



Effectiveness of the two forms of Drugs and Disinfected Solutions on -Oral Microorganism Adhered to Denture Base Materials

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Abstract

Aims: Microbial adherence to different denture base materials causes denture stomatitis. This study sought to assess the effectiveness of certain antibacterial, antifungal drugs and disinfected solutions. **Materials and methods:** the drugs were applied in two forms: gel and mouth rinse against various types of candida and bacterial strains that adhered to the two types of denture base materials the acrylic-chrome cobalt and the heat cure acrylic resin denture base materials. The microbial strains used in this study were isolated, by using two different agar mediums Sabouraud's dextrose agar was used for *Cryptococcus neoformans*, *Candida famata*, and *Candida albicans* while blood agar includes *Staphylococcus aureus*, *Corynebacterium diphtheriae*, *Pseudomonas aeruginosa*, *Lactobacillus acidophilus*, *Actinomyces Spp.*, *Enterococcus Spp.*, *Streptococcus spp.*, and *E. coli*. The susceptibility of candida and bacterial strains against the three types of drugs was determined using nystatin (Nys) in the form of 100000IU /ml suspension and 15gm ointment, and metronidazole (metro) in the form of 250mg tablet diluted in 100000IU/ml of distilled water and 15g 0.75% gel also the chlorhexidine (CHX) 0.20% in the form of both mouthwash and gel used disk diffusion method. The level of inhibition zones was determined in the median and interquartile range due to the small sample size of strain (n=5). The effectiveness of different drugs was examined in the Kruskal-Wallis test. Nonparametric Comparisons for All Pairs. **Results:** The study showed remarkable outcomes related to the effectiveness of the two forms of the three different drugs against fungi and the bacterial strains associated with the fungi in our samples. **Conclusions:** The finding specifies that the gel forms are the most effective.

فعالية الأدوية والحلول المطهرة على الكائنات الحية الدقيقة عن طريق الفم الملصقة بالمواد الأساسية لطقم الأسنان

الملخص

الأهداف: تهدف هذه الدراسة إلى تقييم فعالية بعض الأدوية المضادة للبكتيريا والفطريات والمحاليل المطهرة. **المواد وطرائق العمل:** الأدوية المطبقة في شكلين: هلام وغسول للفم ضد أنواع مختلفة من المبيضات والسلالات البكتيرية الملصقة بنوعين من المواد الأساسية لأطقم الأسنان: كوبالت الأكريليك والكروم ومواد قاعدة طقم الأسنان المصنوعة من راتينج الأكريليك المعالجة بالحرارة. تم عزل السلالات الميكروبية المستخدمة في هذه الدراسة، باستخدام وسطين أجار مختلفين، تم استخدام أجار سابورود الدكستروز لـ *Cryptococcus neoformans*، *Candida famata*، و *Candida albicans* بينما يتضمن أجار الدم *Staphylococcus aureus*، *Corynebacterium diphtheriae*، *Pseudomonas aeruginosa*، *Lactobacillus acidophilus*، *Actinomyces Spp.*، المكورات المعوية النيابية، العقدية النيابية، والإشريكية القولونية. تم تحديد حساسية المبيضات والسلالات البكتيرية ضد الأنواع الثلاثة من الأدوية باستخدام النيساتين (Nys) على شكل معلق 100000 وحدة دولية / مل ومرهم 15 جم، والميترونيدازول (مترو) على شكل أقراص 250 ملجم مخففة في 100000 وحدة دولية / مل من المقطر. الماء و 15 جم 0.75% هلام أيضاً الكلور هيكسيدين 0.20% (CHX) في شكل كل من غسول الفم والجل يستخدم طريقة انتشار القرص. تم تحديد مستوى مناطق التثبيط في المدى المتوسط والربعي بسبب صغر حجم

عينة السلالة (ن = 5). تم فحص فعالية الأدوية المختلفة في اختبار كروسكال واليس. المقارنات اللامعلمية لجميع الأزواج. النتائج: أظهرت الدراسة نتائج ملحوظة تتعلق بفعالية شكلي الأدوية الثلاثة المختلفة ضد الفطريات والسلالات البكتيرية المرتبطة بالفطريات في عيناتنا. الاستنتاجات: تشير النتائج إلى أن الأشكال الهلامية هي الأكثر فعالية.

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INTRODUCTION

Acrylic-based complete or partial removable dentures are commonly utilized in the treatment of edentulism, especially among the elderly population ⁽¹⁾. Since its introduction in 1937, Polymethyl methacrylate (PMMA) has emerged as the predominant material in the fabrication of removable full dentures. This material offers multiple benefits, such as appealing esthetic qualities, minimal water absorption and solubility, sufficient strength, low toxicity, ease of repair, and a straightforward molding process. Cobalt-Chromium (Co-Cr) alloys, known for their applications in orthopedics and dentistry, are primarily classified as base-metal alloys. Co-Cr alloys are extensively used in dentistry for the fabrication of metallic frameworks for removable partial dentures and, more recently, for the fabrication of porcelain-fused-to-metal restorations and implant frameworks. The rising global interest in using Co-Cr alloys for dental applications can be attributed to their inexpensive cost and acceptable physicommechanical characteristics ⁽²⁾.

