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Comparison Between Antibacterial Activity Of Commercial Availabl Neomycin And Pure Synthesis Neomycin

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Dedication

To all people who made this day possible…who believed in us, supported us along the way here, people who always seemed to know their way to hope and so they enrich our world with hope, dreams, wisdom and knowledge and make the world a better place to live.

Abstract

In this research we are studying the activity of aminoglycoside inhibit bacterial growth , we are also will compare between the commercial available Neomycin and pure Neomycin according to their activity and their ability to inhibit growth four different types of bacteria in a several concentrations.

Acknowledgement

An enormous and rather overwhelming debt of gratitude goes to our supervisor Dr. Madher Najem Abdulla.

Especial thanks for Dr. Ayad whom without we couldn’t do all the biological experiments needed in this research.

Finally huge and heartfelt thanks for family and friends for their constant love, support and encouragement over the years.

Contents:

Introduction

This research is to Compare between the antibacterial activity of the commercially available Neomycin and pure Neomycin. It is divided into four sections:
Section one: is about Historical background of Neomycin, Indications,Mechanism of action ,Administration,Adverse Effects,contraindications , Monitoring and toxicity

Section two is about the chemical experiments to prepare stock solutions and dilutions

Section three is about the biological experiments to found the inhibition zone of bacteria against Neomycin
The last Section is about the result and discussion of the research paper depending on the biological and chemical experiments.
This research is based on the hypothesis: Does the pure Neomycin more effective than the commercial Neomycin?

I.THE THEORETICAL PART

1.Historical background of Neomycin

Neomycin is an antibiotic drug that was first discovered in 1949 by microbiologist Selman Waksman and his team at Rutgers University. The discovery of neomycin was part of a larger effort by Waksman to search for new antibiotics from soil microorganisms.

The discovery of neomycin was particularly significant because it was effective against a wide range of bacteria, including many that were resistant to other antibiotics. This made it an important tool in the fight against infectious diseases.

Neomycin was first used clinically in the early 1950s, and it quickly became one of the most widely used antibiotics in the world. It was particularly important in the treatment of tuberculosis, which was a major public health problem at the time.

Over the years, neomycin has been used to treat a wide range of bacterial infections, including those caused by *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. It has also been used in combination with other drugs to treat certain viral infections.

Today, neomycin is still used in medicine, although its use has declined somewhat due to the emergence of antibiotic-resistant bacteria. It is primarily used in topical preparations for the treatment of skin infections, and is also used in some eye drops and ear drops.

(1-2)Indications

Neomycin belongs to a group of antibiotics known as aminoglycosides. Like others in the aminoglycoside family, neomycin works by inhibiting bacterial protein synthesis leading to its bactericidal effect. This group of medications is particularly effective against gram-negative organisms allowing for good coverage of enteric organisms(Jana S, Deb JK,2006) neomycin is poorly absorbed into the systemic circulation, making its use particularly useful within the Gastrointestinal tract.

FDA-approved Indications

Hepatic Coma (portal-systemic encephalopathy): Neomycin is used to manage hepatic encephalopathy (i.e., hepatic coma). Its use is recommended in the acute setting of hepatic encephalopathy rather than chronic due to its side effect profile. (Patidar KR, Bajaj JS.,2013)

- Surgical (perioperative) Prophylaxis: Neomycin is commonly combined with erythromycin or metronidazole as part of Nichols and Condon's bowel preparation

(1-3) Mechanism of Action

Neomycin's mechanism of action is very similar to most aminoglycosides as it binds to the 30s ribosomal subunit interfering with bacterial protein synthesis. The initial steps needed for peptide synthesis are uninterrupted, but elongation fails to occur due to disruption of translational precision. This action thereby disrupts bacteria's translation process, leading to the medication's bactericidal effects.

The pathogenesis of hepatic coma is often due to underlying liver disease leading to elevated ammonia levels in the blood. At high levels, ammonia can cross the blood-brain barriers leading to many of the neurologic manifestations of hepatic coma. In addition, ammonia within the brain leads to increased levels of glutamine and lactate, resulting in neuronal edema. Therefore, therapies aim to decrease ammonia levels by either decreasing ammonia production or increasing ammonia excretion.

