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Research Article

Stability and Antibiotic Activity Vancomycin Ophtalmic Solution Prepared from Vancomycin Dry Injection Against *Pseudomonas aeroginosa and Staphylococcus aereus*

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ABSTRACT

Vancomycin is Glycopeptide antibiotic relatively unstable in aqueous solution and is not commercially available as eyedrops, so measurement of pH and antibiotic activity are needed[1] . This study aim to assess the stability and the in vitro antibacterial potency of vancomycin eyedrops against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively, under different solvent and storage temperatures Stock solutions of vancomycin 100 mg⁄ml was prepared by reconstituting vancomycin dry injection with water for injection (Aqua PI) or NaCl 0,9% and stored at cold and room temperature. The minimum inhibitor concentrations against *P. aeruginosa* and *S. aureus* were measured to evaluate the antimicrobial potency at consentration 30µg / mL, 45µg / mL and 60 µg/ mL . Changes in the pH values and physical characteristics of the solutions were recorded for evaluate stability of solution Vancomycin 60 µg/ mL has antibacterial effect against *S. aureus* The antibacterial potency of Vancomycin in NaCl 0,9% and in aqua PI solution decreased significantly from day 3 and day 4 respectively, storage temperature affected antibacterial potency. The pH value of vancomycin in NaCl 0.9% solution at a room and cold temperature are 4.68-6.66 and 5,41- 6.66 successively, while in aqua PI solution are 2,8-4.50 at room temperature and 3,51- 4.45 at cold temperatures.

Keywords: *Stabiltiy and antibiotic activity, Vancomycin, ophthalmic solution, Pseudomonas aeroginosa and Staphylococcus aereus*

INTRODUCTION

Eye infection is a red and swollen eye condition caused by microbiological agents such as viruses, fungi, parasites or bacteria. Some cases of eye infections caused by microbiological agents that keratitis, corneal ulcers, endophthalmitis, Anterior Uveitis, and Konjungtivis[2]. Eye infections such as conjunctivitis, keratitis, endophthalmitis, dacryocystitis, blephritis, infection of the eyelids, scleritis microbes, canaliculitis, preseptal cellulitis, orbital cellulitis, endophthalmitis and panophthalmitis etc., lead to increased incidence of morbidity and blindness in the world[3]. Under normal conditions, eye impermeable to agents that come from the environment through the blinking reflex, ocular surface cleaning by mechanical, and prevention of the accumulation of microorganisms. Presence of lysozyme, lactoferrin, secretory immunoglobulin and defensins at high levels in tear can prevent bacterial colonization on the eye[4]. Bacteria and viruses can infect the eye and into the posterior part of the eye in several ways, that occured after intraocular surgery, injury to the eyeball, the spread of bacteria from the site of infection in other places. Endophtalmitis can also be caused by infection of the cornea (keratitis) which may lead to complications[3]. The most common bacteria that cause keratitis is *Pseudomonas aeruginosa* (58.8% of cases) and *Staphylococcus aureus* (11.8% of cases). Infections caused by these bacteria cause damage to the cornea progressively[5]. Majority of ocular infection is caused by gram positive organisms which were susceptible to vancomycin followed by gram negative organisms susceptible to amikacin, fluoroquinolone, gram negative coccobacilli to amikacin and tobramycin, and gatifloxacin effective against both type of organisms[6]. Eye infections, including bacterial keratitis requires antibiotics. Antibiotics used to treat infections of the eye must fulfill the following criteria to produce a sufficiently high concentration of drug in the cornea and is able to maintain the antibacterial effect in the long term. Under conditions of microbes causing the infection is not known, it is recommended to use empirical antibiotic while waiting the germs that cause the infection are identified[7]. There are several drug dosage forms on the eye, one of which is eye drops[8]. Eye drops is sterile dosage form of solution or suspension is used with a drop of drugs on the mucous membranes of the eye around the eyelid and eyeball. Eyedrops must meet the requirements, that a sterile, clear, tonicity, should be comparable with 0.9% NaCl, having pH 4,4 that similar with tear and free from foreign particles. Eye drops should not be used more than one month after the packaging is opened, because possibility of contamination[9]. Limitations of sterile ophthalmic antibiotic regimen that is often made ophthalmologist at

