

ANTIBIOTIC IMPREGNATED COTTON GAUZES FOR TOPICAL ANTIBIOTIC TREATMENT

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ABSTRACT—Antibiotics are powerful medicines that are used either to inhibit bacterial growth or to destroy bacteria. There are eight different types of antibiotics and the most suitable antibiotic for a particular disease is prescribed by a clinician. Overuse of antibiotics may cause severe problems such as side effects and development of resistance to such an antibiotic by the respective bacteria. Such an antimicrobial resistance is a potent issue worldwide since it demands the termination of the antibiotic and research into discovering new ones. Bioavailability of different types of antibiotics is different and is a critical factor determining the dose. Usual administrative methods such as oral administration or intravenous administration require high doses depending upon the bioavailability of a given antibiotic. As such, topical antibiotic treatment is getting attention of the researchers and is most suited for treating skin diseases and wounds. In this research, we have chosen four different antibiotics, namely, Cefuroxime, Azithromycin, Metronidazole and Flucloxacillin and 20 mg each of these drugs were dissolved, separately, in 20 mL of ethanol and the antibiotics were separately adsorbed to sterilized cotton gauzes and stored in sterilized plastic bottles closed with lids. Their antimicrobial efficacies on Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* were studied after one week of gauze preparation. Another set was aged under ambient conditions, in plastic bottles closed with lids, and their antimicrobial efficacies were also investigated. Azithromycin shows the largest inhibition zones for both bacteria but better performance for *Staphylococcus aureus* in both cases. Metronidazole and Flucloxacillin show similar inhibition zones for both bacteria in week old samples but Flucloxacillin does not show any activity when aged for 22 months. Cefuroxime shows the same pattern as does by Azithromycin. These findings are explained with the help of mechanisms of antimicrobial action of the antibiotics on bacterial species.

Index Terms— Antibiotic impregnated cotton gauzes, Azithromycin, Cefuroxime, Flucloxacillin, Gram-positive and Gram-negative bacteria, Metronidazole, Topical antibiotic treatment

1. INTRODUCTION

Antibiotics are medicines that inhibit the growth of bacteria or that destroy bacteria. These medicines are used to treat infections or diseases caused by over hundreds of bacterial species that cause various illnesses. Although, there are a large number of antibiotics available in the market, they have been derived from a few types. These types include Penicillins such as penicillin and amoxicillin (1), Macrolides such as erythromycin (E-Mycin) (2), clarithromycin (Biaxin) (3), and azithromycin (Zithromax) (4), Fluoroquinolones such as ciprofloxacin (Cipro), levofloxacin (Levaquin), and ofloxacin (Floxin) (5), Sulfonamides such as co-trimoxazole (Bactrim) and trimethoprim (Proloprim) (6), Tetracyclines (7) such as tetracycline (Sumycin, Panmycin) and doxycycline (Vibramycin) and Aminoglycosides such as gentamicin (Garamycin) and tobramycin (Tobrex) (8). Different antibiotics are effective against different bacterial species and hence proper choice of correct antibiotic to a particular bacterial infection is mandatory and it should be done by a clinician. Having selected the right antibiotic for a particular type of bacterial infection, next important issue is its proper

administration. At the beginning of the antibiotic era, antibiotic therapy was to administer them orally and oral administrations of sulfonamides, penicillins, tetracyclines, and chloramphenicol (9) are typical examples which have been proven to have adequate antibiotic levels in the blood of the patients. However, some antibiotics are acid-labile and hence they are destroyed in the liver while some other antibiotics are less soluble and hence they should be administered intravenously (IV). IV administration generally requires the hospitalization of the patient even for a mild infection and hence this mode of administration is not usually preferred for such cases (9). However, for severe cases where hospitalization is necessary, clinicians prefer the IV mode of administration which is the safest and quickest way to initiate antibiotic treatment. Another route of antibiotic administration is by topical means where the antibiotic is directed to the site of the infection. Topical antibiotics, which are medications applied to skins, are best suited to prevent infections caused by bacteria that get into minor cuts, burns and scrapes. Topical antibiotics (10) are available in many forms which include creams, ointments, powders, and sprays. Bacitracin (11), neomycin (12), mupirocin (13), and polymyxin B (14) are some commonly

