin with (81.35%).

The current study showed that the chloramphenicol is the best of antibiotic in its influence on the bacterial isolates taken from the mouth, followed by the antibiotic Gentamicin and Rifampicin. The antibiotic tetracycline, Ampicillin, Neomycin, Amoxicillin are less effective on the bacterial isolates. One of the results we have noticed that the bacterial isolates showed variation in their resistance to antibiotics of the group of aminoglycosides, the ratio of sensitive to Neomycin 74.57%. The percentage of sensitive to Gentamicin by 81.35%. The resistance to aminoglycosides antibiotics increased notably in recent times, Livermore and Winstanley, 2001 studied Relationships between antibiotic and mechanism are also presented to allow full interpretative reading for those testing wide panels of drugs versus isolates. [18]. Evidenced by the results of the current study, the majority of bacterial isolates possessed prescription relatively high resistance to antibiotics represented β-lactam (Ampicillin, Amoxicillin, Pencillin *G*). The high bacterial resistance to antibiotics *β*-lactam due to several mechanisms, came our results are compatible with Cherian and Manjunath ,2003 during their study that extended spectrum beta lactamase producing enterobacteriaceae in a tertiary care hospital in Trinidad and Tobago [19]. The results of this study also

showed the high resistance shown by the bacterial isolates to streptomycin explains the mechanism of the resistance to this antibiotic. The study also shows an increasing resistance to tetracycline, it is believed that this resistance resulted from the presence of plasmids that encode resistance to the antibiotic which moves significantly. Koo and Woo, 2011 during their study that distribution and transferability of tetracycline resistance determinants in *E. coli* isolated from meat and meat products reported that the high prevalence of tetracycline resistant *Escherichia coli* in meat may be due to the high transferability of tetracycline determinants [20]. It is noted during the study that the lowest resistance showed by the bacterial isolates was to chloramphenicol, Gentamicin and Rifampicin. It appeared that most of the bacterial isolates were sensitive to these adversaries and may be due to response to the majority of the isolates of these two adversaries to being of limited use antibiotics at the present time in hospitals, leading to increased resistance to antibiotics can be passed as determinants responsible for drug resistance to antibiotics by plasmids.

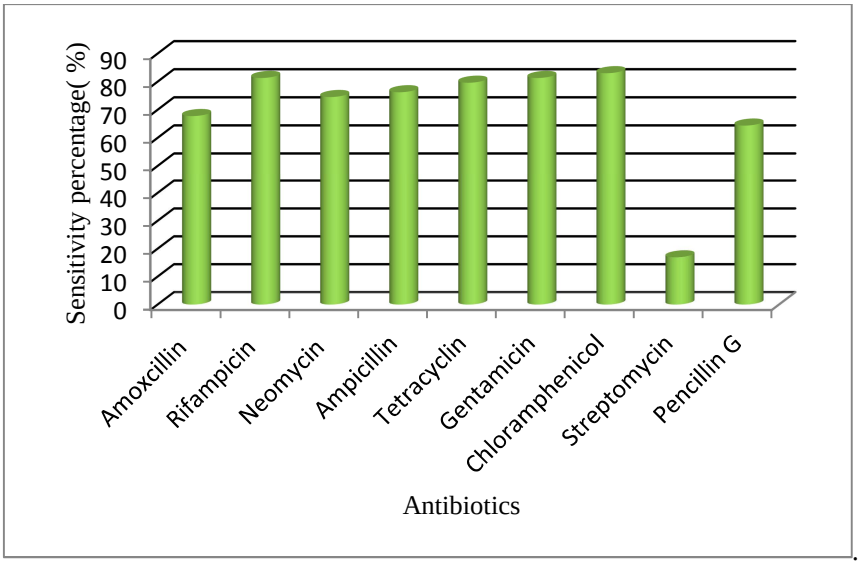

Fig 4 -The percentage sensitivity of bacterial isolates antibiotic

The Sensitivity of Bacteria to Heavy Metal

The results shown in Figure 5 that the resistance and sensitive percentages of bacterial isolates seven heavy metals, where it showed explain the highest resistance to heavy metals, Silver nitrate, Iron chloride, Zinc chloride ,lead acetate (100%), while appeared sensitivity to mercury(100%), cadmium (86.44%) and copper sulfate (1.69%).