Neomycin's bactericidal effects decrease ammonia-producing bacteria residing in the gastrointestinal(GI) tract, thereby decreasing the burden of ammonia on the patient.(Patidar KR, Bajaj JS,2013)

Neomycin is often reserved for patients that cannot tolerate rifaximin. Neomycin is less commonly used due to its more significant side effect profile associated with long-term use. Additionally, one should note that the use of poorly-absorbed antibiotics like rifaximin and neomycin is second-line to synthetic disaccharides (e.g., lactulose, lactitol). In practice, these antibiotics and synthetic disaccharides are often combined.

Due to neomycin's poor GI absorption, it is an effective choice for perioperative bowel prep with the minimal systemic circulation. Neomycin is often combined with erythromycin a day before surgery to decrease bacterial load in the colon to reduce the possibility of surgical site infections. Metronidazole may be an option due to its better tolerability than erythromycin. These antibiotics are part of a regimen in conjunction with mechanical bowel prep and standard IV antibiotic prophylaxis.

There is some controversy about whether these methods of bowel preparation are effective and whether clinicians should use them. A multicentered, randomized, parallel, single-blinded trial conducted in Finland found that when compared to no bowel prep at all, there was no significant reduction in surgical site infections or morbidity when using mechanical and oral bowel preparation for elective colectomies. A 2015 retrospective study found that oral antibiotic bowel preparation significantly reduced surgical site infections, decreased length of stay, and decreased readmission rates.

Pharmacokinetics

Absorption: The lack of absorption from the gastrointestinal tract is the basis of the main use of neomycin as an oral agent to suppress intestinal bacterial flora.

Distribution: As with other aminoglycosides, the quantity of absorbed neomycin

transferred to the tissues increases cumulatively with each dose administered until a steady-state concentration is obtained. The kidney is the primary excretory path of the absorbed drug, and the highest concentration is found in the renal cortex. With cumulative dosings, progressive accumulation also occurs in the inner ear. The release of tissue-bound neomycin occurs slowly over several weeks after discontinued dosing.

Metabolism: There is limited information available on the metabolism of neomycin. It has limited systemic absorption following the drug administration, and metabolism is deemed to be negligible.

Excretion: Neomycin sulfate is excreted primarily through feces (97% of oral dose as unchanged drug).

(1-4)Administration

Neomycin has various routes of administration but has poor GI absorption, like most aminoglycosides (Jana S, Deb JK, 2006) .Oral administration functions to act within the GI tract itself. Alternative preparations include topical use.

Hepatic Coma

One gram of neomycin is administered every six hours for up to six days to treat hepatic encephalopathy.

Surgical (Perioperative) Prophylaxis

One gram of oral neomycin is given with 1 gram of erythromycin base at 2 pm, 3 pm, and 10 pm the day before surgery.

Use in Special Population

Patients with Hepatic Impairment

No dosage adjustments are provided in the manufacturer's labeling.

Patients with Renal Impairment

No dosage adjustments are provided in the manufacturer's labeling. Patients with renal insufficiency can develop toxic blood levels unless doses are properly regulated. If renal insufficiency develops during treatment, the clinician should reduce the dosage or discontinue antibiotics.

Pregnancy Considerations

Neomycin being an aminoglycoside, can cross the placenta. It may cause teratogenicity if administered to a pregnant woman. Hence, neomycin should be given to pregnant women only if needed. It is classified as FDA Pregnancy Category C medicine.

Breastfeeding Considerations

There is a lack of data about the excretion of neomycin into milk; however, other aminoglycoside antibiotics are poorly excreted into breastmilk. In addition, newborn infants absorb small amounts of aminoglycosides; hence systemic effects of neomycin

(1-5)Adverse Effects

According to the package insert, neomycin's common adverse drug reaction is irritation or soreness of the mouth and rectal area. In addition, the side effect profile for the specific drug involves nausea, diarrhea, and clostridium difficile-related colitis. More serious adverse events include nephrotoxicity, auditory ototoxicity, and vestibular ototoxicity (usually irreversible). Neuromuscular blockade is a rare but severe adverse drug reaction induced by neomycin therapy. Therefore neomycin should be avoided in patients with myasthenia gravis.(Elsais A, Popperud TH, Melien Ø, Kerty E.,2013) Methods of decreasing the frequency of such adverse events, especially nephrotoxicity, include once-daily dosing and maintaining adequate hydration status.

(1-6) Contraindications

- Oral neomycin is contraindicated in intestinal obstruction and patients with a prior history of hypersensitivity. Patients with a history of hypersensitivity and serious toxic reactions to other aminoglycosides may have cross-sensitivity to neomycin.
- Neomycin is also contraindicated in patients with inflammatory or ulcerative gastrointestinal disease because of the potential for enhanced gastrointestinal absorption of neomycin.

