# *Effect of Autoclave on Bacterial Growth Isolated from Different Types of Denture Adhesives*

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## ABSTRACT

**Background:** Different types of denture adhesives like powder or paste have been used to enhance the retention and stability in denture wearers, especially in patients with resorbed ridges , these adhesives should be free from microbial contamination in order to use safely inside patient mouth and avoid infection , the purpose of this study is to investigate the bacterial contamination of different adhesives pastes and powders and the effect of autoclaving in reducing the bacterial growth .

**Materials and Methods:**Six types of commonly available denture adhesives (three powders and three pastes) were tested for bacterial contamination by using culture on suitable media that contained either brain-heart infusion (BHI) ,blood agar, nutrient agar,MSA agar and others were inoculated with 0.5 gm samples of each adhesive. plates were incubated at 37C° for 24 hours in pre and post sterilization by using the colony forming unit for estimation the bacterial growth.

**Results :** The results revealed that , In pre sterilization test all adhesive types (powders and pastes ) contained Gram positive and Gram negative bacteria ,the results also revealed that Dentofix (paste type 1) has the lowest bacterial growth in which 40% without bacterial growth , also the results reported that 25% of adhesives were contaminated by *Staphylococcus* species and 23 % of adhesives were contaminated by *Neisseria* species .Dentofix paste was highly significant difference regarding the mean of bacterial count at both dilutions 1/10 and 1/100 among other adhesives with the lowest contamination in comparison to other types of denture adhesives in presterilization step.while after using sterilization by autoclave generally sterilization induce high significant difference in each type of denture adhesive after exposure to autoclave for 15 min at 121 °C 15 pound/inch it decreases the number of bacterial growth in all types. The results showed that powders adhesives had high contamination rate than pastes and their killing rate after sterilization was lower than pastes. Fittydent (paste type 3) was highly affected by sterilization than other adhesives in first dilution 1/10 when killing rate was 78.9% . all types of tested adhesives showing that in second dilution 1/100 sterilization more effective at this dilution and sterilization induce 100% killing in Dentofix ( paste type 1) , Bonyplus ( paste type 2) and Fittydent (paste type 3) .

**Conclusions:** The result of this study concluded that the powders denture adhesive have high bacterial contamination rate than the pastes and using of sterilization by autoclave must be done to increase killing rate.

## KEYWORDS:

denture adhesive, bacterial contamination, sterilization

## المستخلص

تم البحث في عينات نوع باودر واخرى نوع معجون من لواصق طقم الاسنان المتداولة في الاسواق واثبتت الدراسة وجود تلوث بكتيري بانواع مختلفة من البكتيريا في جميع العينات الخاضعة للفحص ومن ثم تم اجراء تخافيف لعينات اللواصق بتراكيز مختلفة من المحلول الملحي ووضعها في جهاز التعقيم بحراره 121 درجة مئوية وبضغط 15 باوند مما ساهم في تقليل معدل التلوث البكتيري وصولا الى مرحلة خلو كل معاجين اللواصق المستخدمة من التلوث مما يؤكد على اهمية خضوع تلك اللواصق الى عملية التعقيم قبل استخدامها من قبل المرضى

## INTRODUCTION:

Denture adhesives are extensively improved the retentive strength of complete denture. [1] Residual ridge resorption is considered a major oral disease which could occur despite of careful prosthetic handling[2]. One of the most undesirable effects of residual ridge resorption is compromised denture retention which is considered a real challenge in complete denture therapy [3,4] .

Many denture wearers regularly use denture adhesives to enhance denture retention, stability and function[5-7].Denture adhesives had been used to aid in complete denture retention long time ago [8,9]. Wilson et al, [10] reported that 30% of the patients wearing dentures used denture adhesives. Another study declared that out of the 20% of the adult population in US who wear dentures at least 22% used denture adhesives [11] , the use of denture adhesives is considered suitable, adjunctive and effective treatment modality in removable prosthodontics. [12,13]

Denture adhesives have been marketed in different formulations such as powders ,pastes or creams for soluble adhesives and strips or cushions for insoluble adhesives. Adhesive ingredients that improve denture support can include a blend of polymer salts, calcium salts and zinc for soluble adhesives and laminated fabric for insoluble adhesives some manufacturers are introducing zinc-free adhesives. In addition to the active ingredients in denture adhesives, a number of non-active components may also be included – such as flavouring additives, colourings, preservatives and

antimicrobial agents[7,14,15].