Microbial adhesion, the initial phase in biofilm formation, occurs during mastication. This microbial biofilm might be home to opportunistic pathogens including

Candida species, which have been linked to precancerous lesions, as well as gram-positive Streptococci, rods, and gram-negative bacteria ⁽³⁾. Denture stomatitis is one of the most prevalent oral illnesses among people who use dental prostheses. Denture stomatitis, a prevalent oral illness among dental prosthesis users, is characterized by inflammation of the mucosa under the prosthesis. The multifactorial etiology of denture stomatitis encompasses bacterial plaque accumulation, resin porosity, trauma from ill-fitting prostheses, and inadequate oral hygiene, all of which predispose the mucosa beneath the prosthesis to infection ⁽⁴⁾. Approximately 65% of upper full denture wearers are impacted by this lesion. Predominantly located in the palatine mucosa, it is usually asymptomatic, yet may present with symptoms like discomfort, halitosis, itching, and burning sensations ⁽⁵⁾. Post-delivery of removable dentures, patient education, maintenance of cleanliness, and regular monitoring are essential to ensure the proper functioning and to prevent complications in the supporting tissues ⁽⁶⁾. However, the preferred therapy for denture stomatitis consists mostly of hygiene instructions for washing the prosthesis by manual technique, which is frequently and generally adopted by

patients, administration of a topical antifungal, and verification of the need for a new prosthesis ⁽⁵⁾.

The direct application of medication, either as an ointment on the lesion or on the sterilized prosthesis, enhances medicine contact time with the lesions. This results in more effective treatment and faster healing furthermore it has been demonstrated that exposure of oral microorganisms to disinfectants and drug solutions in the form of mouth rinse or Immersion of dentures in disinfectants and drug solution suppresses the adhesion ability of oral microbial to buccal epithelial cells and adhesion to the prosthesis ⁽⁷⁾.

While the direct application of medication as ointment or mouth rinse has been described for a long time, the appropriate therapy has yet to be determined, due to the high rate of relapse. As a result, the goal of this piece has been described for a long time, but the appropriate therapy has yet to be determined, due to the high rate of relapse Consequently, this study aims to determine the most effective techniques against oral microbes on the surfaces of heat-cured acrylic and Cobalt-Chromium dentures, using drugs and disinfectant solutions in forms of ointments or mouth rinses

MATERIALS AND METHODS

Specimen Collection: Both male and female patients were selected randomly attending the prosthodontics department/ College of Dentistry/ University of Duhok. For specimen collection selected ten patients

were acrylic-chrome cobalt partial dentures and heat cure acrylic resin complete dentures denture wearer for one to five years and had denture stomatitis with different intensities according to **ethical approval number: (2-25092022)**. The inner surface of the denture base materials was swabbed using a disposable sterile cotton wool swab. These swabs were then cultivated on Sabouraud dextrose agar and Blood agar plates, with incubation periods ranging from 24 to 48 hours at 37°C, based on preliminary trials that determined optimal growth times for the microorganisms of interest ⁽⁸⁾ then identification for candida and bacterial species was done by observing the morphology and color of the colonies, then staining and microscopically identifying them finally on the Vitek 2 microbial ID/AST ⁽⁹⁾. The isolated single colonies of fungi and bacteria were cultivated using spread plate method on separate agar plates and incubated for 24 hours at 37°C to get pure isolated colonies ⁽¹⁰⁾.

As a result of the colonies isolation of candida and bacteria that accompanied the swab, it includes *Cryptococcus neoformans*, *Candida famata*, *Candida albicans* from the Sabouraud's dextrose, and *Staphylococcus aureus*, *Corynebacterium diphtheriae*, *Pseudomonas aeruginosa*, *Lactobacillus acidophilus*, *Actinomyces* Spp., *Enterococcus* Spp., *Streptococcus* spp., and *E. coli* from blood agar .

The drugs that used in testin:

Nystatin (Nys) 100000IU/ml oral-suspension manufactured by: ACI Pharm

PVT.LTD made in India. Nystatin (Nys) 15g Ointment manufactured by: Gulf Pharmaceutical Industries (Julphar) U.A.E. Chlorhexidine (CHX) 0.20% mouthwash, chlorhexidine (CHX) 0.20% Gel both drugs manufactured by: Laboratorios KIN S.A. DIGONAL 200-Spain. Metronidazole (Metro) 15mg 0.75% Gel 5 manufactured by: Philadelephia Pharmaceuticals/ Amman-Jordan. Metronidazole (Metro) 250mg tablet manufactured by: Julphar Ras Al Khaimah U.A.E, diluted in 30ml or 100000IU/ml distilled water 11 .