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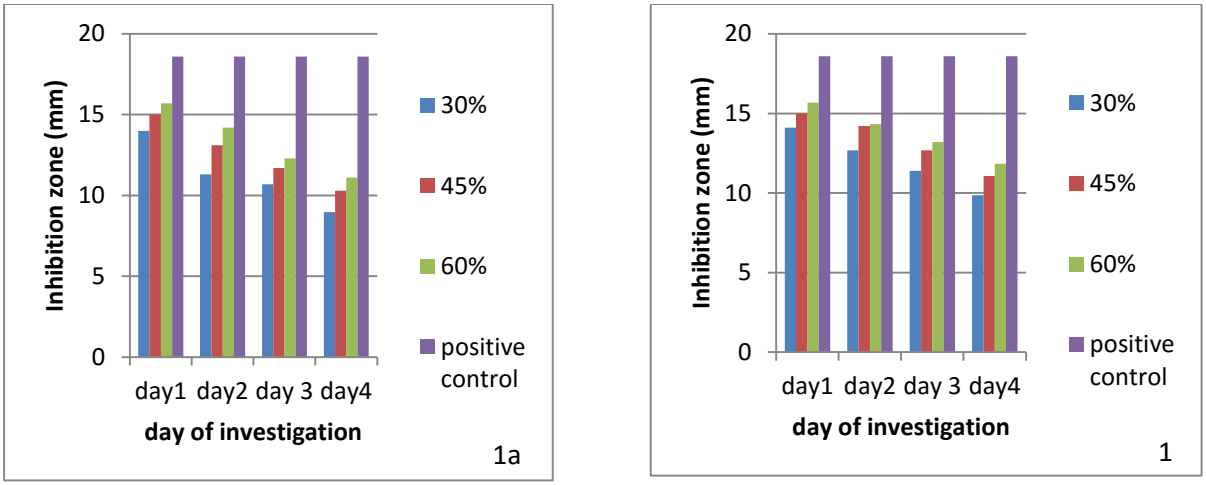

Figure 1: Results of antibacterial activity test (mean of inhibition zone) Vancomycin against *Staphylococcus aureus* in NaCl 0.9% that is stored at room temperature (1a) and cold temperature (1b)

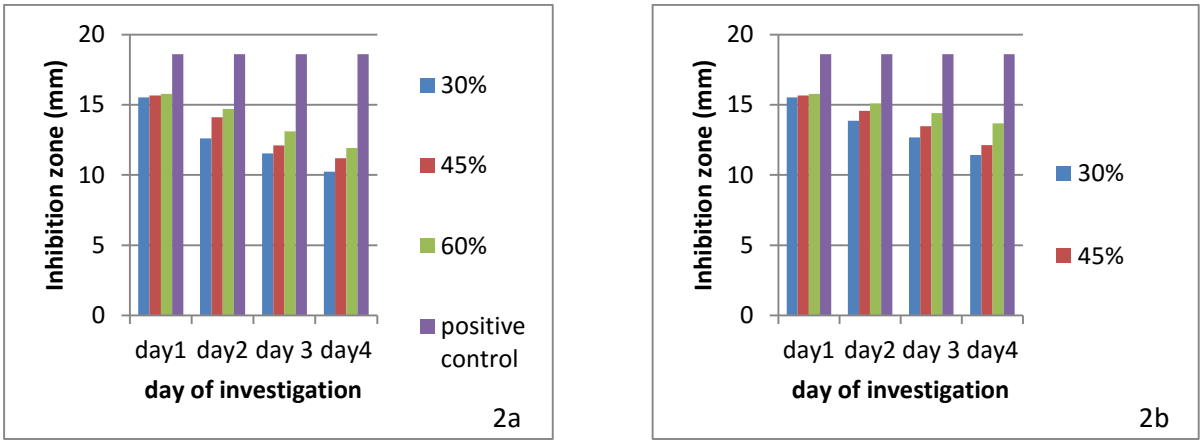

Figure 2: Results of antibacterial activity test (mean of inhibition zone) Vancomycin against *Staphylococcus aureus* in water for injection that is stored at room temperature (2a) and cold temperature (2b)

one of the General hospital in Indonesia prescribe an antibiotic injection was prepared by reconstituting using aqua pro injection or Sodium Chloride 0.9% as ophthalmic preparations. One of antibiotics that is prescribed and used as eye drops is vancomycin for treating the infections due to *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Vancomycin is a glycopeptide class of antibiotics that effective on gram-positive bacteria, works by inhibiting the synthesis of cell wall in bacteria[10]. It has been used for its antibacterial activity against gram positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and resistant enterococci[11]. Injection Vancomycin is often prescribed to be made in the form of eye drops for patients with endophthalmitis. The main problem of the preparation of drugs using water as a solvent is that the tendency interact molecules of drug with water through hydrolysis reaction that makes the dosage form becomes unstable. Therefore, in the manufacture of eye drops need to require special attention in terms of sterility, clarity, pH, and a tonicity value[12]. Antibiotic activity is shown by the effect of inhibition against microorganisms. Decreasing antimicrobial activity could not be demonstrated by chemical methods, so that the microbiological or biological test which is a standard to overcome doubts about the possibility of the loss of antibiotic activity[13].