The retentive ability of denture adhesives presented a much greater retentive force than that of saliva alone. The paste form of adhesives exhibited a higher retentive force than the powder form even the ones of the same brand. [16]

The ideal denture adhesive should be non-toxic, nonirritant and biocompatible with the oral mucosa, odourless and tasteless, should retain the adhesive properties for 12–16 h and should not promote microbial growth [17].

Ruta etal [18] Stated that microbial counts on blood agar were significantly higher for diabetic patients as compared to the non diabetic control group and denture adhesives had no effect on the microbial colonization for both groups.

William D etal conducted that bacterial and fungal contamination were found in all containers of denture adhesives tested. [19] Microwave energy has been used as an alternative method for disinfection and sterilization of dental prostheses Microbial transmission requires blockage by a practical, easy and effective method of dental prosthesis disinfection or sterilization procedures. Microwave energy has currently been used as an alternative to conventional methods, although variations in its efficiency have been reported . Microwave energy reduced microbial contamination of tested denture adhesives but failed to completely sterilized [19-23].Rohrer and Bulard [24] showed that microwave energy can easily kill microorganisms on denture acrylic resin surfaces within 15 min of exposure.

Webb et al. [25] reported that microwaving dentures at 350 W during 6 min may be a more effective method of sterilization than soaking the dentures in 0.02% sodium hypochlorite for 8 h.Moist heat in the form of pressurized steam is regarded as the most dependable method for the destruction of all forms of life, including bacterial spores. This method is incorporated into a device called the autoclave. The use of common infection-prevention methods appears to reduce the bacterial counts. Bacterial contamination was lowest after autoclaving . [26]

Infection control has become a major focus for dental personnel ,the Cross-contamination is a major concern in dentistry, especially in the field of prosthodontics appropriate measures, therefore, should be adopted to avoid this kind of cross-contamination, This study aims to identify the bacterial growth in denture adhesive material ( powder and paste ) and to estimation the effect of autoclave to reduce bacterial growth.

## MATERIALS AND METHODS:

**SUBJECT:**This study was conducted in the department of basic science (microbiology ) , college of dentistry , Al Mustansiriah University from November 2015 to October 2016.

Six brands of commercially available denture adhesives three powder and three pastes were tested for bacterial contamination in pre and post sterilization by autoclave at 121˚ C, pressure 15 pound /inch² for 15 minutes.

The denture adhesive material types as follow:(as seen in Figure -1)

1. Powder Type 1 Protefix –Queisser pharma- Germany
2. Powder type 2 PD denture adhesive – Produits Dentaires S.A-Switzerland
3. Powder type 3 Fit fix -Pyrex polykem – india
4. Paste type 1 Dentofix Helago- Pharma GmbH – Germany
5. Paste type 2 Bonyplus –Bonyfag –Switzerland
6. Paste type 3 Fittydent – Fittydent international GMBH- Austria

For each type of denture adhesive ( 10 ) containers were purchased and assigned number from 1-6.
Normal saline for dilution was used 0.9 Nacl

**Culture media:** The following culture media were used in this study , they were prepared freshly prior to each experiment according to the instruction of the suppliers and sterilization by autoclave .

Brain Heart Infusion Agar, Blood Agar, Nutrient Agar, Chocalate Agar, EMB , MacConckey Agar

**Processing :** 0.5 gram of different type of denture adhesive were weighted for each type was done before and after sterilization by autoclave .the sample was diluted with sterile saline (1/10, 1/100 ) , the dilutions(1/10, 1/100 ) were cultured on BHIA for total bacterial count and the same processing repeated after sterilization by autoclave.

100µ of dilutint (1/10, 1/100 ) was spread in duplicate on BHIA plates and incubated at 37˚ C for 24 hours then colony counter was used in counting small embedded colonies.

The isolates of both gram positive and gram negative bacteria were identified on the basis of morphology of colony , microscopical examination and biochemical tests (gram stain test, catalase test , coagulase , oxidase , TSI , IMVIC test).