Disk diffusion method for testing the susceptibility of fungal strains to the drugs and disinfectant solution:

Preparing thirty petri dishes of Sabouraud agar, ten for each strain five for determination of susceptibility of them to the gel form, and the other five for the solution forms of the used drugs. The disks have been prepared by sterilizing blank disks in the autoclave and then left in six sterilized

closed containers three of them containing Nys, Metro, and CHX agents in gel form and the other three containers containing the three types of drugs in liquid form, the disks immersed inside the containers for about 24 hrs. A loop full of the yeast culture of *Cryptococcus neoformans*, *Candida famata*, and *Candida albicans* was taken and inoculated in three tubes of 4 ml brain heart infusion broth, mixed well. Then the swabs from the broth of each strain were cultured on ten petri dishes Sabouraud agar. After that the prepared disks were placed on the surface of the thirty cultured agars (each five-petri dish of a particular strain has three disks on their surface that are immersed in the solution form of the three drugs and the other five-petri dish of a particular strain have also three disks that immersed in the gel form of the medications) then the thirty petri dishes incubated for 24 hrs at 37°C and the inhibition zone was measured 8 as in Figure1.



Figure 1: Inhibition zones for the antimicrobial gel and solution forms

Disk diffusion method for testing the susceptibility of bacterial strains to the drugs and disinfectant solution

For the determination of susceptibility of bacterial strains to the three types of the drugs in form of both solution and gel by

using disk diffusion method used the same procedure as with the determination of fungi stains but instead of Sabouraud agar Mueller Hinton agars by preparing eighty Mueller Hinton

agars ten for each one of the eight bacterial strains ⁽¹²⁾.

Statistical Analysis

The level of inhibition zones was determined in the median and interquartile range due to the small sample size of strain (n=5). The effectiveness of different drugs on inhibition zones of the fungi and bacteria found in dentures was examined in the Kruskal Wallis test. Nonparametric Comparisons for All Pairs Using the Dunn Method for Joint Ranking was used for pairwise comparisons. The statistical

calculations were performed using JMP Pro 17.1.0 (JMP®, Version 17.1.0. SAS Institute Inc., Cary, NC, 1989–2023).

RESULTS

Susceptibility of Fungi to the three different types of Drugs

The study showed remarkable outcomes related to the effectiveness of the two forms of the three different drugs against fungi, as shown in Table 1 and Figure 2 the highest median of inhibition zones in the gels form of the three medications than in solutions form.

Table 1: Effectiveness of different drugs on inhibition zones of the fungi adhered to denture base materials

Strain (n=5)	Inhibition zone (mm) Median (Interquartile Range)						P
	Chlor-hexidine 0.20% gel	Chlor-hexidine 0.20% mouth-wash	Metro-nidazole 15g 0.75% Gel	Metro-nidazole 250g tablet	Nystatin 15g Ointment	Nystatin 100000IU/ml oral-suspension	
<i>Cryptococcus neoformans</i>	24 (1.5)	13 (2.5)	9 (2)	1 (1.5)	20 (3)	15 (2)	<0.0001
Pairwise comparisons Nystatin 15g Ointment < Metro-nidazole 250g tablet (p=0.0052) Metro-nidazole 15g 0.75% Gel < Chlor-hexidine 0.20% gel (p=0.0059) Metro-nidazole 250g tablet < Chlor-hexidine 0.20% gel (p=0.0001)							
<i>Candida famata</i>	20 (2)	16 (1.5)	8 (2.5)	16 (2)	22 (2)	10 (1.5)	<0.0001
Pairwise comparisons Nystatin 15g Ointment > Metro-nidazole 15g 0.75% Gel (p=0.0002) Metro-nidazole 15g 0.75% Gel < Chlor-hexidine 0.20% gel (p=0.0066) Nystatin 100000IU/ml oral-suspension < Nystatin 15g Ointment (p=0.0043)							
<i>Candida albicans</i>	16 (2)	8 (3)	12 (4.5)	11 (3)	21 (5)	16 (2.5)	0.0001
Pairwise comparisons Nystatin 15g Ointment > Chlor-hexidine 0.20% mouth-wash (p=0.0001) Nystatin 15g Ointment > Metro-nidazole 250g tablet (p=0.0285) Nystatin 100000IU/ml oral-suspension > Chlor-hexidine 0.20% mouth-wash (p=0.0342) Nystatin 15g Ointment > Metro-nidazole 15g 0.75% Gel (p=0.0460)							
Kruskal Wallis test was performed for statistical analyses. Nonparametric Comparisons For All Pairs Using the Dunn Method For Joint Ranking was used for pairwise comparisons.							