Boxed Warnings

- Nephrotoxicity
- Ototoxicity
- Neuromuscular blockade and respiratory paralysis
- Neurotoxicity manifested by numbness, muscle twitching, and seizures
- Concurrent use with other aminoglycosides and potent diuretics

(1-7) Monitoring

A baseline serum BUN/creatinine should be obtained with subsequent periodical follow-up blood tests during chronic therapy to monitor for effects on renal function. Since elderly patients may have impaired renal function, which may not be evident in the results of routine screening tests like BUN or serum creatinine, a creatinine clearance determination is more advantageous.

Prompt discontinuation of the drug should occur with any signs of renal or otologic damage. (Wargo KA, Edwards JD,2014) .

(1-8) Toxicity

Nephrotoxicity with the use of aminoglycoside antibiotics occurs primarily through renal tubular toxicity. Additional mechanisms are a decrease in glomerular filtration and a reduction of blood flow to the kidneys. If discontinued, this damage is usually

temporary. Patient-specific risk factors for increased toxicity include age, impaired renal function, and dehydration. In contrast, treatment-specific risk factors are often related to prolonged therapy or high dosage, and they should always merit consideration before administering neomycin. It is also essential to consider concurrent medications that impair renal function, such as NSAIDs, diuretics, iodine contrast media, and other aminoglycosides.

The possibility of ototoxicity is a serious consideration with neomycin, as hearing loss is often permanent. The feared complication is bilateral; high-frequency sensorineural hearing loss is secondary to cochleotoxicity (Guthrie OW.,2008) .The clinician should discontinue therapy with neomycin at the earliest sign of changes in hearing to reduce the extent of cochlear damage. Therefore, it is imperative to inform patients and raise their awareness about the potential side effects of this medication use.

According to the manufacturer's labeling, there is no antidote for neomycin, but hemodialysis can remove neomycin from the blood.

II.PRACTICAL PART

(2-1)Chemistry:

We made a solution with 0.25 g/L concentration and then diluted it to reach a concentration of 128 mg/ml.

c1 * v1 = c2 * v2
250 mg * v1 = 128 * 5
v1= 2.56
D.W =v2 - v1
D.W= 5 - 2.56=2.44ml

To obtain the desired concentrations
that are (128 µg/ml , 64 µg/ ml , 16 µg/ ml , 8 µg/ ml)
we will dilute the solution at a ratio of 1:2.

1)
c1*v1 = c2 * v2.
128 * v1 =64 * 4
v1 = 2
D.W = 4-2 = 2ml

2)
c1*v1 = c2 * v2
128 * v1 =32 * 4
v1 = 3
D.W = 4 -3 = 1 ml

3)
c1*v1 = c2 * v2
128 * v1 =16 * 4
v1 = 0.5
D.W = 4-0.5 = 3.5 ml

4)
c1*v1 = c2 * v2
128 * v1 =8 * 4
v1 = 0.25
D.W = 4-0.25 = 3.75 ml

(2-2) Biology

1) We took bacteria kept in the refrigerator, which are ineffective, and we activated them through food (we took four types of bacteria: *Pseudomonas Staphylococcus aureus, Klebsiella , Escherichia coli*), and we kept them in the incubator at a temperature of 37 C.
2) On the next day, we measured the absorbance of the bacteria solutions using a spectrophotometer, then we diluted the solutions until the absorbance value for all solutions reached 0.5, which means absorbance 0.5 = 1.5×10^6 cells in each cubic milliliter.
3) After that, we dissolved the acara powder in water with heating, in order to increase the solubility, and after the solution was completed, we put it in the autoclave for sterilization, then we put it in the pit reddish
4) In each pit reddish we made 4 holes, in each hole we will inject 100 µl of the drug solution into it to allow time for the drug solution to spread in the agar (in this step we put a label on each pit reddish where it contains information for the concentration of the solution, the type of bacteria and the number of the tril)
5) After two hours or more, we coated the face of the agar with a thin layer of bacteria, then closed the pit reddish and kept it in the incubator for a full day.
6) The next day we measured the inhibition zone around each hole .

