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RESEARCH ARTICLE

COMPARISON OF THE EFFCINEY OF SOME MOUTH WASHES FROM DIFFERENT ORIGINS AGAINST LACTOBACILLUS AND STREPTOCOCCUS MUTANS THAT ISOLATED FROM GINGIVITIS & DENTAL CARIES.

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Abstract

The study include isolation and diagnosis of Streptococcus mutans and Lactobacillus spp. Which cause gingivitis and dental caries and culturing on Mitis Salivarius Agar media for *Streptococcus mutans* and cultring Lactobacillus on (MSR).

(30) sample was taken from patients suffering gingivitis and dental caries by paper point and transferred to laboratory by transporting media and (MSR),(MSA) and incubated in unarobic condition under (37)for 72 hours.

The result aeal that *Streptococcus mutans*(9) samples and its ratio (56.25%),while of (7) samples the bacteria Lactobacillus and its ratio (43.75%) .

Comparis of (8) types of mouth washes from different origin in as listid(1) and there effects on isolated bacteria.

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Introduction:-

In the different sites of the mouth, the bacteria are able of cohabiting in saprophytism, depending directly on many factors such as pH, availability of nutrients and natural of mucous surface. The establishment and maintenance of oral microbiota is related to inter bacterial co aggregation and biofilm formation, which constitute the primary etiologic agents of oral diseases(Tanzer,et al .,2001). The plaque (biofilm)related infection such as dental caries and Periodontal disease represent two most common types of dental disease. The dental plaque provides ground for the inhabitancy of pathogenic bacteria that lead to the tooth decay, where bacterial processes change sugar in food left on tooth to acid that demineralization hard tooth structure from calcium and progressively break down(Prashant, et al .,2007).

The demineralization which caused particularly by *Streptococcus* bacteria occurs within dental plaque that adheres to the tooth surfaces and become colonized by other bacteria such as Lactobaciccussp, some species of gram negative bacteria, yeas and that responsible to secondary infection in mouths (Gamal,M.E.S.,2014).

Dental caries is a multifactorial disease. A number of lifestyle, environmental andhereditary factors contribute to its development. These include the frequent intake offermentable carbohydrates, poor oral hygiene, high counts of cariogenicmicroorganisms, the inadequate use of fluoride and impaired salivary function (Koneman,et al .,2006).mutans streptococci are considered majorpathogens in the initiation of caries due to their acidogenic and

acidic properties and their ability to adhere to the tooth surface and to other bacteria (6-8). Lactobacilli, which are highly acidogenic, are more common in deep caries lesions and indicate high fermentable carbohydrate consumption and disease progression rather than initiation (Taciano, et al., 2010).

There are several types of mouth wash which all perform a particular function. There are mouth washes which help to strengthen your teeth, antiseptic mouthwashes which deal with tooth decay and bad breath and herbal mouthwashes which do not contain alcohol.

The types of mouthwash available include :

1. Fluoride mouthwashes contain sodium fluoride which helps to strengthen the teeth as well as adding extra protection against tooth decay. However, fluoride is present in toothpaste and tap water and is more than adequate for our needs. Be careful about consuming excessive amounts of fluoride.
2. Cosmetic mouthwashes do not offer the same protection as other types and are used more as a means of disguising bad breath (halitosis). They help to keep your teeth clean but don't reduce the risk of tooth decay.
3. Antiseptic mouthwashes contain chlorhexidine gluconate, a chemical which stops the growth of bacteria and is suitable for people with bad breath (halitosis).
4. They are effective in that they can prevent the build up of plaque to a certain degree but they should be used in conjunction with a toothbrush and dental floss.
5. Avoid overuse of these as the high level of chlorhexidine can cause discoloration of teeth over a long period of time. But if you don't experience this then your dentist or dental hygienist will be able to easily treat it.
6. Natural mouthwashes are alcohol free (and contain no fluoride) and work in much the same way as conventional mouthwashes.
7. Some people find that a home-made mouth wash, such as those made from a pinch of salt and warm water, are useful following a tooth extraction. They can also treat a mouth infection or injury.
8. Total care mouthwashes contain antibacterial ingredients which help to reduce the build up of plaque and prevent gum disease. (Demian, *et al.*, 1960; Cochran, 2009; Conneally, 2010)
9. So the purpose of this study was aimed to isolate and identify oral pathogenic bacteria from patients suffering from gingivitis and dental caries and make a comparison between the effect of antibacterial activity of eight types of mouthwashes from different origins on these bacteria and the formation of biofilm.
10. The purpose of this study was to examine the bactericidal activity of selected herbal products and other products for mouth washing, possessing antibacterial activity as declared by the manufacturers, used in treatment of inflammation and for disinfection of the mouth.

