INTERNATIONAL JOURNAL OF PHARMACOLOGY AND THERAPEUTICS
ISSN 2249 – 6467

Research Article

A FIELD SURVEY AND AN INVITRO STUDY OF NEOMYCIN OINTMENTS

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ABSTRACT

The present study was a survey and invitro study of Neomycin ointments. Neomycin and its Combination with other drugs in the form of ointments have been in use for many years for alleviating skin infections. The combinations used along with Neomycin in the present work are Bacitracin, Polymyxin B Sulphate, sulphacetamide sodium, Betamethasone valerate and Hydrocortisone Acetate. We have included a field study of 250 doctors and 250 pharmacists, probing them with related questions and ourselves, determining the product efficiency in our laboratory through an antibacterial assay. Formulation I is a combination of Betamethasone valerate, Neomycin sulphate and Chlorocresol as preservative has been found to be the doctor' favorite and also the most sought after one of the pharmacies, further substantiated by our practical assay that it is the most effective, out of the five formulations under consideration. Formulation III and Formulation V have been ranked second and third against other formulations and synergism in these ointments is definitely present, as we can also see from the results of our experiment. Inference from the present work is that combination of drugs in these ointments is definitely with reason and not just a part of any marketing strategy by the manufacturers.

Key words: Neomycin, Polymyxin B Sulphate, Betamethasone valerate, Antibacterial.

INTRODUCTION:

Neomycin is a wide spectrum amino glycoside antibiotic produced by the growth of *Streptmyces fradiae*[(1)] of the family *Streptomycetaceae*. Neomycin consists almost entirely a pair of C_{23} H_{46} N_6 O_{13} epimers designated as Neomycin-B and

ISSN 2249 – 6467

Neomycin-C [2]. Neomycin and its combination with other drugs in the form of ointments have been in use for many years for alleviating skin infections [3]. The other amino glycoside antibiotics [4] include amikacin, gentamicin, neomycin, streptomycin, and tobramycin. All are bactericidal and active against some Gram-positive and many Gram-negative organisms [5]. Gentamicin, because of its low cost, remains the aminoglycoside of choice in hospitals with low levels of resistance among Enterobacteriaceae . Gentamicin is commonly used for the treatment of infections caused by gram-negative and some gram-positive bacteria [6]. It has been studied in the treatment of peritonitis [7], early-onset sepsis in neonates [8], urinary tract infection [9], febrile episodes in neutropenic patients[10], osteomyelitis[11], and otitis media[12]. Gentamicin is often used in combination with penicillin, vancomycin, or rifampin[13]. Tobramycin hasbeen shown to be more effective in the treatment of infections caused by Pseudomonas aeruginosa[14]. Tobramycin is commonly used to treat pulmonary complications in individuals with cystic fibrosis [15] and has also been studied in the treatment of neonatal bacterial infections [16], peritonitis [17], and renal impairment with bacterial infections[18]. Amikacin, tobramycin are also active against Pseudomonas aeruginosa; streptomycin is active against Mycobacterium tuberculosis and is now almost entirely reserved for tuberculosis[19].

The currently available screening methods for the detection of antimicrobial activity of compounds fall into three groups, including bioautographic, diffusion, and dilution methods. The bioautographic and diffusion methods are known as qualitative techniques since these methods will only give an idea of the presence or absence of substances with antimicrobial activity. On the other hand, dilution methods are considered quantitative assays once they determine the minimal inhibitory concentration [20].

The plethora of neomycin ointment available in the market appeared to us to be a matter of intrigue, as to whether it was really necessary to have so many of them, or was it a part of the marketing strategy of some companies to promote their product using a newer combination [21,-22]. This formed the basis of our study in which we have selected five most important Neomycin ointments available commercially. As part of our work, we have included a field study of 250 doctors and 250 pharmacists in and around Visakhapatnam area, probing them with related questions and ourselves, determining the product efficiency in our laboratory through an antibacterial assay [23].

MATERIALS AND METHODS:

Drugs:

The various commercially available ointments of the antibiotic neomycin, whether in combination or individually were obtained from Apollo Pharmacy.

