

# Neomycin sulfate: A new antifungal and antiadherent agent against *Candida albicans* cells on heat-cured acrylic resin denture base material. An *in vitro* study

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## ABSTRACT

Neomycin sulfate is an antibacterial agent. In this research, the antifungal and antiadherent effect of neomycin sulfate on *Candida albicans* that colonize the heat-cured acrylic resin denture base material was evaluated.

The results showed a significant effect compared with the control group. This effect is similar to the effect of the known antifungal agents (fluconazol and chlorhexidine) and lesser than (nystatin).

**Key Words:** *Candida albicans*, adherence, neomycin sulfate.

## الخلاصة

يعتبر النيومايسين واحد من المواد المضادة للجراثيم ، وفي هذا البحث تم تقييم النيومايسين من حيث تأثيره المضاد على الفطريات والمضاد لالتصاق خميرة المبيضات البيضاء المستوطنة على مادة قاعدة الطقم من الراتنج الاكريلي والمبلمر حرارياً ، واعطى نتائج معنوية بالمقارنة مع مجموعة السيطرة ومساوية او مشابهة لتأثير المواد المعروفة مثل الفلوكونازول والكلورهيكسيدين وتأثيراً اقل من تأثير النستاتين.

## INTRODUCTION

*Candida albicans* colonization as denture material is widely recognized as the main cause for the developing of denture stomatitis,<sup>(1, 2)</sup> and the adherence of *Candida albicans* on acrylic denture surface is therefore considered as the first step in the pathogenicity of candida-associated denture stomatitis which is the most prevalent form of oral candidiasis in denture wearers.<sup>(3)</sup>

The use of antifungal agents has been shown to play an important role in the control of denture plaque and prevent the candida-associated denture stomatitis.<sup>(4)</sup>

Neomycin is aminoglycosides derivatives: A group of complex chemical compounds, inhibit bacterial protein synthesis.

Aminoglycosides are effective against many aerobic Gram (-ve) and some Gram (+ve) microorganisms.<sup>(5)</sup>

Neomycin is produced by the growth of *Streptococcus fradiae*.<sup>(6)</sup> It has potency equivalent to not less than 600µg of neomycin/ mg and consists almost entirely of a pair of C<sub>23</sub>H<sub>46</sub>N<sub>6</sub>O<sub>13</sub> epimers designed as neomycin B and neomycin C and the ratio of B to C has been observed to vary widely among different production lots. Ointments often formulated as neomycin-polymyxin-bacitracin combination applied to infected skin lesions or in the nares for suppression of staphylococci.<sup>(7)</sup>

The aim of this research is to evaluate the antifungal and antiadherent effect of neomycin sulfate.

## MATERIALS AND METHODS

Neomycin sulfate solution was prepared from the neomycin sulfate powder in the form of active ingredient, which was obtained from SDI (The State Enterprise for Drug Industries and Medical Appliances) according to its potency. The solution was kept at  $25 \pm 1$  °C.<sup>(8)</sup>

The unstimulated whole saliva was collected in clean tubes.<sup>(9)</sup>

The antifungal assay was carried out using broth microdilution method.<sup>(10)</sup> The optical density value was measured using spectrophotometer at 590nm wavelength.

Brain Heart Infusion broth was used in this assay for culturing *Candida albicans* which was isolated on Saboroud's dextrose agar from the inner surface of upper complete denture.<sup>(9)</sup>

The heat-cured acrylic resin samples were prepared and wetted in phosphate buffer saline 24 hours at  $37 \pm 1$  °C before the adherence experiment.<sup>(11)</sup>

The control solutions were used for the comparison as a negative control. The treating solutions were prepared from fluconazole, nystatin and chlorhexidine according to their potencies respectively (64µg/ml, 100,000 IU and 2%).

The antiadherent effect was carried out according to Kassab<sup>(9)</sup> and Taylor *et al.*<sup>(11)</sup> and the results were observed by the fluorescent Microscope (Olympus/Japan).

Regarding the statistical analysis, the experimental design used by the computer program (SAS) was One Way Analysis of Variance at levels of significance 0.01 and 0.05. The means were compared using Duncan's New Multiple Range Test to determine the difference between the studied factors.

## RESULTS

A significant antifungal effect of neomycin sulfate was represented in Table (1) although it was significantly less than nystatin and fluconazole but not significant to chlorhexidine.

A significant antiadherent effect of neomycin sulfate in saliva uncoated and saliva coated acrylic resin samples was demonstrated in Tables (2) and (3).

The statistical analysis by Duncan's New Multiple Range Test demonstrated that all times of immersion were effective in eradicating *Candida albicans* cells that adhered to saliva uncoated (Table 2) and saliva coated acrylic resin samples (Table 3) and there is no significant differences between them.

The results also demonstrated that few cells of *Candida albicans* still remained adhered to acrylic resin samples after their treatment with neomycin sulfate solution as shown in Figure (1).