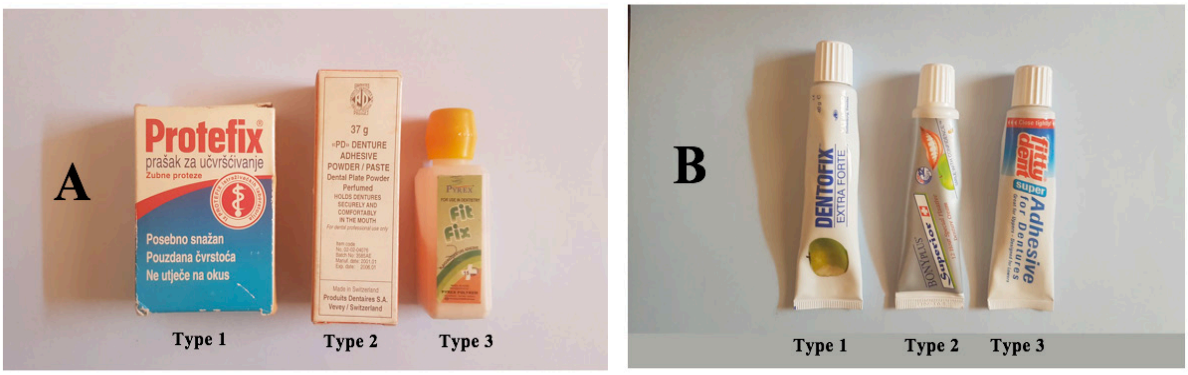


**Figure (1) Types of adhesive materials A( powders) B ( pastes)**

**Statistical analysis:**

The data were analyzed by using the statistical package of social sciences (SPSS) version 20,the data were summarized in number and percentage and figures and analyzed by using a nova and paired sample t test value≤0.05 considered significant.

## RESULTS

Table-1- Demonstrated that the Gram positive bacteria was presented in 65% of adhesives, Gram negative in 20% of adhesives, no growth was reported in 6.7% of adhesives.

**Table-1-State of growth, number and percentage of each type of bacteria for all adhesives.**

| *Type of bacteria and state of growth* | | *Number of adhesives* | *%* |
|---|---|---|---|
| Growth positive | Gram positive | 39 | 65 |
| | Gram negative | 12 | 20 |
| | mixed | 5 | 8.3 |
| Growth negative(no growth) | | 4 | 6.7 |
| Total | | 60 | 100 |

In Figure -2-demonstrated that the Gram positive bacteria was presented in 90% of paste type 2 ,in 70% of powder type 2 and paste type3 while Gram negative presented in 40% of powder type 3,mixed type presented in 30% of powder type 1 and no growth was reported in 40% of paste type 1.