available topical antibiotics. Topical antibiotics are particularly attractive for treating many primary cutaneous bacterial infections but they may also be used in treating secondarily infected eczematous dermatitis, such as atopic dermatitis, nummular dermatitis, or stasis dermatitis. Topical antibiotic treatments are of best choice in infection prophylaxis in superficial skin wounds, particularly when used with a dressing that occludes the wound. In fact, medicated gauze dressing impregnated topical ointment containing Neomycin, Polymyxin and Bacitracin, which are synergistic, are commercially available for wounds with high risk of infections. Topical antibiotic treatment has the advantage that the antibiotic is not distributed all over the body but directed only to the required site thus having minimum bio-burden, negligible absorption through intact skin, absorption of wound discharges allowing easy drainage of wound exudates and permitting ventilation to the area, possibility of having multiple antibiotic combinations, low risk of cross resistance facilitating inhibition of bacterial repopulation or resident epidermal flora after eradication of bacteria from stratum corneum, negligible maceration of the lesion and the possibility to have high antibiotic concentration at the wound site. These antibiotic wound dressings are recommended for burns and scalds, donor and recipient graft sites, skin loss wounds, lacerations, abrasions, abscesses, pressure sores, ulcers and for infected lesions caused by susceptible bacteria.

For the antibiotics that have been administered either through IV or orally the bioavailability is an important criterion determining doses. Bioavailability is defined as the percentage of a dose of the drug that reaches the systemic circulation. The bioavailability of antibiotics administered through IV is 100%, particularly, for rapid IV administration for antimicrobial therapy of a patient having a severe infection. Some antibiotics such as the fluoroquinolones, metronidazole, tetracycline, minocycline, doxycycline, linezolid, and trimethoprim-sulfamethoxazole have good bio-availabilities and hence for these drugs oral (PO) and IV doses are similar (15). However, antibiotics such as penicillin G that is susceptible to gastric acid has less than 30% bio-availability, penicillin V that is more stable towards gastric acids has around 60%-70% bio-availability and amoxicillin offers a greater oral bioavailability of 74%-92%. Bioavailability of oral ampicillin is only 30% to 55%. Oral cephalosporins, such as cefaclor, cefadroxil, cefprozil, cephalexin, ceftibuten, and loracarbef (technically a carbacephem), are stable towards gastric acids and hence they have high bioavailability of 80%-95% but the bioavailability of cefixime is much lower at 40%-50%. Besides, foods taken may stimulate the gastric acid secretion and hence affect the bioavailability of acid-labile antibiotics such as penicillin G. The bioavailability of erythromycin and azithromycin is only about 40% and it is further lowered in

the presence of food. Interaction of certain drugs such as fluoroquinolones and tetracyclines that can chelate with metal ions such as Al^{3+} , Ca^{2+} and Mg^{2+} leads to lower absorption and hence lower bioavailability. Even for antibiotics with high bioavailability, their absorption can be impaired for patients having lower blood circulation due to diseases such as hypotension. Factors such as ileus, colitis, bowel ischemia, and changes in gastric pH can also influence the gastrointestinal drug absorption. As such, oral administration demands high doses of antibiotics to meet with required levels of their bioavailability. Frequent intake of high doses of antibiotics has detrimental effects such as increased risk of side effects and adaptation of microbes to these antibiotics. Antibiotic resistance of pathogens is a serious problem demanding the termination of such drugs and the discovery and use of new ones. However, discovery of new antibiotics does not happen at the rate of developing antimicrobial resistance to currently used antibiotics.