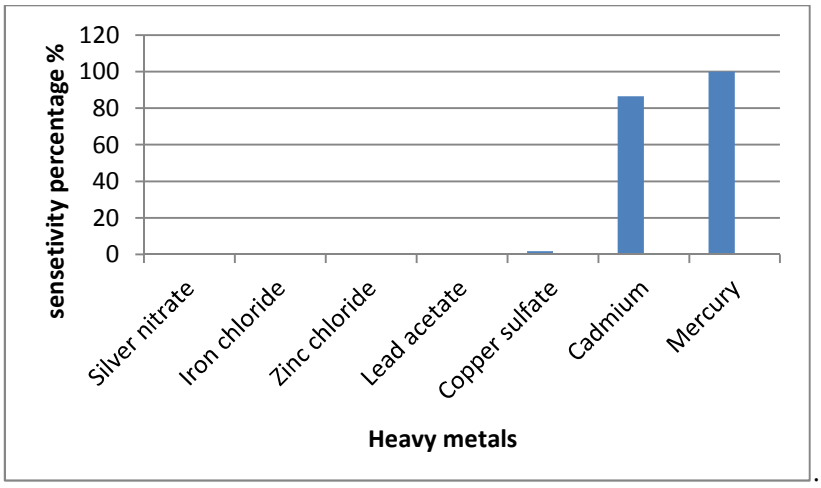

Fig 5 - The percentage of bacterial isolates sensitive of heavy metal

The results of this study also showed the high resistance shown by the bacterial isolates to silver nitrate. Starodub and Trevors ,1990 reported that Thirty nine percentage of bacteria *E. coli* resistance silver [21]. That is proof of bacteria resistance silver of an agent modification at intervals the microorganism ordering. Silver resistance is stable at intervals a microorganism population and transmissible to sensitive recipient strains by conjugation or

transformation in vitro. Silver ,2003 found link between Bacterial silver resistance and molecular biology by uses misuses of silver compounds [22] Bacterial resistance to silver will arise , Gupta et al 1999, reported that Molecular basis for resistance to silver cations in *Salmonella* and they are found that the high resistance silver determinant from a hospital burn ward Salmonella plasmid contains nine open reading frames, arranged in three measured and divergently transcribed RNAs [23].We have noticed that the bacterial isolates showed resistance for lead , One of the mechanisms that microorganisms use to avoid the toxicity of heavy metals of no biological perform is to limit their movement across the cell envelope . Jarosławiecka and Piotrowska 2014 studied that the main mechanisms of lead resistance, cell exclusion, and ion efflux to the cell exterior and noted the cytoplasm membrane be a natural barrier for lead, since the practical teams of many macromolecules are concerned in binding this metal in gram-negative microorganism, [24] . Evidenced by the results of the current study, the majority of bacterial isolates possessed prescription relatively high sensitive to Mercury. Barkay and Miller, 2003 studied bacterial mercury resistance from atoms to ecosystems so reported that one or more proteins apparently involved in transport genes conferring occur on chromosomes, plasmids, and transposons and their operon arrangements can be quite diverse, structural genes, several of which are modular. proteins protects host cells from this toxic metal, [25]. The data obtained during this study clearly shows that with sensitive microorganism of cadmium. This may be due largely to reasons related to less concentration from cadmium increase the rates of sensitive isolates. Cohen et al 1990 , studied that the effect of zinc and cadmium ions on *Escherichia coli* and they were noted that exposure of *E. coli* to various concentrations of these ions resulted in an increase of the total protein and the metal binding proteins amount in the cells. The activity of alkaline phosphates was raise in the presence of these ions [26]. The heavy metal Copper was less effective on the bacterial isolates. Michels and Wilks, 2005 reported that Copper alloy surfaces have intrinsic properties to destroy a large variety of microorganisms during their study that effect Copper alloys human infectious disease [27].

CONCLUSION

Results of the study showed that the rate of tooth caries was highest in the second age group 44%, the results of tests proved the antibiotic sensitivity, the optimal antibiotic for the tooth caries are chloramphenicol (83.05%), Gentamicin and Rifampicin (81.35%). The results of this study showed an increase in the proportion of resistance all heavy metals except mercury (100%), cadmium (86.44%) and copper sulfate (1.69%).

Acknowledgement
The authors gratefully acknowledge University Malaysia Pahang (UMP), Malaysia for the financial supported by grant GRS 140318 that enables the authors to accomplish this work.

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