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Table 1: The various categories of Neomycin or combination of Neomycin with other drugs which were considered for present work are as follows:

Formulation I	Formulation II	Formulation III	Formulation IV	Formulation V
Neomycin sulphate I.P. (0.5% w/w) Betamethasone valerate I.P. (0.12% w/w)	Neomycin sulphate I.P. (0.5% w/w) Chlorocresol I.P. (0.1% w/w)	Neomycin sulphate I.P. (0.5% w/w) Polymyxin B Sulphate U.S.P. (0.5% w/w) Bacitracin zinc I.P. (0.5% w/w)	Neomycin sulphate I.P. (0.5% w/w) Bacitracin zinc I.P (0.5% w/w) Sulphacetamide I.P. (0.2% w/w) Sulphacetamide sodium (0.24% w/w)	Neomycin sulphate I.P. (0.5% w/w) Polymyxin B Sulphate U.S.P. (0.5% w/w) Bacitracin zinc I.P. (0.5% w/w) Hydrocortisone I.P. (0.2% w/w)

Test microorganisms used:

Antimicrobial assays were performed on three species of microorganisms,which include gram positive bacteria *Staphylococcus aureus* , gram negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli* .The test microorganisms were obtained from Microbial Type Culture Collection (MTCC), Santosh hospital, Chennai. All the organisms used were pure cultures, preserved as stab cultures at a temperature of 4^0 C. The tests were per-formed in triplicates for each microorganism evaluated. The final results were presented as the arithmetic average

Media

Liquid Media, Nutrient Broth:

Suspend 8 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2-8°C. The color is amber, slightly opalescent. The dehydrated medium should be homogeneous, free-flowing and beige in color. If there are any physical changes, discard the medium. Nutrient broth [24- 26] consisting of the following ingredients was used to cultivate the organisms obtained from stab cultures

Composition: Peptone (Hi-media labs, Mumbai) 0.5% w/w, Beef extract (Hi-media labs, Mumbai) 0.3% w/w, Sodium chloride (Fischer in organics & Aromatics, Chennai): 0.5% w/w, P^H : 7.2- 7.4.

Solid Media, Nutrient Agar:

It was prepared like exactly nutrient broth and addition of agar to the medium [24- 26]. It was used for the purpose of antibacterial assay. It consists of the following ingredients.

Composition: Agar (Scientific chemicals, Chennai): 1.5% w/w, Peptone (Hi-media labs, Mumbai): 0.5% w/w, Yeast extract (Loba-chemie pvt.ltd. Mumbai): 0.5% w/w, Beef extract (Hi-media labs, Mumbai): 0.5% w/w, Sodium chloride (Fischer in organics & Aromatics, Chennai): 0.5% w/w, P^H : 7.2- 7.4.

Preparation of inoculum

Nutrient Agar was used as the media for culturing of bacterial strains. A loopful of bacterial pure culture was inoculated in 20ml sterile nutrient broth medium in the test tubes aseptically and this process was repeated for all the bacterial strains. The tubes were incubated at 37^0 C for 24 hr. growth was observed in all

the test tubes and this was further used in the experiment.

Antibacterial Activity: Agar Well Diffusion Method

About 1ml of the inoculum was poured in the sterilized nutrient agar media when media attains a temperature of 30-40^{0}c, mixed well, and 20ml of this media was poured in all the petriplates and allowed to solidify and then four wells of 8 mm were made in each petriplate with the help of a sterile cork borer and the wells were filled with the corresponding ointments. Sterile nutrient agar plates served as control. The plates were incubated at 37^{o}C for 24 hrs and the zones of inhibition were measured. The assessment of activity [27- 30] was done on the basis of the zones of inhibition found on Nutrient Agar plates against incubation of organisms with the corresponding ointments. The entire process was carried out aseptically in the laminar airflow. The experiment was performed in triplicate.

FIELD SURVEY METHODOLOGY:

Collection of data from Pharmacist's with a view to comparing with Doctor's opinion

About 250 Pharmacist's were also interviewed to have a true picture of the sales of formulations under consideration. This enabled us to get an overall idea. The questions put across to the chemists were as follows:

Table 2: Questionnaire for Taking Doctor's* Opinion on the Commercially Available Neomycin Ointments

1. What is the specific use of each of these available forms?
2. Would you prefer a cream, a powder, or an ointment? Does this depend on the nature of the ailment or the part of the body affected?
3. Which of the formulation is most widely used?
4. a) If you have to choose one from these five formulations, which would you choose?
   b) What will be the criterion for the choice?
5. Does the name of the manufacturer play a role in the choice of a formulation?
6. Does the cost factor affect the preference of a particular formulation over its counterparts?
7. Do you have any alternatives to Neomycin in the cases in which these five formulations could also be used? If so, what are they?
8. Do you think the combination of Neomycin with other drugs is necessary?
9. Do you think that there is a synergism between polypeptide antibiotics and Neomycin
10. How would you rank Neomycin in an order of 1 to 10

*Number of Doctors met was 250, specializing in various fields, in and around Visakhapatnam city.