The way to establish the organism susceptibility to antibiotics by inoculating agar plates with culture and allowing mediated antibiotic diffuses order. The effectiveness of antibiotics will be indicated by inhibition zones[13]. Based on this background it is necessary to investigate whether Vancomycin injection that prepared by reconstituting using aqua pro injection or Sodium Chloride 0.9% as ophthalmic preparations has stability and high potency against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

From the results of the study is expected to provide information on the efficacy of vancomycin ophthalmic preparations that is made by diluting Vancomycin injection with water for injection or NaCl 0.9% against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Results of this study can be the basis of guidelines for the manufacturing and storaging of ophthalmic preparations of` Vancomycin.

MATERIAL AND METHODS

Material

Table 1: Stability test and pH value of Vancomycin ophthalmic solution in aqua PI or NaCl 0,9% stored at cold and room temperature

Solvents	Temperature	Day of testing	Concentration		
			30 µg	45 µg	60 µg
NaCl 0,9%	room	1	6,662	6,662	6,663
		2	6,234	6,234	6,237
		3	5,417	5,418	5,418
		4	4,675	4,677	4,677
	cold	1	6,662	6,662	6,663
		2	6,451	6,452	6,455
		3	5,911	5,913	5,913
		4	5,412	5,412	5,413
Water for injection	room	1	4,497	4,497	4,499
		2	3,825	3,825	3,828
		3	3,135	3,211	3,213
		4	2,810	2,811	2,813
	cold	1	4,497	4,497	4,499
		2	4,076	4,078	4,080
		3	3,903	3,906	3,909
		4	3,511	3,513	3,515

Vancomycin / Vancep® dry injection (0.5 gram Vancomisin), Aqua pro injection, NaCl 0.9%, Nutrient Agar (OXOID) and Nutrient Broth (OXOID), *Water for Injection*(WFI) 25 mL, Natrium Klorida 0,9 % 500 mL, Mueller Hinton (MH) Agar, Cetrimite Agar, Mc Farland standard 0,5, gliserol, analytical grade paper disks (diameter 6 m), Vankomisin 30 µg disc standard, *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853), obtained from the Laboratory of Microbiology, Indonesia University.

Methods

Sterilization of Equipment and Materials

All tools and materials that is used for microbiological testing sterilized using an autoclave at a temperature of 121^0C for 15 minutes. However, for materials made of rubber was sterilized by soaking in 70% alcohol.

Preparation of disks.

Blank, analytical grade paper disks (diameter 6 m) were impregnated with the antibiotic solutions by pipette delivery method. The sterile discs were placed in petri-dishes approximately 5mm apart. Using a mechanical pipettor with a fixed volume delivery of 0.02 ml, the disks were loaded with antibiotic solutions. The disks were allowed to dry in a clean incubator at 35°C for 1-2 hours.

Preparation of Inoculum

Three well-isolated colonies selected from agar plate culture. The top of colony is touched with a loop, and the growth is transferred into a tube containing 5 mL nutrient broth for *Staphylococcus aureus* and Cetrimide for *Pseudomonas aeruginosa* and following incubated at 35^0C[14]. the number of bacteria was adjusted to standard Mc Farland 0.5 (according to the number of bacteria 10^7-10^8 / mL[15].

Preparation of vancomycin concentration

Vancomycin injection vials (500 mg) was obtained from Eli Lilly was dissolved in NaCl 0,9% and aqua PI using aseptic techniques to give concentrations of 100 mg⁄ml for stock solution.

Then the stock solution was diluted with respective solvents to obtain a concentration of 30µg / mL, 45µg / mL and 60µg / mL.

Stability Test

Vancomycin that has been diluted with NaCl 0,9% or aqua PI divided into two treatments, which were stored at room temperature (24^0C) and stored at cold temperatures (20-80^0C). pH measurements were taken from each treatment everyday.