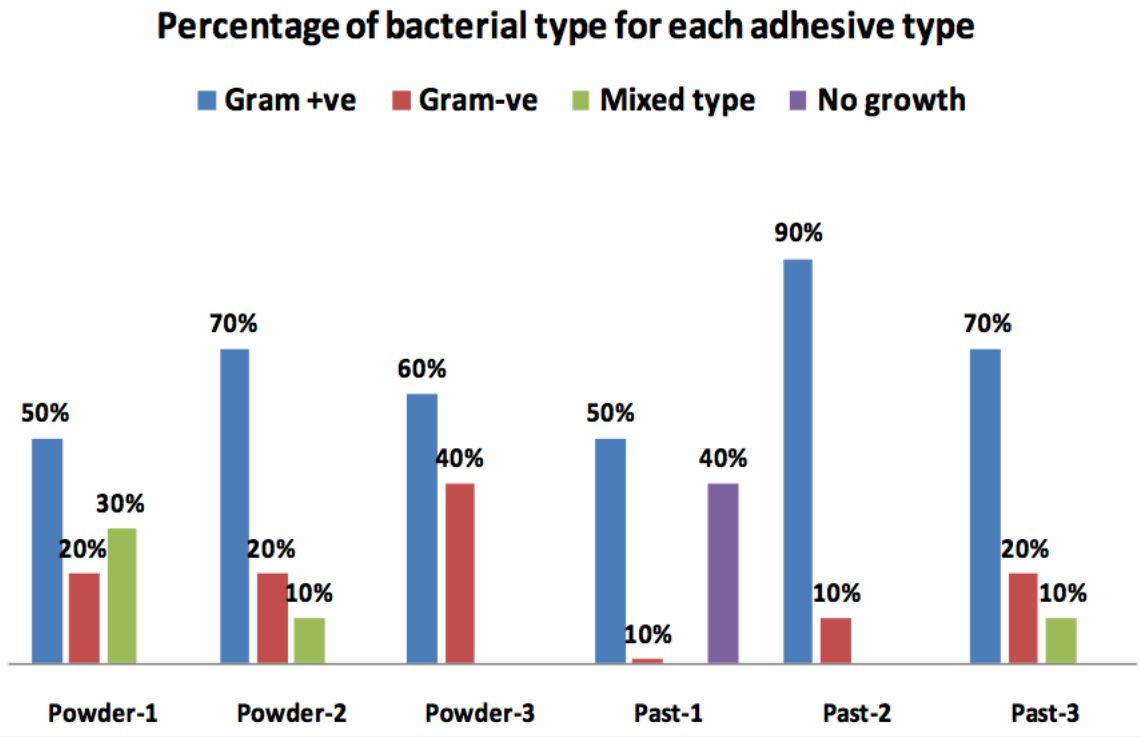


**Figure-2-Percentage of each type of bacteria for study adhesives**

Table-2- illustrated that 25% of adhesive were contaminated by Staphylococcus species, 23% by Neisseria species, others contaminated by other species.

**Table-2-Distribution of bacterial species for study adhesives.**

| *Bacterial species* | *Number of adhesive* | *%* |
|---|---|---|
| E.coli | 3 | 5 |
| Klebsiella species | 3 | 5 |
| Neisseria species | 14 | 23 |
| No growth | 4 | 7 |
| Staphylococcus and Neisseria | 7 | 12 |
| Staphylococcus and streptococcus species | 7 | 12 |
| Staphylococcus species | 15 | 25 |
| Streptococcus species | 7 | 11 |
| Total | 60 | 100 |

Table-3- showed that 30% of powder type1contaminated by Staphylococcus and Neisseria species and 30% contaminated by Staphylococcus and Streptococcus species. For powder type 2, 20% contaminated by Neisseria species, 20% by staphylococcus and Neisseria species, 20%by staph. and 20%by Strep. spescies. Regarding powder type 3, 60% contaminated by staphylococcus species and 40% by Neisseria species. For past adhesives, Paste type 1 contaminated by Neisseria species in 40% of sample while Past type-2- contaminated in 30% by Staph. Species and 30% by strep. Species. Staph. and strep. Species reported in 30% of sample of Paste type 3 while other species presented in different percentages.

**Table-3- distribution of bacterial species according to adhesive type.**

| *Adhesive types* | *Bacterial species* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *E..coli* | *klebsiella species* | *Neisseria species* | *No growth* | *Staph. and Neisseria* | *Staph. And strep. species* | *Staph.spe-cies* | *Strep. species* |
| | *%* | *%* | *%* | *%* | *%* | *%* | *%* | *%* |
| powder-1 | 10 | 0 | 10 | 0. | 30. | 30. | 10 | 10 |
| powder-2 | 0 | 10 | 20 | 0. | 20. | 10. | 20 | 20 |
| powder-3 | 0 | 0 | 40 | 0. | 0. | 0. | 60 | 0. |
| past-1 | 0 | 0 | 40 | 40. | 0. | 0. | 10 | 10. |
| past-2 | 10 | 0 | 20 | 0. | 10. | 0. | 30 | 30 |
| past-3 | 10 | 20 | 10 | 0. | 10. | 30. | 20 | 0 |

Table-4-demonstrates a high significant statistical difference by using Anova test among adhesives (p=0.001) regarding to mean of bacterial colony count after dilution to 1/10 and paste type 1 presented with lowest contamination in comparisons to other adhesives (mean=12.2±SD10.9), but on multiple comparisons between each two types, no statistical significant difference was found between (powder1 and past 3, p=0.495), (powder3 and past 2, p =0.852) and (past 3and powder 1, p =0.495)