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Table 3. Questionnaire for Taking Pharmacist's* Opinion on the commercially available Neomycin Ointments

1.	Which of these five formulations sells best?
2.	Do you stock according to demand or decide it by the data and details listed out by the Marketing Executives?

***Number of Pharmacists met was 250, specializing in various fields, in and around Visakhapatnam city.

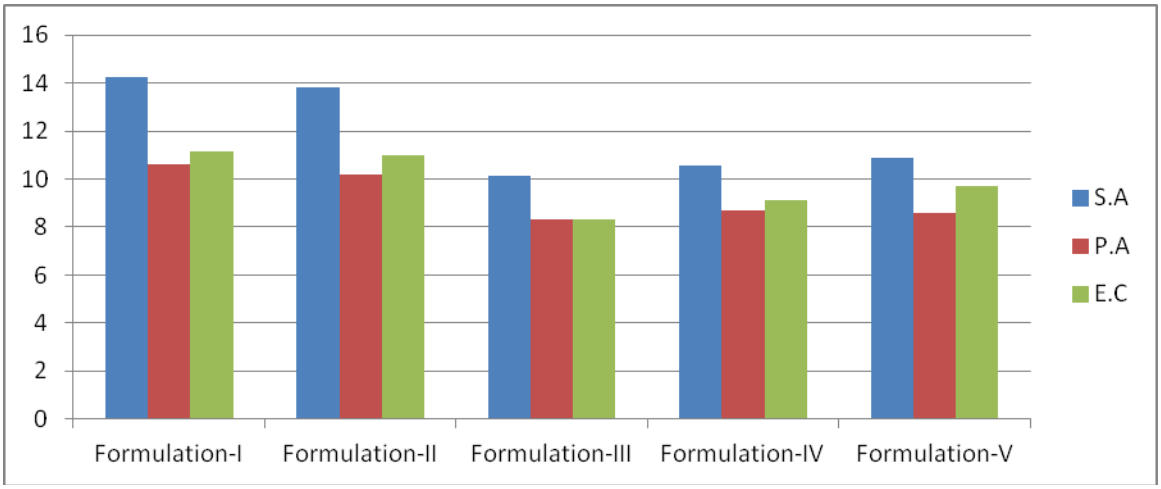

Figure 1 Assay of ointments against different microorganisms

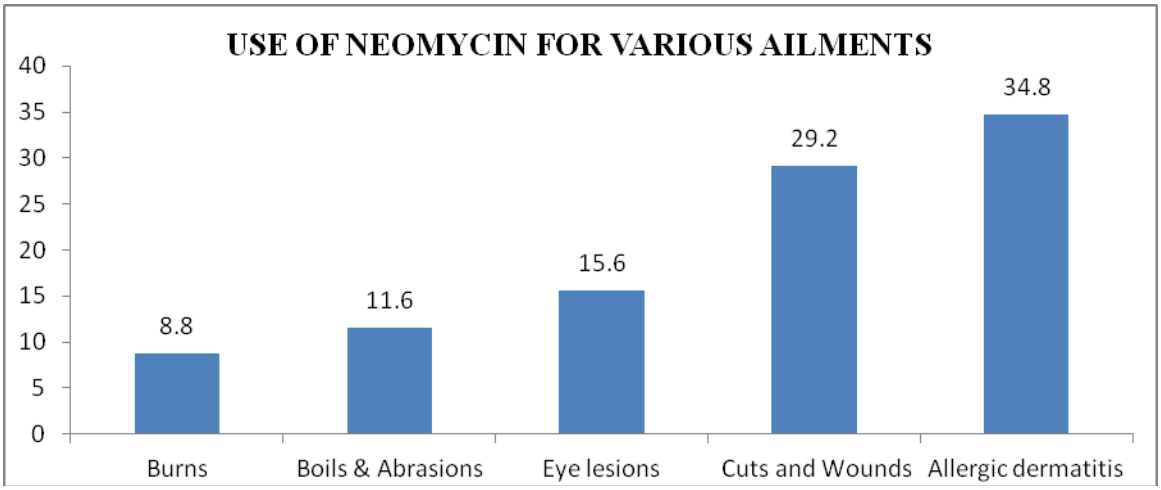

Figure 2 A bar diagram was plotted between % usage of Neomycin on Y-axis Vs Ailments on X-axis.