Materials & Methods:-

1-Specimens collection and bacterial identification:

Thirty swabs were obtained from patients clinically diagnosed by dental physicians to have gingivitis and dental caries. The infected area of the tooth was swabbed with sterile cotton wool and transferred to a sterile screw-capped tube that contained 5.0 ml of Reduced.

Transport Fluid (RTF) with aseptic. The swabs were streaked on general and selective media MRS (deMan Rogosa Sharpe agar), Lactobacillus, MSA (Mitis salivarius agar), Streptococcus mutans. The plates were incubated at 37°C for 36-72 h in the presence of 5% CO₂. and the number of colonies was counted based on the colony characteristics and confirmed by gram staining. (Collee, et al., 1996; Brown, 2005).

Mouthwashes collection

Eight mouthwash products (table 1) from Mosul (Iraq) pharmacies were used in this study

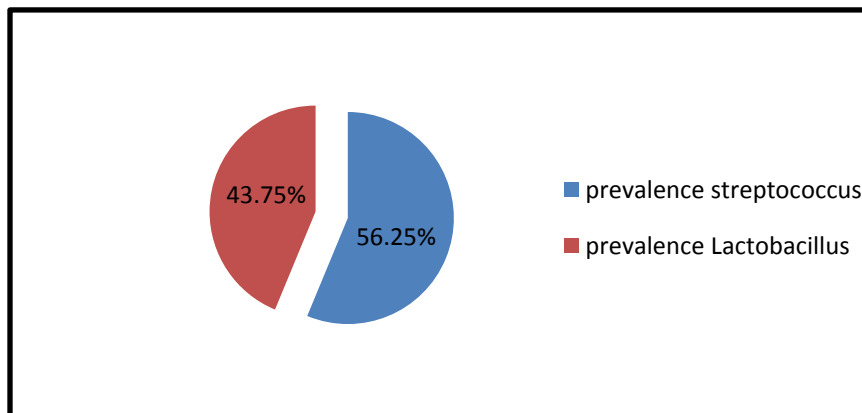


Table 1:-Types, compositions and manufacturer of the mouthwashes used in this study

Name	Composition	Manufacture
Clardine	Chlorhexidinedigluconate B.P 0.2 gm	United Arab Emirates
Biofresh(K)	Chlorhexidinegluconate 0.12%, Sodium saccharin, , Sorbitol,Aroma, Aqua, polyoxcetelaen glycol.	Syria
Zac	Chlorhexidinegluconate 0.12%,Sodium fluoride 0.05%	Amman-Jorden
Colgate	Sodium fluoride 0.05% w/w(225ppmF), Aqua, Glycerin,Alcohol,Propylene glycol, Sorbitol,Tetrapotassium Pyrophosphate,Polysorbate 20,Terasodium Pyrophosphate, Zinc Citrate,PVM/MA Copolymer,Aroma, sodium Benzoate, Sodium fluoride, Sodium saccharin,CI 42090	Sao Paulo-Brazil
Kin B5	Sorbito, Aqua, Glycerin,PEG-40 Hydrogenated Castor Oil, Propyleneglycol, xylitol,Panthenol (0.5%), Zinc lactate (0.38%), Sodiummethylparaben ,Citric acid, Aroma,Disodium EDTA, Niacinamide(0.10%),Cetylpyridinium Chloride(0.05%), Menthol, Sodium fluoride(0.05%)(226ppm F), Sodiumpropylparaben, Sodiumsaccharin,Limonene	Barcelona-Spain
Breath RX	Sorbitol, propylene glycol,PEG-40 Hydrogenated Castor Oil,Poloxamer407, xylitol,zinc glyconate, Aroma (mint, thymol, eucalyptus oil) ,CocamidopropylBetaine, sodium saccharin, cetylpyridinum chloride, Citric acid,CI42090	United State of America
LACALUT aktiv	Aqua, Glycerin,PEG-40 Hydrogenated Castor Oil,Olafur, , Aroma, Aluminum,Lactate, Zinc Sulfate,ChlorhexidineDigluconate,PotassiumAcesulfame, Propyleneglycol, ,Limonene(hydroxyethyl)aminopropyl-N-hydroxyethyloctadecylamine-dihydrofluoride Fluoride 225ppm	Germany
Water& Saline	Nacl(500µ)	Iraq