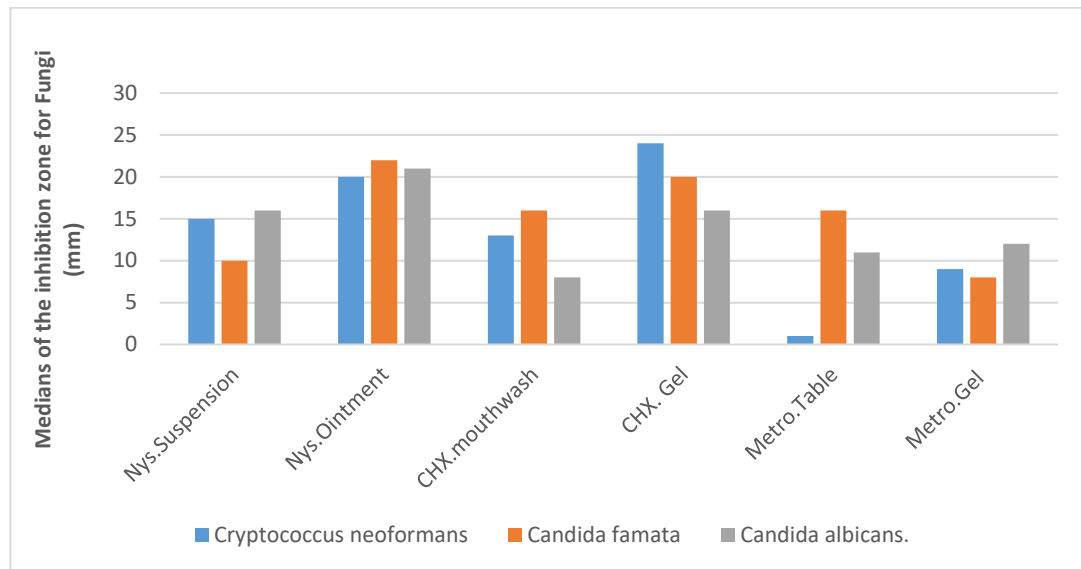


Figure 2: Susceptibility of Fungi to the two forms of three different types of Drugs

Cryptococcus neoformans demonstrated the highest sensitivity to Chlorhexidine 0.20% gel (CHX gel) with a median (Interquartile Range) of the inhibition zone (M= 24mm), followed by nystatin 15g ointment (Nys ointment) with (M=20mm). Also, it appeared to have a good response to the action of both Nystatin 100000IU/ml oral-suspension (Nys solution) with (M=15mm) and chlorhexidine 0.20% mouthwash (CHX solution) with (M=13mm), while its lowest response was to metronidazole 15g 0.75% gel (Metro gel) with (M=9mm) and metronidazole 250g tablet (Metro solution) with (M=1mm). The comparison between them is illustrated in Table.(1)

Candida famata showed high sensitivity to both Nys ointment and CHX gel, with median (M) inhibition zones of 22mm and 20mm, respectively. Whereas the strain showed approximately the same response toward Metro solution and CHX solution with (M=16mm), (M=16mm) respectively.

The lowest sensitivity was shown toward Nys solution and Metro gel with median (M) inhibition zone of (10mm) and (8mm) in order .

Candida albicans exhibited remarkable sensitivity toward the gel and solution forms of Nys with (M=21mm), (M=16mm) in sequence, while the sensitivity of this strain slightly decreased toward CHX gel with Median inhibition zones of (16mm) and prominent decreased toward CHX solution with (M=8mm), whereas the two forms of Metro drug displayed nearly the same effect toward *candida albicans* of (M=12mm), (M=11mm) for both Metro gel and Metro solution respectively .

The pairwise comparisons among all the different drugs toward each fungus are illustrated in Table.(1)

Susceptibility of Bacterial strains to the three different types of Drugs

Tables 2 and Figure 3 summarize our findings, indicating that, in general, the gel

form of the three medications demonstrated greater efficacy against the bacterial strains associated with the fungi in our samples, compared to the solution forms. However, some bacterial strains exhibited resistance towards both forms.

Staphylococcus aureus showed the highest sensitivity toward Metro solution with a median (M) inhibition zone of (24mm) then showed lesser sensitivity to CHX gel with (M=20mm). The sensitivity of the *Staphylococcus aureus* decreased toward CHX solution and Nys ointment with (M=10mm) and (M=9mm) in sequent, while this bacteria showed resistance toward the effect of Metro gel and Nys solution with (M=1mm) and (M=0) respectively .

Corynebacterium diphtheria displayed the greatest sensitivity to CHX gel, with a median inhibition zone of (19mm), followed by Metro solution, with a median of (14mm). While its sensitivity decreased toward the remaining forms of the three drugs, toward CHX solution with (M=9mm), and (M=7mm) toward Metro gel, Nys solution, and Nys ointment.