ISSN 2249 – 6467

Table 4: Assay of ointments against different microorganisms

S. No	Formulation Number	*Staphylococcus aureus* (S. A)	*Pseudomonas aeruginosa* (P. A)	*Escherichia coli* (E. C)
1.	I	14.25	10.63	11.16
2.	II	13.83	10.18	11.00
3.	III	10.13	8.33	8.3
4.	IV	10.55	8.67	9.1
5.	V	10.87	8.59	9.7

(Avg. Zone Inhibition Diameter in mm)

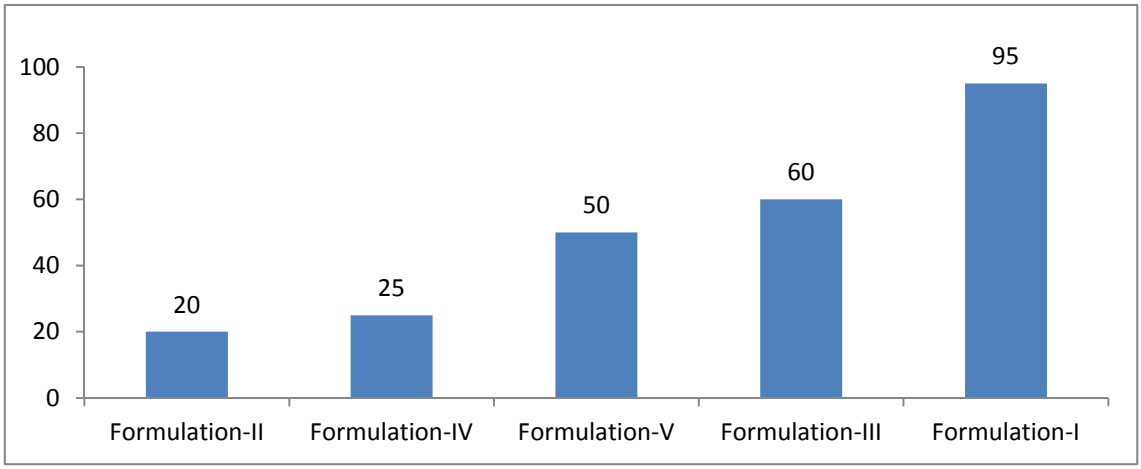

Figure 3. A bar diagram was plotted between No. of Doctors on Y- axis Vs Formulations on X-axis

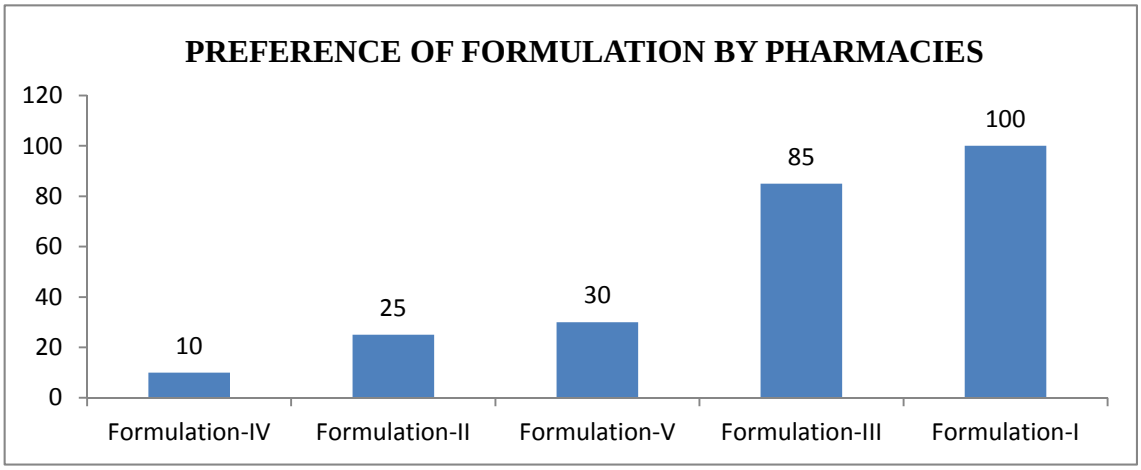

Figure 4. A bar diagram was plotted between No. of Pharmacies on Y- axis Vs Formulations Number on X-axis.