Table (1): Duncan's New Multiple Range Test for the antifungal effect of the control, antifungal agents and neomycin on *Candida albicans* cells

Drugs/Potency	Absorbance Mean (nm) $\pm$ SD	Duncan's Group
Control (+v) ( <i>Candida albicans</i> culture)	$0.62 \pm 0.2$	A*
Fluconazole (64µg/ml) control (-ve)	$0.15 \pm 0.05$	BC
Nystatin (10,000 IU) control (-ve)	$0.05 \pm 0.004$	C
Chlorhexidine (2%) control (-ve)	$0.26 \pm 0.06$	B
Neomycin (600µg/mg)	$0.3 \pm 0.1$	B

\*Different letters mean significant difference exists.

nm: Nanometer

SD: Standard deviation **Abdul-Rahman GhY, Kassab NH**

µg: Microgram

mg: Milligram

IU= International Unit

Table (2): Duncan's New Multiple Range Test for the antiadherent effect of neomycin on *Candida albicans* cells in saliva-uncoated samples

Acrylic Resin Samples	Time of Imersion (h)	Number of Adhered <i>Candida albicans</i> Cells/mm <sup>2</sup> (Mean ± SD)	Duncan's Grouping*
	Control	7.4 ± 1.9	A
Saliva (-ve) <sup>o</sup>	Neomycin T <sub>1</sub> (1h)	1.14 ± 0.5	B
	Neomycin T <sub>2</sub> (24hs)	0.4 ± 0.3	B
	Neomycin T <sub>3</sub> (48hs)	0.19 ± 0.13	B

\*The different letters mean significant difference exists.

<sup>o</sup>Saliva coated samples.

h: Hour; T: Time; mm: Millimeter

SD: Standard deviation

Table (3): Duncan's New Multiple Range Test for the antiadherent effect of neomycin on *Candida albicans* cells in saliva-coated samples

Acrylic Resin Samples	Time of Immersion (h)	Number of Adhered <i>Candida albicans</i> Cells/mm <sup>2</sup> (Mean ± SD)	Duncan's Grouping*
	Control	29.1 ± 2.7	A
Saliva (+ve) <sup>oo</sup>	Neomycin T <sub>1</sub> (1h)	5.25 ± 0.25	B
	Neomycin T <sub>2</sub> (24hs)	5.2 ± 0.18	B
	Neomycin T <sub>3</sub> (48hs)	5.03 ± 0.2	B

\*The different letters mean significant difference exists.

<sup>oo</sup>Saliva coated samples.

h: Hour; T: Time; mm: Millimeter

SD: Standard deviation

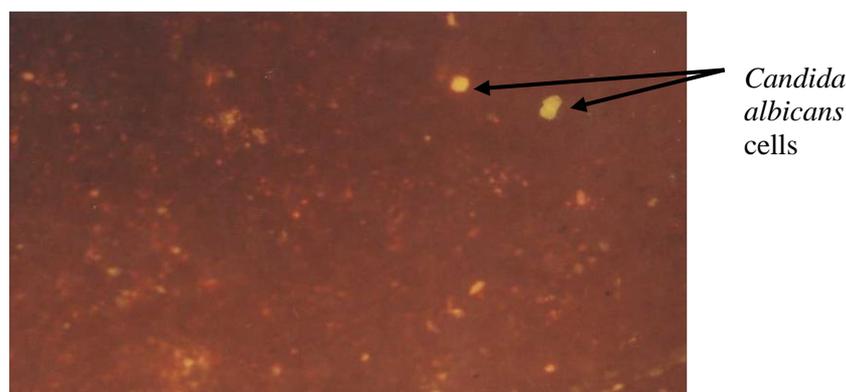


Figure (1): The adherent of cells of *Candida albicans* on acrylic resin samples after treatment with neomycin sulfate at (40×) magnification power

## DISCUSSION

One of the important oral diseases caused by *Candida albicans* is denture stomatitis which is the most common intra-oral abnormality among individuals who wear dentures.<sup>(12)</sup>

The superficial infections caused by *Candida albicans* species may be treated with topical applications of clotrimazole, miconazole, econazole, ketoconazole, oxiconazole, ciclopiroxolamine, nystatin or amphotericin B.<sup>(7)</sup>

The ability to remove or reduce the colonized yeasts and fungal biofilm from acrylic resin may be more important clinically than the basic characteristics of denture cleansers. Therefore, a substance which prevents adhesion and invasion by *Candida* may function as effective antifungal agent.<sup>(13)</sup>

Neomycin sulfate which possesses antibacterial effect against Gram (-ve) mainly and some Gram (+ve).<sup>(14)</sup> In this research the antifungal and antiadherent effect of neomycin sulfate on the *Candida albicans* cells that adhered on acrylic resin denture base material surface was noticed significantly. This effect provokes the idea of using neomycin sulfate in prevention and treatment of denture soaking solution or both of them.

### CONCLUSION

This research represent the first one in prosthetic dentistry which demonstrated the antifungal effect of neomycin sulfate and its ability to eradicate fungal cells especially *Candida albicans* cells that adhered to acrylic resin denture base material surface.

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