Antibiotic potency test by microbiological assay

Vancomycin antibiotic potency test performed using bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* using Disc diffusion methods. Medium Mueller Hinton (MH) Agar to both bacteria that are still liquid is poured into each sterile petri dish as much as 20 mL and allowed to solidify. Furthermore if the medium has become solid, then made of four zones, each of the two zones to solvent using aqua pro Injection and Sodium Chloride 0.9%. Sterile 6 mm paper disks (Becton Dickinson and Company, USA) with the vancomycin solution in aqua PI or in NaCl 0,9% (30µg / mL, 45µg / mL and 60µg / mL) or positive control (vankomycin) were then placed on the inoculated plates. The plates were incubated at (35 ± 2) °C for 16–24 h. Antibacterial activities were evaluated by measuring the diameters of zones of inhibition in mm against the test organism.

Data analysis

Stability of vancomycin observed by measurement of pH, and the data obtained by antibiotic potency test performed by calculating the diameter of inhibition zone. Data was analyzed with Anova using SPSS 20 for windows..

RESULTS AND DISCUSSION

Results of stability test and pH value of Vancomycin ophthalmic solution was prepared by reconstituting vancomycin dry injection with water for injection (Aqua PI) or NaCl 0,9% stored at cold and room temperature can be seen in Table I.

Ophtalmic solution is sterile solution, especially free from foreign particles, suitably compounded and packaged for instillation into the eye[16]. Preparation of an ophthalmic solution requires careful consideration of such factors as inherent toxicity and isotonicity value, buffering agent, preservative, sterilization and proper packaging were needed to make good ophthalmic preparation[1]. Stability testing ophthalmic preparations began with the number of requests preparation of eye drops from dry injection of vancomycin to aseptic dispensing division. Based on screening of prescription obtained from the aseptic dispensing at a general hospital in Jakarta during January to December 2014, Vancomycin was prescribed as many as 136 sheets. Solvent choice may also important because, as well as influencing antibiotic stability, it can affect patient tolerance according to the level of irritation induced by non-favourable physicochemical properties, including pH. pH is one of the most important factors in the stability of a product. Many pH:stability profiles are published or can be obtained and can be used to determine the pH of maximum stability of a drug. After the pH range is determined, buffers can be prepared to maintain the pH for the expected shelf-life or duration of therapy of the product[16]. pH value of vancomycin in aqua PI solution solution at room and cold temperature was 2.81 - 4.50 and 3,51 - 4,50 respectively, while in NaCl 0,9% was 4,68-6,66 at room temperature and 3,51-4,5 at cold temperature. Study was done by McLellan et al, 2008 showed that pH Vancomycin ophthalmic solution was prepared by reconstituting vankomycin injection with NaCl 0,9% in room temperature was 3,49 at day 0 and 3,71 at day 60[17]. The pH vankomicyn ophthalmic solution with benzalkonium chloride stored at room temperature was 3,59 at day 0 and 3,79 at day 60. Vancomycin is stable over the pH range 3-5[18]. the maximum stability region of vancomycin is pH 3.0-5.7[19]. Ophthalmic solutions are generally formulated in the range of pH 4-8[20]. while the pH value of normal tears is about 7.4. Eye irritation may occur outside the physiological pH range[11]. Results antibacterial activity Vancomycin against *Staphylococcus aureus* in NaCl 0.9% and aqua PI were stored at room and cold temperature can be seen in Figure I and Figure 2, vancomycin did not have antibacterial activity against *Pseudomonas aeeroginosa*. Observations