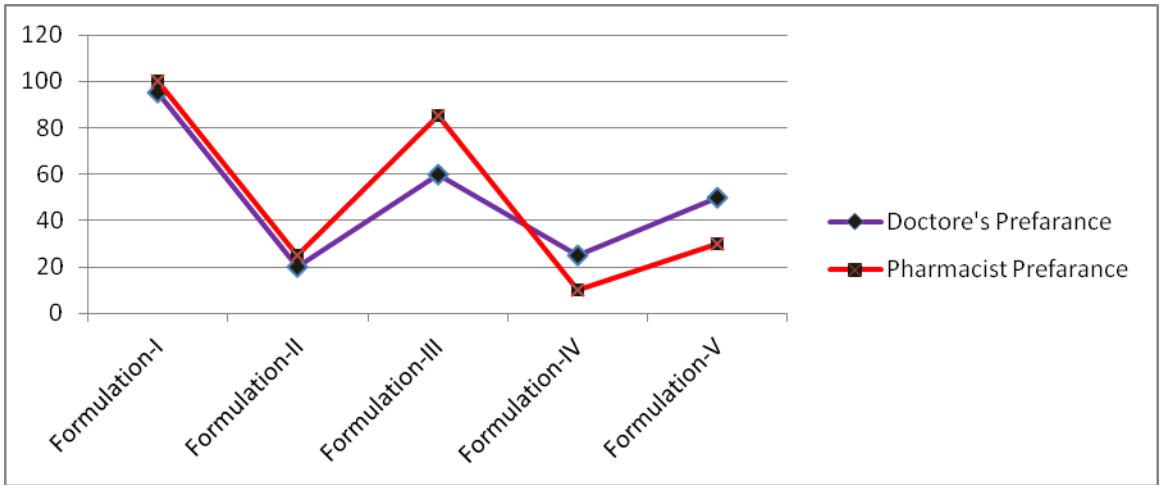

Figure 5 Comparison between Doctor's preference and Pharmacist preference of the five Neomycin Formulations

RESULTS & DISCUSSION:

The present study was a combination of field work and practical experiment. Field work included a survey of about 250 doctors (table 2) and 250 pharmacists (table 3) based on the questionnaire given previously. Interpretation of these interviews with regard to Neomycin ointments under consideration is summarized as follows.

The neomycin based ointments are employed by the doctors[14] for the following ailments namely Cuts and wounds, Infected wounds with inflammation, Allergic dermatitis, Boils and abrasions, Eye lesions, After suturing, Skin ulcers and burns, Conjunctivitis, Itching and eczema (Figure 2). Ointments and creams are preferred for dry lesions, whereas powders are for the treatment of wet lesions.

Formulation I is the most widely used and also the foremost choice of the doctors, followed by III, V, IV and finally by II. Out of the 250 doctors, 95 (38%) were preferred formulation-I, 60 (24%) preferred formulation-III, 50 (20%) preferred formulation-V, 25 (10%) preferred formulation-IV and 20 (8%) preferred formulation-II (Figure 3). The doctors attach importance to the name of the manufacturer in choosing the products. Alternatives mentioned in lieu of neomycin are – Erythromycin, Incamycin, Gentamycin, Framycetin, Mupirocin and Fusidic acid, of which, Gentamycin and Framycetin are seem to be more popular[13]. Combination of Neomycin with other antibacterial drugs [21-22], particularly Polymyxin B sulphate, definitely affects the performance in their options. Price is one of the considerations given by doctors while prescribing the ointments.