**Table-4-Mean of bacterial colony count at 1/10 dilution for study adhesives.**

| *Adhesive type* | *N* | *Mean* | *Std. Deviation* | *95% Confidence Interval for Mean* | | *F test d.f.=59* | *P value* |
|---|---|---|---|---|---|---|---|
| | | | | *Lower Bound* | *Upper Bound* | | |
| powder-1 | 10 | 36.2 | 8.2 | 30.3 | 42.1 | 32.873 | 0.001(HS) |
| powder-2 | 10 | 51.4 | 8.5 | 45.2 | 57.5 | | |
| powder-3 | 10 | 28.6 | 4.0 | 25.7 | 31.4 | | |
| past-1 | 10 | 12.2 | 10.9 | 4.3 | 20.0 | | |
| past-2 | 10 | 29.2 | 2.2 | 27.6 | 30.7 | | |
| past-3 | 10 | 38.4 | 5.0 | 34.8 | 41.9 | | |

Table-5-demonstrates that there is a high significant statistical differences by using Anova test among adhesives(p value=0.001) regarding mean colony count of bacteria after dilution to 1/100 and the

paste type 1 presented with lowest contamination in comparisons to other adhesives (mean=5.5±SD5.7), but on multiple comparisons between each two types, no statistical significant difference was found between(powder-1 and powder 3 ,p=0.068), (powder1 and past 3,p =0.694) ,(past 2and powder 3,p =0.455) and (powder3 and past 3,p=0.149) .

**Table -5-Mean of colony count of bacteria at 1/100 dilution for different adhesive types**

| Adhesive type | N | Mean | Std. Deviation | 95% Confidence Interval for Mean | | F test | P value |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | | |
| powder-1 | 10 | 22.9 | 9.0 | 16.42 | 29.38 | 37.964 | 0.001 |
| powder-2 | 10 | 39.2 | 6.6 | 34.47 | 43.93 | | |
| powder-3 | 10 | 18.2 | 4.6 | 14.87 | 21.53 | | |
| past-1 | 10 | 5.5 | 5.7 | 1.39 | 9.61 | | |
| past-2 | 10 | 16.3 | 1.3 | 15.34 | 17.26 | | |
| past-3 | 10 | 21.9 | 2.9 | 19.76 | 24.04 | | |

Table-6-showed that sterilization induce high statistical significant differences in each type of adhesive (p≤0.05) and past 3 highly affected by sterilization than other adhesives

**Table -6-Mean difference of bacterial colony count (pre and post sterilization) in each adhesive at dilution of 1/10.**

| Adhesive types | Mean differences | Killing% | SD | 95% Confidence Interval of the Difference | | Paired sample T test | p-value |
|---|---|---|---|---|---|---|---|
| | | | | Lower | Upper | | |
| powder-1 | 23 | 63.8 | 4.0 | 20.08 | 25.92 | 17.81 | 0.001 |
| powder-2 | 32 | 62.7 | 6.2 | 28.35 | 37.24 | 16.69 | 0.001 |
| powder-3 | 18 | 62 | 2.9 | 15.16 | 19.43 | 18.33 | 0.001 |
| past-1 | 8 | 66.6 | 7.5 | 3.28 | 14.11 | 3.63 | 0.005 |
| past-2 | 21 | 72.4 | 2.7 | 19.76 | 23.63 | 25.32 | 0.001 |
| past-3 | 30 | 78.9 | 5.4 | 26.23 | 33.96 | 17.61 | 0.001 |

Table-7- demonstrated there is a high statistical difference by using anova test among adhesives regarding mean difference induced by sterilization, the highest mean difference was noticed in powder type 2(mean difference=32.8±6.2SD), but on multiple comparisons no statistical significant difference was found (between powder 1 and paste 2, p=0.5), (between powder 2 and past 3.p=0.2),(between powder 3 and past 2,p=0.6).