Results of the inhibition zone of both the commercial and pure Neomycin:

Commercial Neomycin

		Escherichia coli	*Staphylococcus*	*Pseudomonas*	*Klebsiella*
1	A	14 mm	20	21	15
	B	Zero	14	16	11
	C	Zero	11	14	Zero
	D	Zero	Zero	12	Zero
2	A	14	14	20	15
	B	Zero	15	18	11
	C	Zero	9	16	Zero
	D	Zero	Zero	13	Zero
3	A	14	18	23	15
	B	Zero	15	16	11
	C	Zero	9	14	Zero
	D	Zero	Zero	13	Zero

A =128 µg/ml **B= 64 µg/ ml** **C =16 µg/ ml** **D = 8 µg/ ml**

Original Neomycin

		Escherichia coli	*Staphylococcus*	*Pseudomonas*	*Klebsiella*
1	A	21 mm	25 mm	24 mm	18 mm
	B	14 mm	22 mm	20 mm	16 mm
	C	Zero	15 mm	18 mm	12 mm
	D	Zero	13 mm	17 mm	Zero
2	A	16 mm	27 mm	26 mm	17 mm
	B	14 mm	19 mm	25 mm	14 mm
	C	Zero	16 mm	18 mm	11 mm
	D	Zero	12 mm	16 mm	Zero
3	A	17 mm	23 mm	25 mm	19 mm
	B	12 mm	18 mm	25 mm	15 mm
	C	Zero	16 mm	19 mm	14 mm
	D	Zero	13 mm	17 mm	Zero

A =128 µg/ml **B= 64 µg/ ml** **C =16 µg/ ml** **D = 8 µg/ ml**

The standard deviation :

original neomycin

Escherichia coli

Conc.A: 21 , 16 , 17

To calculate the standard deviation of these values, we can use the following
formula:

σ = sqrt [Σ (Xi - μ)2 / N]

Where:

Xi = Each value in the dataset

μ = The mean of the dataset

N = The number of values in the dataset

Σ = The sum of the values

So, first, we need to calculate the mean of the dataset:

μ = (21 + 16 + 17) / 3 = 18

Next, we can calculate the variance of the dataset:

Variance = Σ (Xi - μ)2 / N

= [(21 - 18)2 + (16 - 18)2 + (17 - 18)2] / 3

= (9 + 4 + 1) / 3

= 14 / 3

Finally, we can calculate the standard deviation by taking the square root of the
variance:

σ = sqrt(14 / 3)

≈ 1.91

Therefore, the standard deviation of the values 21, 16, and 17 is approximately
1.91.

the standard deviation of conc. b: 14, 14, and 12 is approximately 0.94

the standard deviation of conc. c and conc.d : the values 0, 0, and 0 is 0.

This

means that there is no variation in the dataset, as all the values are identical.

Staphylococcus aureus :

Conc. A:

the standard deviation of the values 25, 27, and 23 is approximately 1.63.

Conc.B:

the standard deviation of the values 22, 19, and 18 is approximately 1.83.

Conc.C:

the standard deviation of the values 15, 16, and 16 is approximately 0.47.

Conc. D:

the standard deviation of the values 13, 12, and 13 is approximately 0.47.

Pseudomonas aureus :

Concentration A

the standard deviation of the values 24, 26, and 25 is approximately 0.82.

Concentration B :

the standard deviation of these values is approximately 2.36.

Concentration C :

the standard deviation of these values 18 , 18, 19 is approximately 0.47.

Concentration D :

the standard deviation of these values 17, 16 , 17 is approximately 0.47.

Klebsiella :

Concentration A:

the standard deviation of these values 18, 19, 17 is approximately 0.82.

Concentration B :

the standard deviation of these values 16, 14, 15 is approximately 0.82.

Concentration C :

the standard deviation of these values 12 , 11, 14 is approximately 1.15.

Concentration D :

the values 0, 0, and 0 is 0. This means that there is no variation in the dataset, as
all the values are identical.

commercial neomycin :

Escherichia coli

the standard deviation of all concentration in the three trials is zero.

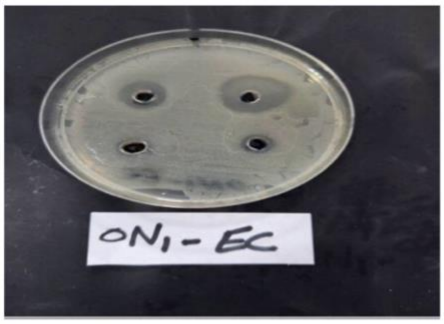
Staphylococcus aureus :

Concentration A: the standard deviation of these values 20, 14, 18 is approximately 2.49.
concentration B : the standard deviation of these values 14, 15, 15 is approximately 0.47.
concentration C : the standard deviation of these values 11, 9, 9 is 1.00.
concentration D : the values 0, 0, and 0 is 0. This means that there is no variation in the dataset, as all the values are identical.