Out of the 250 pharmacists, 100 (40%) were preferred formulation-I, 85 (34%) preferred formulation-III, 30 (12%) preferred formulation-V, 25 (10%) preferred formulation-II and 10 (4%) preferred formulation-IV (Figure 4). The order of selling of formulations could be as Formulation I > III > V. Pharmacists also mentioned that they get the stock according to patients demand.

The results of the Antibacterial assay of the Neomycin ointments against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* are shown in the (table 4) & (figure 1). *Pseudomonas aeruginosa is* virtually resistant. Formulation I is the best effective against *Escherichia coli* and

Staphylococcus aureus, followed by II, V, IV, and finally III.

CONCLUSION:

Formulation I is a combination of Betamethasone valerate, Neomycin sulphate and Chlorocresol as preservative. It has been found to be the doctor' favorite and also the most sought after one of the pharmacies, further substantiated by our practical assay that it is the most effective, out of the five formulations under consideration. Polymyxin B sulphate combination with Neomycin in both Formulation III and Formulation V is responsible for the fact that they have been ranked second and third in preference against the other formulations. So synergism in these ointments is definitely present, as we can also see from the results of our experiment.

It can be inferred from this research work that combination of drugs in these ointments is definitely with reason and not just a part of any marketing strategy by the manufacturers. The doctors' opinions and that of the pharmacists and the results obtained in our laboratory are in conformity with one another and this proves why neomycin formulations have stayed and will stay in the years to come.

ACKNOWLEDGEMENTS:

The authors take pleasure in expressing their grateful thanks to the Department of Pharmaceutical sciences, Gitam Institute of Pharmacy, Gitam University, Visakhapatnam for providing us the necessary facilities and work conditions.

REFERENCES:

1. Umezawa H, Hooper IR, Amino-Glycoside antibiotics, Handbook of experimental pharmacology, Vol. 62. Springer, Berlin, 1982, p. 368.
2. Richard P Saik, Carol A Walz, Jonathan E Rhoads, Evaluation of a bacitracin-neomycin surgical skin preparation, The American Journal of Surgery, Vol.121(5), 1971, p. 557–560.
3. Edson RS, Terrell CL, The Aminoglycosides, Mayo Clinic Proceedings, Vol. 74(5), 1999, p. 519-28.
4. Peter A Lio, Elaine T Kaye, Topical Antibacterial Agents, Medical Clinics of North America, Vol. 95(4), 2011, p. 703–721
5. Singhal S, Sharma S C, Singhal K C, Adverse reactions to gentamycin inpatients with ear, nose or throat infections, Indian Journal of Physiology and Pharmacology, Vol. 36, 1992, p. 189–192.
6. Gendeh B S, Said H, Gibb A G, Aziz N S, Kong N, Zahir Z M, Gentamicin ototoxicity in continuous ambulatory peritoneal dialysis, The Journal of Laryngology & Otology, Vol. 107, 1993, p. 681–685
7. Agarwal G, Rastogi A, Pyati S, Wilks A, Pildes R S, Comparison of once-daily versus twice-daily gentamicin dosing regimens in infants > or = 2500 g, Journal of Perinatology, Vol. 22, 2002, p. 268–274
8. Chong C Y, Tan A S, Ng W, Tan-Kendrick A, Balakrishnan A, Chao S M, Treatment of urinary tract infection with gentamicin once or three times daily. Acta Paediatrica, Vol. 92, 2003, p. 291–296.
9. El Bakri F, Pallett A, Smith A G, Duncombe, AS, Once-daily versus multipledaily gentamicin in empirical antibiotic therapy of febrile neutropenia following intensive chemotherapy, the Journal of Antimicrobial Chemotherapy, Vol.45, 2000, p. 383–386.
10. Haydon R C, Blaha J D, Mancinelli C, Koike K, Audiometric thresholds in osteomyelitis patients treated with gentamicin-impregnated methylmethacrylate beads (septopal). Clinical Orthopaedics and Related Research, Vol. 295, 1993, p., 43–46
11. Indudharan R, Valuyeetham K A, Raju S S, Role of glucocorticoids in ototopical antibiotic-steroid preparations in the treatment of chronic suppurative otitis media, Archives of Medical Research, Vol. 36, 2005, p. 154–158
12. Edson R S, Terrell C L, The aminoglycosides: Streptomycin, kanamycin, gentamicin, tobramycin, amikacin, netilmicin, and sisomicin, Mayo Clinic Proceedings, Vol. 62, 1987, p. 916–920.
13. Siegenthaler W E, Bonetti A, Luthy R, Aminoglycoside antibiotics in infectious diseases,