Pseudomonas aeruginosa showed high sensitivity to CHX gel, with a median of (22mm), and slightly less sensitivity to Metro gel, with a median of (16mm). *Pseudomonas aeruginosa* responsiveness decreased to (15mm) M toward CHX solution and (8mm) M toward Nys ointment.

This bacteria almost showed resistance toward both Metro solution and Nys solution with (M=2mm) and (M=1mm) respectively.

Lactobacillus acidophilus was sensitive to Nys ointment and CHX gel showing means and SDs of (8mm) and (9mm) respectively, also it appeared to be resistant toward Nys solution and Metro gel with medians (1mm) for both. While showed moderate sensitivity toward CHX solution of (M=11mm) and low sensitivity toward Metro solution of (M=7mm) .

Actinomyces Spp. showed resistance to both Nys solution and Metro gel, with medians inhibition zone of (1mm). It was affected moderately by Nys ointment and Metro solution with (M=9mm), and (M=8mm) respectively. The highest response of the bacterial strain was toward the action of CHX solution and gel with a median of (21mm), (14mm) in sequence .

Enterococcus Spp. exhibited notable sensitivity to CHX solution and gel, with means and SDs of (11mm) and (20mm) respectively, while it showed less sensitivity to both Nys ointment and Metro solution with (M=9mm) and (M=8mm) in sequence, this bacteria resisted the effectiveness of both Metro gel and Nys solution with Median of (1mm) for both .

Streptococcus spp. showed the highest response to Nys solution, with a median of (14mm). Whereas Nys ointment, CHX gel, CHX solution, and Metro gel have low effects on this species one by one with medians of (9mm), (8mm), (6mm), and (8mm) respectively. *E. coli* was most sensitive to CHX gel, with a median of (20mm), followed by Metro gel with (15mm). It had lesser sensitivity toward both

CHX solution with (M=10mm) and Metro solution (M=10mm), whereas E. coli showed the lowest response to the activity of Nys ointment with (M=8mm) and resistance toward Nys solution with (M=2mm) .

The pairwise comparisons among all the different drugs toward each bacterial strain are illustrated in Table (2)

Table 2: Effectiveness of different drugs on inhibition zones of the bacteria adhered to denture base materials

	Median (Interquartile Range)						
Strain (n-5)	Chlor-hexidine 0.20% gel	Chlor-hexidine 0.20% mouth-wash	Metro-nidazole 15g 0.75% Gel	Metro-nidazole 250g tablet	Nystatin 15g Ointment	Nystatin 100000IU/ml oral-suspension	P
<i>Staphylococcus aureus</i>	20 (3)	10 (3)	1 (2)	24 (2)	9 (2.5)	0 (1.5)	<0.0001
Pairwise comparisons Metro-nidazole 250g tablet > Metro-nidazole 15g 0.75% Gel (p=0.0023) Metro-nidazole 15g 0.75% Gel <Chlor-hexidine 0.20% gel (p=0.0470) Nystatin 100000IU/ml oral-suspension <Chlor-hexidine 0.20% gel (p=0.0121) Nystatin 100000IU/ml oral-suspension <Metro-nidazole 250g tablet (p=0.0004)							
<i>Corynebacterium diphtheriae</i>	19 (3)	9 (2.5)	7 (3.5)	14 (1.5)	7 (1.5)	7 (2)	0.0004
Pairwise comparisons Nystatin 100000IU/ml oral-suspension <Chlor-hexidine 0.20% gel (p=0.0313) Metro-nidazole 15g 0.75% Gel <Chlor-hexidine 0.20% gel (p=0.0179) Nystatin 15g Ointment <Chlor-hexidine 0.20% gel (p=0.0023)							
<i>Pseudomonas aeruginosa</i>	22 (3)	15 (2)	16 (2.5)	2 (2.5)	8 (2.5)	1 (1)	<0.0001
Pairwise comparisons Nystatin 100000IU/ml oral-suspension <Metro-nidazole 15g 0.75% Gel (p=0.0258) Metro-nidazole 250g tablet <Chlor-hexidine 0.20% gel (p=0.0019) Nystatin 100000IU/ml oral-suspension <Chlor-hexidine 0.20% gel (p=0.0004)							
<i>Lactobacillus acidophilus</i>	9 (2)	11 (3.5)	1 (1.5)	7 (3.5)	8 (2.5)	1 (1.5)	0.0003
Pairwise comparisons Nystatin 100000IU/ml oral-suspension <Chlor-hexidine 0.20% gel (p=0.0454) Metro-nidazole 15g 0.75% Gel <Chlor-hexidine 0.20% mouth-wash (p=0.0058) Nystatin 100000IU/ml oral-suspension <Chlor-hexidine 0.20% mouth-wash (p=0.0022)							
<i>Actinomyces Spp.</i>	21 (3.5)	14 (3)	1 (1)	8 (2)	9 (2.5)	1 (1.5)	<0.0001
Pairwise comparisons Nystatin 100000IU/ml oral-suspension <Chlor-hexidine 0.20% mouth-wash (p=0.0409) Metro-nidazole 15g 0.75% Gel <Chlor-hexidine 0.20% mouth-wash (p=0.0285) Nystatin 100000IU/ml oral-suspension <Chlor-hexidine 0.20% gel (p=0.0011) Metro-nidazole 15g 0.75% Gel <Chlor-hexidine 0.20% gel (p=0.0007)							
<i>Enterococcus Spp.</i>	20 (2.5)	11 (2.5)	1 (1.5)	7 (1.5)	7 (3)	1 (1)	<0.0001
Pairwise comparisons Metro-nidazole 15g 0.75% Gel <Chlor-hexidine 0.20% mouth-wash (p=0.0164) Nystatin 100000IU/ml oral-suspension <Chlor-hexidine 0.20% gel (p=0.0020)							