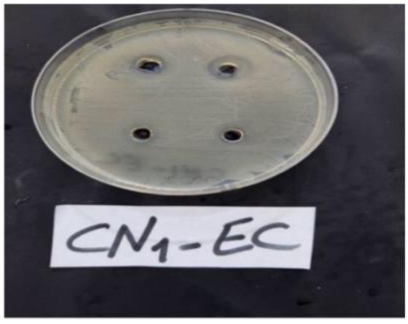
Pseudomonas :

Concentration A: the standard deviation of the values 21, 20, and 23 is approximately 1.1057.
Concentration B : the standard deviation of the values 16, 18, and 16 is approximately 0.9431.
Concentration C : the standard deviation of the values 14, 14, and 16 is approximately 0.9431.
Concentration : the standard deviation of the set {13, 12, 12} is approximately 0.4714.

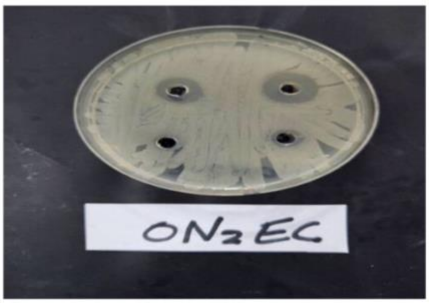
Klebsiella :the standard deviation of all concentration in the three trials is zero.



Original Neomycin trial 1



Commercial Neomycin trial 1



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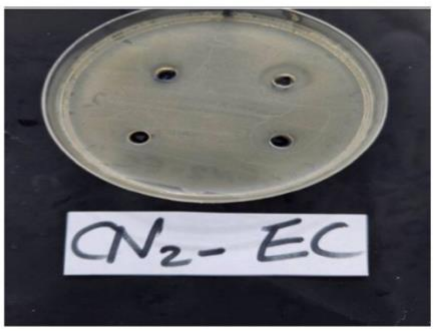

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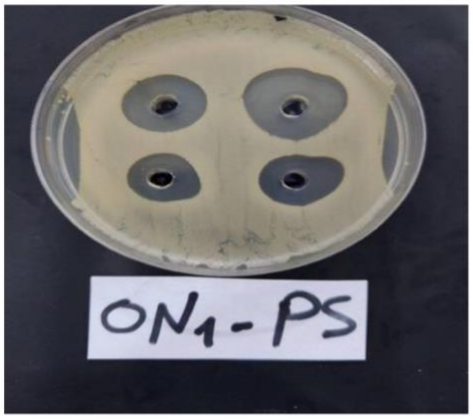

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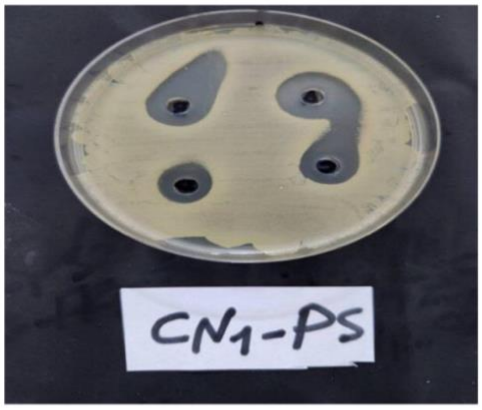

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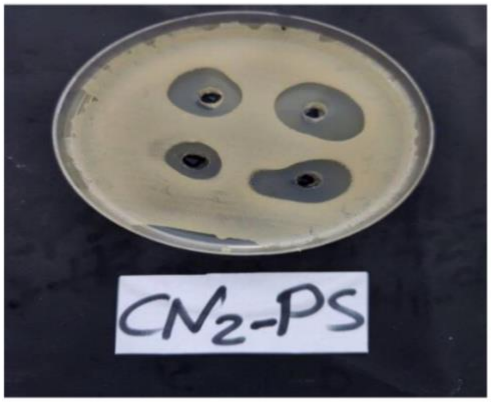
Original Neomycin trial 2