formation inhibition zone was made up until the inhibitory zone is not formed again. The inhibition zone occured only until the fourth day, although on fifth day inhibition zone was formed but very weak potential for antibiotics activity. A good inhibition zone of Vancomycin for antibiotic activity is greater than 12 mm (sensitive)[13]. Figure 1 show the diameter inhibition zones Vancomicyn solution in NaCl 0.9% stored at room temperature and cold temperatures, inhibition zones that were formed on the fourth day at the dose 30 μg are 8.90 mm and 9.80 mm, respectively. Diameter inhibition zones 9 mm or less indicates resistance. While at the dose 45 mg and 60 μg inhibition inhibition zones were fomed in the middle / intermediate[13]. The zones of inhibition for Vancomicyn in aqua PI greater than 10 mm either stored in cold temperature or room temperature, its showed antibacterial activity at dose 30 μg/mL, 45 μg/mL and 60 μg/mL (Figure 2). Vancomycin has antibacterial effect if inhibition zone exhibited greater than 10 mm[13] and according to the Clinical and Laboratory Standards Institute, 2015[21]. vancomycin sensitive againist *Staphylococcus aureus* when diameter inhibition zone greater than 15 mm. Vancomycin sensitive to *Staphylococcus aureus* if the diameter of inhibition zone greater than 15 mm. Thereby Vancomycin that is reconstituting with NaCl 0.9% either stored at room temperature or cold temperatures has efficacy as antibiotics only until fourth day, and can not be used as eye drop again[21]. Vancomycin does not have effect on *Pseudomonas aeruginosa* growth either reconstituting with aqua PI or NaCl 9%, this can be seen from the absence of inhibition zone formed for these bacteria. *Pseudomonas aeruginosa* is a gram-negative bacteria, while vancomycin is only active against gram-positive bacteria[22]. *P. aeruginosa* is a ubiquitous organism present in many diverse environmental settings, and it can be isolated from various living sources, including plants, animals, and humans. The ability of *P. aeruginosa* to survive on minimal nutritional requirements and to tolerate a variety of physical conditions has allowed this organism to persist in both community and hospital settings[23]. Majority of ocular infections are associated with bacterial etiology, which was more due to gram-positive organisms than Gram negative organism. Most of the Gram-positive organisms were susceptible to vancomycin and cefazolin, whereas Gram-negative organisms were susceptible to amikacin and gatifloxacin[3]. According to Riviera and Boucher, 2011 Vancomycin is an antibiotic with activity on Gram-positive spectrum is effective for the treatment of *Staphylococcus aureus* and *Enterococcus infections*[24]. Vancomycin is a glycopeptide; it inhibits early stages in cell wall mucopeptide synthesis and it exhibited greatest potency against ocular Gram-positive isolates[3]. Whereas in the study done by Khosravi A D et al., vancomycin had good coverage 95% against Gram-positive. *Staphylococci aureus* had 100% susceptibility to vancomycin but in in the study done by Khosravi et al, 2007 the isolates of *S. aureus* were resistant to vancomycin. *Coagulase negative Staphylococci* was mostly susceptible (93%) to vancomycin and gatifloxacin[25]. Vancomycin is Glycopeptide antibiotic - originally identified in the 1950, but now widely used due to the increasing incidence of infections due to Gram-positive organisms which are resistant to β-lactam antibiotics. Vancomycin interferes with lipid phosphodisaccharide-pentapeptide complex; no competition between penicillin and vancomycin for binding sites and no cross-resistance. In this study, vankomicyn doesn't have antibiotic activity for *Peudomonas aeroginosa* that can be seen from no inhibition zone was formed. *P. aeruginosa*, which