The American Journal of Medicine, Vol.80(Suppl. 6B), 1986, p. 2–14.
14. Bates R D, Nahata M C, Jones J W, McCoy K, Young G, Cox S, Barson W J , Pharmacokinetics and safety of tobramycin after once-daily administration in patients with cystic fibrosis. Chest, Vol. 112, 1997, p. 1208–1213.
15. de Hoog M, van Zanten G A, Hoeve L J, Blom A M, van den Anker J N, A pilot case control follow-up study on hearing in children treated with Tobramycin in the newborn period, International Journal of Pediatric Otorhinolaryngology, Vol. 65, 2002, p. 225–232.
16. Nikolaidis P, Vas S, Lawson V, Kennedy-Vosu L, Bernard A, Abraham G, Oreopoulos D G, intraperitoneal tobramycin ototoxic in CAPD patients, Peritoneal Dialysis International, Vol. 11, 1991, p. 156–161.
17. Gorse G J, Bernstein J M, Cronin R E, Etzell P S , A comparison of genetilmicin and tobramycin therapy in patients with renal impairment, Scandinavian Journal of Infectious Diseases, Vol. 24, 1992, p. 503–514.
18. Waksman S A, Lechevalier H, Neomycin, a new antibiotic active against streptomycin resistant bacteria including tuberculosis organisms. Science, Vol.109, 1949, p. 305.
19. Vanden Berghe DA, Vlietinck A J, Methods in Plant Biochemistry, Screening methods for antibacterial and antiviral agents from higher plants., Academic Press, London, p. 47-69
20. Sydney D Rubbo, Julien Reich, Shirley Dixson, The use of a combination of neomycin, bacitracin, and polymyxin in endodontia, Oral Surgery, Oral Medicine, Oral Pathology, Vol.11(8),1958, P. 878–896.
21. Richard P Saik, Carol A Walz, Jonathan E Rhoads, Evaluation of a bacitracin-neomycin surgical skin preparation, The American Journal of Surgery, Vol. 121 (5),1971, p. 557–560.
22. Smritilekha Bera, George G Zhanel, Frank Schweizer, Antibacterial Activities of Aminoglycoside Antibiotics-Derived Cationic Amphiphiles. Polyol-Modified Neomycin B-, Kanamycin A-, Amikacin-, and Neamine-Based Amphiphiles with Potent Broad Spectrum Antibacterial Activity, Journal of Medicinal Chemistry, Vol. 53 (9), 2010, p.3626–3631.
23. Acar J F, Goldstein F W, Disc Susceptibility Test, Antibiotics in Laboratory Medi-cine, 4th Edition, Williams & Wilkins, Baltimore, 1996, p. 1-51.
24. Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Antioxidant and Antimicrobial Activity of Essen-tial Oil and Methanol Extracts of Achillea millefolium subsp. millefolium Afan. (Asteraceae), Journal of Ethnopharmacology, Vol. 87(No. 2-3), 2003, p. 215-220.
25. American Public Health Association,Standard methods of water analysis, 1923, 5th ed., American Public Health Association, Washington, D.C.
26. Marshall RT, Standard methods for the microbiological examination of dairy products, 16th ed., 1993, American Public Health Association, Washington
27. M.D. Cole, Key antifungal, antibacterial and anti-insect assays—a critical review, Biochemical Systematics and Ecology, Vol. 22(8), 1994, p. 837–856.
28. deserti Derbel S, Bouaziz M , Dhouib A, Sayadi S, Chaieb M , Chemical composition and biological potential of seed oil and leaf extracts of Henophyton, Comptes Rendus Chimie Vol.13(4), 2010, p. 473-478.
29. Davis W W, stout, Disc Plate Method of Microbiological Antibiotic Assay, Applied Microbiology, Vol. 22(4), 1971, p. 659–665.
30. Bera S, Zhanel GG, Schweizer F, Antibacterial activity of guanidinylated neomycin B- and kanamycin A-derived amphiphilic lipid conjugates, Journal of Antimicrobial Chemotherapy, Vol. 65(6), 2010, p. 1224-1227.

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