Metro-nidazole 15g 0.75% Gel <Chlor-hexidine 0.20% gel (p=0.0004)							
<i>Streptococcus spp.</i>	8 (2.5)	6 (4)	8 (2.5)	1 (1)	9 (4)	14 (2.5)	0.0006
Pairwise comparisons Nystatin 100000IU/ml oral-suspension >Metro-nidazole 250g tablet (p=0.0002) Nystatin 15g Ointment >Metro-nidazole 250g tablet (p=0.0377)							
<i>E. coli</i>	20 (5)	10 (2.5)	15 (3)	10 (3.5)	8 (4)	2 (3)	0.0001
Pairwise comparisons Nystatin 15g Ointment <Chlor-hexidine 0.20% gel (p=0.0375) Nystatin 100000IU/ml oral-suspension <Metro-nidazole 15g 0.75% Gel (p=0.0054) Nystatin 100000IU/ml oral-suspension < Chlor-hexidine 0.20% gel (p=0.0001)							
Kruskal Wallis test was performed for statistical analyses. Nonparametric Comparisons For All Pairs Using Dunn Method For Joint Ranking was used for pairwise comparisons.							

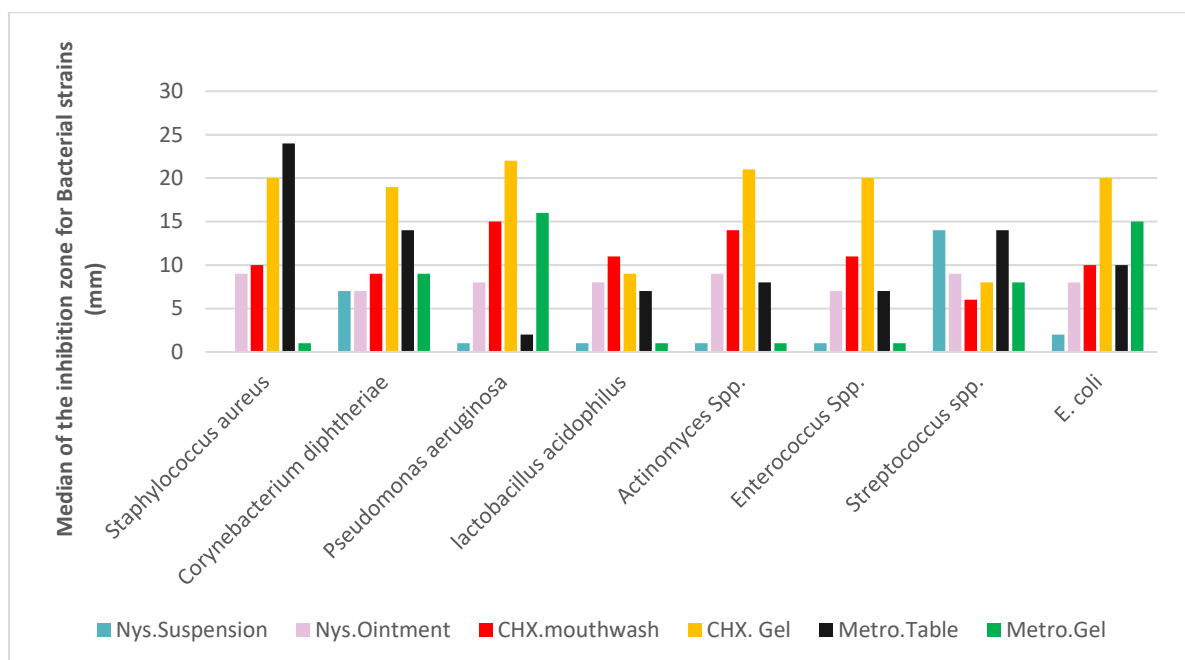


Figure 3: Susceptibility of Bacterial strains to the two forms of the three different types of Drugs