**Table -7-Mean difference of colony count of bacteria (pre and post) using of sterilization for all adhesives at dilution 1/10.**

| Adhesive types | N | Mean differences | Std. Deviation | 95% Confidence Interval for Mean | | F test | p-value |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | | |
| powder-1 | 10 | 23 | 4.0 | 20.0 | 25.9 | 28.988 | 0.001 |
| powder-2 | 10 | 32 | 6.2 | 28.3 | 37.2 | | |
| powder-3 | 10 | 18 | 2.9 | 15.1 | 19.4 | | |
| past-1 | 10 | 8 | 7.5 | 3.2 | 14.1 | | |
| past-2 | 10 | 21 | 2.7 | 19.7 | 23.6 | | |
| past-3 | 10 | 30 | 5.4 | 26.2 | 33.9 | | |

Table-8- showed a high statistical mean difference for all adhesives in regard to pre and post using of sterilization (p≤0.05).results also showed that sterilization more effective after second dilution where sterilization induce 100% killing in past1,2 and 3

Table -8-Mean difference of bacterial colony count (pre and post sterilization) for each adhesive at dilution of 1/100

| Adhesive types | Mean differences | Killing % | SD | 95% Confidence Interval of the Difference | | Paired sample T test d.f..=9 | P-value |
|---|---|---|---|---|---|---|---|
| | | | | Lower | Upper | | |
| powder-1 | 22 | 95.6 | 8.0 | 15.7 | 27.2 | 8.4 | 0.001 |
| powder-2 | 35 | 89.7 | 9.8 | 28.4 | 42.5 | 11.4 | 0.001 |
| powder-3 | 16 | 88.8 | 4.2 | 13.4 | 19.5 | 12.2 | 0.001 |
| past-1 | 6 | 100 | 5.7 | 1.3 | 9.6 | 3.0 | 0.014 |
| past-2 | 16 | 100 | 1.3 | 15.2 | 17.20 | 36.6 | 0.001 |
| past-3 | 22 | 100 | 2.9 | 19.7 | 24.0 | 23.0 | 0.001 |

## DISCUSSION

Denture adhesives are available in formulations of creams, powders, pads/wafers or liquids [27]. Potential benefits of denture adhesives are reported to be improved quality of life, improved chewing ability, increased denture stability, improved comfort and confidence, reduced food collection under dentures, alleviation of denture sore spots, improved mastication and increased patient acceptability of the prosthesis and use in denture post-insertion care [28]

Denture adhesives pastes and powders are the most commonly used . sometimes dentists prescribe a specific type of denture adhesive to be used by the patient while in most instances the patient himself purchase any type of denture adhesives from the market .

In this study an attempt is made to investigate the bacterial contamination in different types of denture adhesives powders and pastes available in markets which most commonly ordered by the patients and the effect of autoclave in sterilization process.

The results of this study discuss that the manufacturing companies of the tested denture adhesives pastes and powders did not undergo well control standardization for their products and this show growth of Gram positive or Gram negative or both , in addition to that there was a difference in the percentage of bacterial growth between different tested denture adhesives pastes and powders and this may be attributed to the difference in their content of antimicrobial agents as hexachlorophene, sodium tetraborate , sodium borate, or propylhydroxybenzoate and ethanol[29-31], in the same time there may be difference in antimicrobial activity of these presetvative materials. Hexachlorophene also known as Nabac is an antiseptic agent. The compound is a white to light tan crystalline powder which is either odorless or processes a slightly phenolic odor. Hexachlorophene is very useful anti-bacterial agent. The action of adding sodium borate is to prevent or retard bacterial growth while propylhydroxybenzoate and ethanol these substances act as preservatives. [32-33]

The Bonyplus (paste type 2) , Fittydent (paste type 3) , PD ( powder type 2) , Fitfix (powder type 3) and Protefix( powder type 1) may have less antimicrobial constituents in their composition when compared to Dentofix paste that has 40% no growth.

The tested adhesives pastes and powders showed presterilization in (Table 2 )that 25% of adhesives were contaminated by *staphylococcus* species . This may be due to the fact that staphylococci are frequent commensal bacteria on the human skin and mucus surfaces , thus are among most likely germs to infect [34] .Also *staphylococcus* have thick cell wall that is essential to cell survival and growth[35] which give advantage to resist killing by antimicrobial agent added to these adhesives .