Bacteria Sensitivity test of mouthwash.

Sensitivity test bacteria against mothwash table (1) to the method Bauer- Kirby on Muller-Hinton agar mediawas done by disc diffusion method. For anaerobicgrowth the plates were incubated at 37 degree C in anaerobic jarpresence of 5% CO₂ for 18-24 hrs and then plates wereexamined for a zone of inhibition.(Bauer,1966;Vadepitt et al.,2003).

**Figur:1** Ratio of bacteria isolated from dental caries

The figure (1) show the number of total samples that taken in this study and it's(30)in numbers from patients suffer from caries and gingivitis at different ages &gender.

The ratio of *Streptococcus mutans* is (56.25%) while the ratio of Lactobacillus is (43.75%) and this differentiation.

belong to presence of these bacteria in the mouth as normal flora but with decrease in the immunity, which convert to pathological microorganism and cause gingivitis & dental caries in presence of other factors like acidic PH, food debris and dental plaque. This specific types colonization of *Streptococcus mutans* colonize dental surface and cause damage to hard tooth structure in the presence of fermentable carbohydrate e.g sucrose and fructose.

Results & Discussion:-

Table 2:-Effect of various mouthwashes on the growth of *Streptococcus mutans*

Effect of mouthwashes								Samples Ber
Water& Saline	LACALUT aktiv	Breath RX	Kin B5	Colgate	Zac	Biofresh(K)	Clardine	
6	6	6	6	6	6	6	6	1
6	10	8	6	6	6	6	10	2
6	9	6	6	6	6	6	9	3
6	8	6	6	6	6	6	9	4
9	14	10	12	6	11	6	9	5
9	12	6	6	6	6	6	10	6
9	10	6	6	6	6	6	10	7
6	6	6	6	6	6	6	9	8
6	6	6	6	6	6	6	9	9

The table(2) show the effect of mouth washes and normal saline on bacteria *Streptococcus mutans* that is isolated from patients who suffer from dental caries and gingivitis.

(9) sample of isolated bacteria *Streptococcus mutans* used to show the effect of these mouth washes which used by dentist and the result are differ according to pathogenicity of bacteria and type of mouth wash which used the Lactalut active dental mouth wash with Germanic manufacturer show the best effect from the other types of used mouth washes because the presence of effective components of this mouth wash like , chlorhexidinedigluconate, propylene glycol, limonene(hydroxyethyl) , amino propyl-N-hydroxyethyloctadecyl amine-dihydrofloride, fluoride 225 ppm which regards as anti carogenicity effects .

Table 3:-Effect of various mouthwashes on the growth of *Lactobacillus*

Effect of mouthwashes								Samples Number
Water& Saline	LACALUT aktiv	Breath RX	Kin B5	Colgate	Zac	Biofresh(K)	Clardine	
6	9	6	6	6	6	6	9	1
10	14	9	10	9	12	7	9	2
9	14	12	14	6	6	6	9	3
6	14	14	10	9	10	12	25	4
9	6	6	12	6	6	6	9	5
9	12	6	6	6	6	6	6	6
9	12	6	6	6	6	6	6	7

Then (Clardine) mouth wash with (UAE) manufacturer because content of chlorhexidine, digluconate ppm(0.2)gm. Then the effect of (Breath RX) mouth wash with (USA) manufacturer with limited effectiveness on the bacteria that is used in this study because it ,not contained chlorhexidine which regards bacteriostatic agent and treat gingivitis .

It noted from the results that isolation No.(5) of bacteria *Streptococcus mutans* most of mouth washes used had an effect on that isolation and this indicate the sensitivity of this isolation toward these mouth washes.