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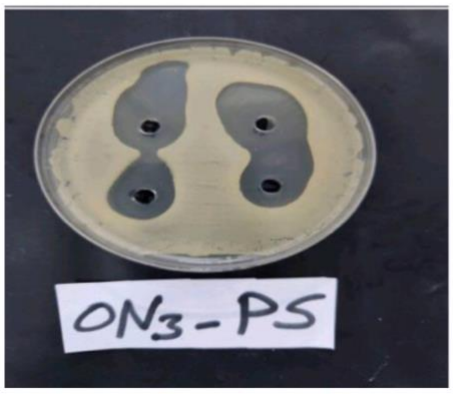
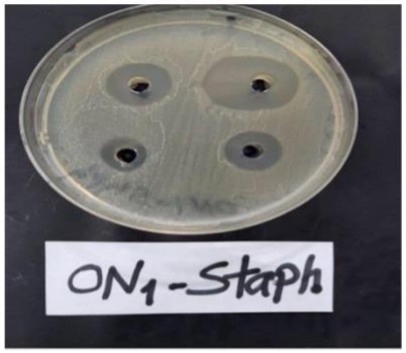
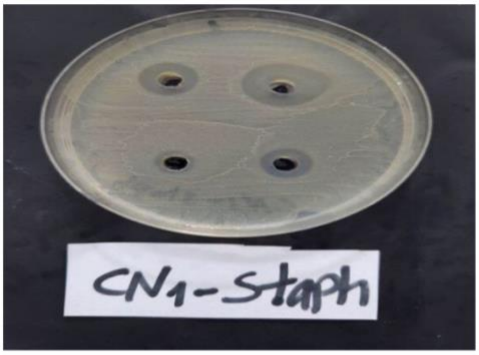


Original Neomycin trial 3

Commercial Neomycin trial 3



Original Neomycin trial 1



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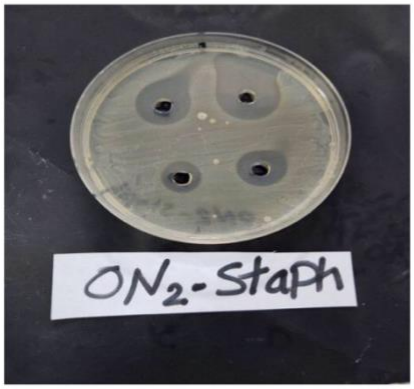
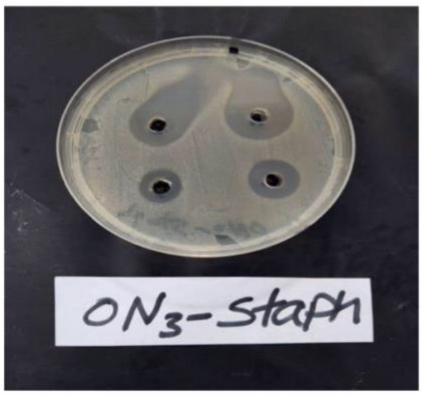
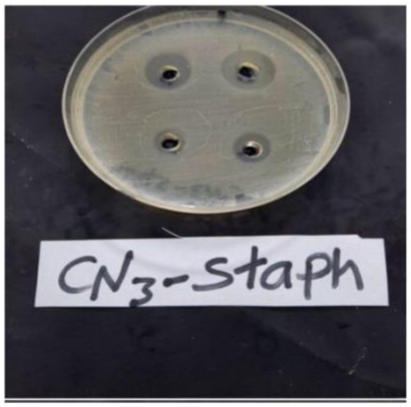


Original Neomycin trial 2

Commercial Neomycin 2



Original Neomycin trial 3



Commercial Neomycin trial 3

Results and Discussion

The data showed that the aminoglycosides have antibacterial activity but it showed very high activity on *Pseudomonas aeruginosa* and high inhibition activity against *Escherichia coli*. Also the data showed that the original Neomycin is more effective than the commercial Neomycin
And it might be for any of the following reasons:
1- Problems in storing conditions and the expire dates
2- Wrong procedure in our experiments led to in accurate data(which is a low possibility because we did three trails just to confirm the results)
3- not accurate concentrations of the drug by the exporting company.

In summary, pure synthesis neomycin is typically more pure This means that there are fewer impurities in the pure synthesis form, which can make it more effective in treating infections. and IT undergoes more rigorous quality control testing than commercial neomycin This can help to ensure that the product is of a high quality and free from impurities. but it is also more expensive and less widely available.

Recommendations

- Neomycin is highly recommended for inhibition of *Pseudomonas aeruginosa* bacteria as it showed high activity on this type of bacteria
- recommendation to companies or who show interest in this subject to do synergistic study by which the Neomycin will be combined with another drug and so the Neomycin role will be working on the cell wall while the other drug will be directed to the DNA and so the the whole combination will help to inhibit bacterial growth in a very short time and with a very small amount of dose .

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