constitutes 43.7% of the Gram-negative bacteria were highly sensitive towards amikacin (30; 96.8%), ciprofloxacin (26; 83.9%), ceftriaxone (21; 67.7%), doxycycline (17; 54.8%), and chloramphenicol (16; 51.6%)[26]. Vancomycin has antibacterial effect if inhibition zone greater than 10 mm[13]. and according to the Clinical and Laboratory Standards Institute 2015, Vancomycin sensitive againist *Staphylococcus aureus* when inhibition zone greater than 15 mm [2]1. Results from statistical test showed that no significant difference antibacterial potency between positive control and vancomycin ophthalmic solution at dose 60 µg/mL reconstituting with NaCl 0,9% either stored at room or cold temperatures until day 3 investigation and no significant difference between positive control and vancomycin ophthalmic solution at dose 60 µg/mL reconstituting with aqua PI that stored at cold temperature until day 4 investigation.

CONCLUSIONS

Vancomycin ophthalmic solution has antibacterial effect against *S. aureus* The antibacterial potency of Vancomycin 60 µg/mL in NaCl 0,9% and in aqua PI solution decreased significantly from day 3 and day 4 respectively, storage temperature affected stability of pH and antibacterial potency.

REFERENCES

1. Alrich, D S. Ophthalmic Preparations. Ophthalmic Preparations. Stimuli to the revision process 2013; 39(5): 1–21
2. Sherwal BL and Verma AK. Epidemiology of Ocular Infection Due to Bacteria and Fungus – A Prospective Study. JK science 2008; 10(3): 127–131.
3. A Mulla, S., et al. Ocular Infections : Rational Approach To Antibiotic Therapy Correspondence. National J of Medical Research 2012; *2*(1): 22–24.
4. Sack RA, Nunes I, Beaton A and Morris C. Host-Defense Mechanism of the Ocular Surfaces, Bioscience Reports 2002; 21(4), 463–480.
5. Bataineh H, Hammory Q and Khatatba A. Bacterial Keratitis: Risk Factors and Causative Agents. Sudan JMS 2008; 3 (1): 7-10
6. Summaiya M, DN Khokhar and BS Ravdiwala, Ocular Infections: Rational Approach To Antibiotic Therapy. National J of Medical Research 2012; 12(1): 22-24
7. Kowalski PR, Karenchak ML and Romanowski GE. Infectious disease: changing antibiotic suspectibility. Journal of Clinical North Amerika 2003:16:1-9
8. Lukas S. *Steril Formulatin*. Edn 1. Andi: Yogyakarta, 1996
9. Kumar P, Bhowmik D, Paswan S and Srivasta S. Recent Challenges and Advances in Ophthalmic Drug Delivery System. www.thepharmajournal.com 2012; 1(.4): 19-31.
10. Schlossberg, David, and Rafik Samuel. *Antibiotics manual: a guide to commonly used antimicrobials*, People Medical Punlishing House: USA, 2012
11. Khangtragool A. Methocel E4M: Preparation and Properties as a Vehicle for the Ocular Drug Delivery of Vancomycin. Chiang Mai J. Sci 2014; 41(1) : 166-173.
12. Allen V L. Compounding, stability and beyond-use dates. Current & Practical Compounding Information for the Pharmacis 2013; 7 (3): 1-6.
13. Harmita and Maksum Radji. *Buku Ajaran Analisis Hayati*, Pharmacy Departement, Indonesia University, 2005.
14. Lalitha, K,M. Manual of Animicrobial Suspectibility Testing. www.ijmm.org. accessed at 28 September 2015
15. Hendra U. *Penuntun Praktikum Mikrobiologi Kedokteran*, Medical Faculty Indonesia University, Jakarta, 2012
16. Allen V L. Compounding, stability and beyond-use dates. Current & Practical Compounding Information for the Pharmacis 2013; 7 (3): 1-6.
17. McLellan C, Pasedis S and Dohlman HC. Testing the longterm stability of Vancomicyn Ophtalmic Solution. International J of Pharmaceutical Compounding, 2008; 12(5): 456-459.
18. Trissel L.A. Handbook on Injectable Drugs, 14th Edn., Bethesda, MD.: American Society of Health-System Pharmacists, 2007.
19. Mathew M and Gupta VD. Stability of Vancomycin hydrochloride solutions at various pH values as determined by high-performance liquid chromatography, Drug. Dev. Ind. Pharm. 1995; 21(2): 257-264.
20. Ali Y and Lehmussaari K. Industrial perspective in ocular drug delivery. Adv. Drug. Deliv. Rev 2006; 58(11): 1258-1268.
21. Clinical and Laboratory Standards Institute: USA. Performance Standards for Antimicrobial Susceptibility Testing. Twenty-Fourth Informational Supplement 2015; 34 (1): M100-S24.
22. Martindale W and Reynolds JEF. *Martindale: The Extra Pharmacopoeia*, 30th Edn., Singapore: Info Access & Distribution, 1994.
23. Lister,D.P., Wolter, J.,D and Hansoni, D.N. Antibacterial-Resistant *Pseudomonas aeruginosa*: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms. Clinical Microbiology Reviews 2009; 22 (9); p. 582–610.
24. Rivera M A and Boucher WA . Current Concepts in Antimicrobial Therapy Against Select Gram-Positive Organisms: Methicillin-Resistant S*taphylococcus aureus*, Penicillin-Resistant Pneumococci, and Vancomycin-Resistant Enterococci. Mayo Clin Proc 2011; Dec; 86(12): 1230–1243.
25. Khosravi AD, Mehdinejad M, Heidari M. Bacteriological findings in patients with ocular infection and antibiotic susceptibility patterns of isolated pathogens. Singapore Med J 2007; 48 (8) : 741
26. Tesfaye1 T. et al. Bacterial Profile and Antimicrobial Susceptibility Pattern of External Ocular Infections in Jimma University Specialized Hospital, Southwest Ethiopia. American Journal of Infectious Diseases and Microbiology 2013; 1(1): 13-20