DISCUSSION

Denture stomatitis and palatal inflammation which is similar to oral candidiasis are the most commonly encountered oral infections among denture wearers ⁽¹³⁾. It is critical to practice regular oral hygiene, including a combination of mechanical and chemical methods as the most appropriate choice in terms of denture cleaning ⁽⁵⁾. The most significant aspect in the prevention and

treatment of denture stomatitis is the quality of prosthesis cleaning, which requires frequent inspection in a dental clinic for the evaluation of the prosthetic and the buccal cavity as a preventive measure to avoid and regulate the triggering variable, in addition to prosthesis hygiene guidelines, topical antifungal and antibacterial treatments should be used ⁽¹⁴⁾. As topical medications are favored over systemic medicines owing to the

renal and hepatotoxicity linked to the latter, as well as their efficacy against oral candidiasis ¹⁵.

Nystatin Drug (ointment & suspension) against Candida and Bacterial strains

According to the Nys medication, the ointment form showed the highest effect on the three candida species than the suspension form as Nys is an antimicrobial agent with both fungicidal and fungistatic properties. In addition to its superior efficacy on the three species as compared to metro gel, CHX gels form a close second, this finding advocated the results of Sartawi *et al.* ⁽¹⁶⁾ *et al.*, 2021 and Shaikh *et al.* ⁽¹⁷⁾ they were summarized that nystatin is the standard topical treatment for oral candidiasis, with the total inhibition of both the binding and colonization of *C. albicans*. In addition to that the finding of this article agreed with Czajka *et al.* ¹⁸, 2023 which reported that the topical application of Nys ointment form was the treatment of oral yeast infection associated with *Cryptococcus neoformans* and *Candida famata*. The uncommon presence of nystatin-resistant fungi and its affordable cost are the other advantages of this drug compared to other antifungals, Nys suspension also illustrated higher effectiveness on the three strains of candida than CHX and metro solution, as the means have been shown in the descriptive analysis of about (15, 10.2, 15.8) for *Cryptococcus*, this finding near to that showed by study of Rai *et al.* ⁽¹⁹⁾, 2022 and Labban *et al.* ⁽²⁰⁾ they reported that using nystatin is still the mainstay for the treatment of oral candidiasis, because of its increased efficacy, can be the treatment regimen of

choice for denture stomatitis. Although Nys suspension demonstrated effectiveness toward the three species of candida but slightly less effect than the ointment this agrees with the Lyu *et al.* ⁽²¹⁾, 2016 study which showed also that the efficacy of Nys suspension in treating denture stomatitis was inferior to that of miconazole also Gonoudi *et al.* ²² study showed the greater satisfaction with the use of garlic rather than nystatin in denture stomatitis.

While the two forms of nystatin administration had the least effect on the oral bacteria that were paired with the candida strains, the ointment kind of nystatin had a higher effect than the mouth wash type since most of those bacteria shown resistance against Nys. This finding was acceptable because nystatin has no antibacterial properties which is close to Baldino *et al.* ²³ study that reported Nys alone displayed lower CFU/mL for *Streptococcus mutans* than Chlorhexidine/nystatin formulation. On the other side, the effectiveness of the drug on some oral bacterial strains disagreed with the lack of studies dealing with the same oral bacterial strains found with candida in the swab of wounds rather than in denture stomatitis as in Lotan *et al.* ⁽²⁴⁾ in its case series of 5 patients with nosocomial.

Candida infection of burns and chronic wounds that all patients had wound cultures with *C. albicans*, *Escherichia coli*, *C. glabrata*, *Enterococcus spp.* and *Staphylococcus aureus* growth, the wounds were treated with 100 000 units/mL of Nys and normal saline of mafenide acetate the results were improvement in burn

wound healing and successful graft take but have also shown that Nys was the most effective agent to prevent *C. albicans* from invading deeper tissue that means nystatin should be used in combination with a topical antibacterial agent as it has light activity against bacteria. This slight difference in results was that the recent study took the biofilm from denture stomatitis patients not from wound. From this article's finding and all other studies, the comparison related to bacterial species was difficult because almost all of the articles deal with the candida species as they are prevalent among all microbial with denture stomatitis biofilm but as summarized the outcome illustrates that Nys of its two forms showed a high effectiveness toward candida species and a lower toward various kinds of bacterial species. Besides that, the ointment form occupies a superior effect than the suspension form.