The effect of (Biofresh-K-) mouth wash and (Colgate) mouth wash on this isolation was not shown due to the components and properties of each mouth wash ,the (Biofresh-K-) contain only 0.12% chlorhexidinedigluconate which is not enough to work on this bacteria and the (Colgate) mouth wash not contain chlorhexidinedigluconate and only contain 0.05% sodium fluoride .

Results reveal that the normal Saline which is used frequently by dentists is very weak and it had no effect or little on the isolates under study. The reason is back to lower the proportion of sodium chloride NaCl with 0.05μ

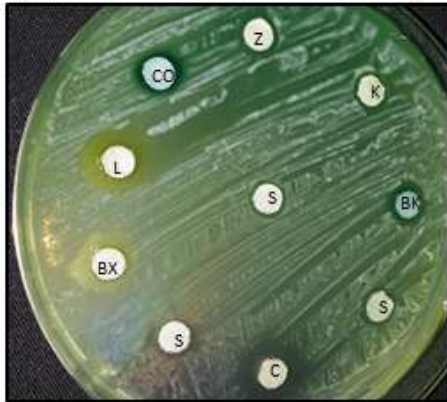
Results prove that common mouthwash solutions have variable antibacterial activity depending on their major active components.

Only mouthwash solutions containing chlorhexidine gluconate or cetylpyridinium chloride exhibited activity against majority, but not all tested bacterial strains in their biofilm state.

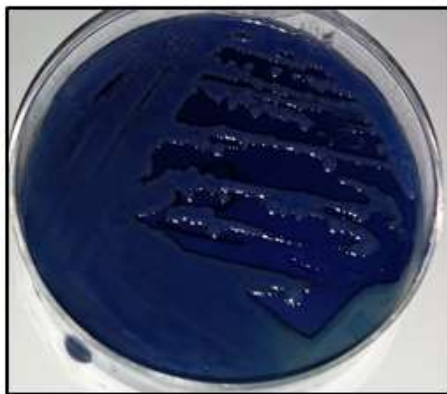
Additionally, bacteria are generally less susceptible to all mouthwash. Results of this study show that mouthwash solutions possess variable antibacterial activity depending on their chemical composition. For example, mouthwash solutions containing the antiseptics chlorhexidine are effective on most oral bacterial strains. This correlates with previous studies and is related to chlorhexidine's mode of action as it works on different sites of the bacteria (Gamal, M.E.S., 2014; Tanzer *et al.*, 2001). Additionally, mouthwash solutions containing sodium bicarbonate 2% were shown to be effective against most of the tested solutions in their biofilm as compared to planktonic state bacterial strains, which is in agreement of previous studies (Atlas, R.M. 1995).



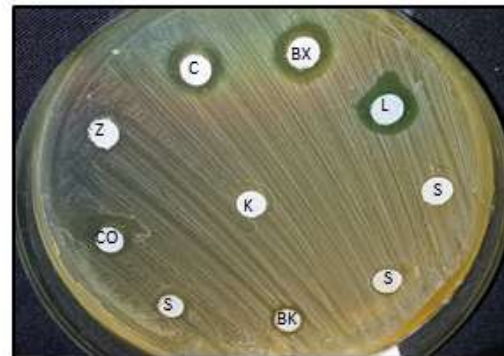
Lactobacillus on MSR media



Effect mouth wash on Lactobacillus



Sterptococcus mutans on MSA



Effect mouth wash on Sterptococcus

On the other hand, mouthwash solutions containing other ingredients such as cetylpyridinium chloride, sodium fluoride, hexetidine, povidone-iodine, eucalyptol, menthol, methylsalicylate, and thymol showed activity against some bacterial strains, but not others. For example, cetylpyridinium chloride (0.05%) mouthwash was shown to possess antimicrobial activity against all tested bacterial strains except *P. aeruginosa* and *K. pneumoniae*. In fact, cetylpyridinium chloride is a quaternary ammonium compound known for its use as a cationic surface active agent that has antibacterial activity (Hoit, *et al.*, 2001). Moreover, the MIC values for cetylpyridinium chloride (0.05%) mouthwash suggest that it has high activity against *S. aureus*. This is in agreement with previously published work on cetylpyridinium chloride products.

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