The results also revealed that 23% of denture adhesives were contaminated by *Nisseria* species . the presence of *Nisseria* species contamination is explained by the fact that *Nisseria* species possess many dynamic polymeric protein filaments called type IV pili, which allow the bacteria to adhere and move along surfaces. The bacterial movement is mediated by twitching motility powered by the extension and retraction of the type IV pilus .Type IV pili are thin filaments that extend from the poles of a diverse group of bacteria, enabling them to move at speeds of a few tenths of a micrometer per second. They are required for twitching motility.[36]

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The finding of the present study presterilization at 1/10 and 1/100 dilutions showed presence of bacterial growth of different species in most of the denture adhesives pastes and powders ,these findings come in agreement with the results of Stafford and Russell [37] About the effectiveness of antimicrobial agent in pastes and powders on the growth of three microorganisms , *Nisseria pharangitis*, *Streptococcus mitis* and *candida albicans* , there results showed

no inhibitory effect was noted for any of the tested adhesives.

Also the results of this study come in agreement with Bartels [38] who tested the inhibitory effect of denture adhesives on *staphylococcus aureus* and *E – Coli* , the bacterial growth was not affected by contact with the denture adhesives.

This study come in agreement with Ekstrand *et al* [39], who tested the microbial contamination in 19 commercially available adhesives incubated for 48 hours in an anaerobic chamber and found that most of the denture adhesives tested were contaminated by bacteria but seven of the brand did not show growth.

The results also come in agreement with Gates *etal and* Maia *etal* [19,40] *who* investigated four denture adhesives and found that not only did each one contain fungal and bacterial contaminants, but also that each one could initiate fungal or bacterial growth when plated on the appropriate media. The percentages of plates exhibited growth were 66%, Some commercially available denture adhesives showed microbial contamination and some had significant inhibitory effect on *C. albicans* growth .

The role of dilution is an apparent in( table 4 and 5) presterilization when the Dentofix ( paste type 1) has mean 12.2 of bacterial colony count at dilution 1/10 and this mean reduced to 5.5 at second dilution 1/100. this may be due to the fact that Dentofix paste is the only adhesive that has percentage of no bacterial growth and the dilution reduces the bacterial colony count , also Dentofix may contain the highest antimicrobial agent in comparison to other tested adhesives. .

The Bonyplus (paste type 2)and Dentofix (paste type 1) contains methyl cellulose in their composition which is white granules, its viscosity increasing by the presence of organic salts [40]. This mean their viscosity increase in NaCl diluents solution and become less dissolve in dilution 1/10 and according to that become less affected by autoclave sterilization with less killing percentages.

While the findings of this study indicated that Fittydent (paste type3 )was the highest respond to sterilization at 1/10 dilution , this may be due to its content of sodium carboxy methyl cellulose which prepared by treating alkali cellulose with sodium chloroacteate, its solubility in water depends on degree of substitution , this water soluble material [41] makes Fittydent paste more soluble in less dilution so has the best dissolution and its diluents was homogenous and autoclave sterilization was most effective , this explained by the highest killing percentage in comparison to other adhesives as shown in (Table 6).

All paste showed 100% killing percentage at dilution 1/100 poststerilization and this reflects the effect of second dilution on reducing bacterial colony count , in addition to increase level of dissolution of Dentofix , Bonyplus and Fittydent pastes at 1/100 dilution , which makes autoclave sterilization more efficient as shown in (Table 8).

The whole tested denture adhesives powders show lower killing rate at both dilutions( 1/10 and 1/100) poststerilization when compared to adhesive pastes and this relates to the composition of powders which mostly contains vegetable gum which are largely carbohydrates that swell to more than their original volume when added to water , it increases the viscosity of water when mixed with . [42,43]its content of carbohydrates make the dissolution of powders in NaCl diluents being weak and according to that the sterilization become less efficient .

The autoclave is sterilizing by heat at 121C˚ under pressure of 15 pounds[44] , The extra pressure in an autoclave means that water boils at a temperature higher than its normal boiling point, roughly 20°C hotter so it holds and carries more heat and kills microbes more effectively. device in its function kills all kinds of bacteria such as fungi, spore forms, viruses and any agent that can transmit bacteria to liquids and equipment in use

## CONCLUSIONS

Bacterial growth were found in most containers of denture adhesives pastes and powders tested . Care should be taken when prescribing and using denture adhesive , the use of autoclave sterilization revealed high killing rate of all types of bacteria were isolated , this study conducted that the pastes adhesives is better sterilized and more recommended to use than the powders.

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