Chlorhexidine gluconate Disinfectant (gel & mouthwash solution) against Fungi and Bacterial strains

Chlorhexidine digluconate is an antiseptic agent with a broad antibacterial spectrum, which acts against Gram-positive and Gram-negative bacteria and some fungi; its formulations have been widely used to chemically control dental biofilm ⁽²⁵⁾. This study found that the two forms of CHX have the same as the two forms of the Nys in their powerful effect in inhibiting the ability of candida species to grow; in addition, the use of CHX in the form of gel exhibited more anti-biofilm activity than mouthwash type as CHX

causes several cellular alterations, such as the fragmentation of the cell wall and the degeneration of the cytoplasm, resulting in the fungicidal effect of the drug. These findings matched Sajjan, *et al.* ²⁶ reviews and Scheibler *et al.* ²⁷ studies concluded that application of CHX gel to the fitting surface of maxillary denture reduced inflammation and significantly reduced fungal activity. Also, rinsing with CHX together with soaking the denture overnight the same solution eliminated *Candida albicans* on the denture surface which is approximately near to the outcome of the gel form toward the three candida strains in this article.

The finding of the recent study disagrees with Akay *et al.* ²⁸ study which concluded that CHX disinfectant solutions weren't meant to be used as the first line of defense against biofilm since their efficiency was less than that of sodium hypochlorite solutions when they evaluated the effectiveness of the most used of antimicrobial agent.

Furthermore, CHX in its two forms shows the highest effect among bacterial species except with *Lactobacillus acidophilus* and *Streptococcus spp.* So, research conducted by Sajjan, *et al.* ⁽²⁶⁾ reviews showed a nearly similar outcome that CHX in the form of mouthwash and gel is effective in reducing the level of microorganisms; it was found to be significantly effective in reducing *Streptococcus*, *Actinomyces*, and other bacterial species related to peri-implantitis. Also, Baldino *et al.* ²³ study matched the current study which concluded that the

combination of the drugs did not produce significantly different results compared to chlorhexidine alone. Considering its broad spectrum of action, chlorhexidine alone was already expected to be efficacious against *S. mutans* and *C. albicans* biofilm. It is worth noting the slight differences in results of the current research with the other research mentioned, as their sampling from oral candidiasis in addition to denture stomatitis while this study from only denture stomatitis. In summary, CHX is not only an excellent antiplaque agent but it also possesses very good antimicrobial properties. Its broad antimicrobial spectrum can be considered a boon for overall oral health. A wealth of research supports its use in various forms in treating denture stomatitis.

Metronidazole drug (gel & mouthwash solution) against Candida and Bacterial strains

In dental practice, metronidazole is the next most widely given antibiotic after amoxicillin. Metronidazole is very effective in treating infections that involve anaerobic or microaerophilic microorganisms because of the mode of action of metronidazole that it passes through the cell target's membrane by passive diffusion, and its nitro group is converted to nitro radicals by ferredoxin or flavodoxin the redox capability of their electron transport components, which are liable for nitro group reduction and the generation of toxic metabolites^{29, 30}. In this study in contrast to Nys and CHX, the use of the metro in the form of mouth rinse showed a moderate effect in reducing both *candida albicans* and *candida*

famata while *cryptococcus neoformans* appear it's resistant against it. Also, the outcome of this article advocated the Sivakumar *et al.*³¹ studies on the use of metro in Pediatric Dentistry which stated that metro is the drug of choice in strictly anaerobic infections and it is a concentration-dependent, not time-dependent, antibiotic, also study recommended that these finding might have better results if used double or triple concentration.

On the other hand, the bacterial strains associated with denture stomatitis of this article demonstrated resistance only toward *Pseudomonas aeruginosa* and *Streptococcus* species. While the drug demonstrated a high effect on all other bacteria, particularly the solution forms the gel form showed less effect and several bacteria showed resistance as *Actinomyces* species and *Enterococcus* species. This outcome appeared to match Wolff *et al.*^{32 studies} in oro-facial infection which reported that metronidazole should not be used to treat actinomycotic infections without added antimicrobial agents since metronidazole is not active against the pathogenic actinomycetes, also, this finding are close to Cooper *et al.*¹¹ review article, deal with treating dentoalveolar infections in a general dentistry practice context, which conclusion is that metronidazole, either alone or in combination, did not produce better clinical outcomes in terms of clearance or symptomatic alleviation when compared to other antibiotics. In summary, because of the poor use of Metro in research related to denture stomatitis, despite the acceptable result in this study for effectiveness against *candida albicans* the drug

in its two forms has a chance to be either an alternative or additional medication in the treatment of denture stomatitis especially if used double or triple concentration.

CONCLUSION

In summary, existing evidence indicates that the eradication of denture stomatitis disease is likely achievable through the topical application (ointment or gel) of antimicrobial agents on both the denture and the mucosal surface. This approach allows direct contact with the inflamed area. Additionally, employing oral rinsing techniques and immersing dentures in antimicrobial solutions hold promise in preventing treatment failure. Subsequently, future investigations should focus on exploring the potential impact of prolonged use of these antimicrobial agents on the physical and mechanical properties of denture base materials.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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