Most of these problems may be eliminated by not administering antibiotics orally or by means of IV but by impregnating them in suitable matrices such as sterilized cotton gauzes and using them to treat wound sites by directly applying them on the wounds. In this research, we have chosen four antibiotics, namely, (i) Cefuroxime {3-(carbamoyloxymethyl)-7-[[2-(furan-2-yl)-2-methoxyiminoacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid} which is a broad spectrum antibiotic with 37% bioavailability when taken under fasting conditions but with 52% bioavailability when taken after foods and is used for treating infections with gram-negative and gram-positive organisms, gonorrhoea, and haemophilus, (ii) Azithromycin {(2R,3S,4R,5R,8R,10R,11R,12S,13S,14R)-11-[(2S,3R,4S,6R)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy-2-ethyl-3,4,10-trihydroxy-13-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,6,8,10,12,14-heptamethyl-1-oxa-6-azacyclopentadecan-15-one} with 37% of bioavailability and food independent absorption, which is used to treat mycobacterium avium intracellular infections, toxoplasmosis, and cryptosporidiosis and so on (iii) Metronidazole (2-methyl-5-nitro-1H-imidazole-1-ethanol) used to treat amebiasis, trichomonas infection, vaginitis, giardiasis, anaerobic bacteria and Treponema infections and also used against protozoa such as Trichomonas vaginalis, amebiasis, and giardiasis, and (iv) Flucloxacillin {(2S,5R,6R)-6-[[3-(2-chloro-6-fluorophenyl)-5-methyl-1,2-oxazole-4-carbonyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid}, a penicillin beta-lactam antibiotic used in the treatment of bacterial infections caused by susceptible, usually gram-positive, organisms. These antibiotic sterilized cotton gauzes were separately prepared and their antimicrobial efficacies were evaluated for Gram-negative and Gram-positive bacteria after a week

of preparation and after 22 months of preparation. In this manuscript, we describe the preparation of antibiotic impregnated cotton gauzes and their antimicrobial activities. We propose that this method is superior for wound healing than oral or IV administration since the amount of antibiotic requirement is far less in this method than that is required in conventional methods. This method also facilitates lowered side effects of drugs and maintains only the required doses only at the site of the wound. This method would also lead to lowered adaptation of microbes to antibiotics thus eliminating antimicrobial resistance.

There is a recent report on the development and characterization of cotton and organic cotton gauze coated with biopolymers such as chitosan and antibiotic drugs for wound healing (16). They have impregnated large amount of antibiotics (250 mg) per gauze and the antibiotics used are tetracycline hydrochloride, chloramphenicol and rifampin and tested against *S. aureus* and *Proteus* bacteria and found to have excellent antibiotic performances. Apart from this, there are no such reports on antibiotic-impregnated cotton gauzes and our study is the first of such for the four antibiotics which we have chosen.

2. EXPERIMENTAL

2.1. Preparation of Antibiotic-impregnated Cotton Gauzes

Antibiotic solutions were prepared by dissolving 20 mg of each antibiotic (Azithromycin, Flucloxacillin, Metronidazole and Cefuroxime) separately in 98% ethanol, under ambient laboratory conditions. Cotton gauzes used were sterilized and 1 cm x 1 cm pieces of cotton gauzes were separately dipped in antibiotic solutions overnight. And then they were taken out of the solutions and kept in labelled, sterilized, separate plastic containers with closed lids. A set of samples were tested for antimicrobial efficacy after one week. The other set was kept in the plastic containers for 22 months. At the end of 22 months, the second set was also tested in order to investigate the durability of the antimicrobial efficacy of antibiotic-coated cotton gauzes. A sterilized plain cotton gauze was used as the control. *Escherichia coli* and *Staphylococcus aureus* were used in this study to represent, respectively, Gram-negative and Gram-positive bacteria.

2.2. Preparation of Bacterial Sub-culture

Parallel Streak Plate Method

A portion of 15.0 ml of sterile Muller Hinton agar (Critrion, USA) was poured into petri plates and allowed it to solidify in the petri plate. Single colonies of *E. coli* and *S. aureus* were gently touched by a sterilized wire loop and 8.0

cm long parallel lines were streaked on the surface of the agar plate. The cultured petri plates were incubated in an incubator (SLS, Britan) at 37 °C for 24 hours.

2.3. Preparation of Bacterial Culture Broth

5.0 mg of Peptone (Winlab, UK), 0.08 g of sodium chloride (Loba Chemie, India) and 0.03 g of yeast (Winlab, UK) were dissolved in distilled water (10.0 ml) and autoclaved. A loop of fresh colonies of *E. coli* and *S. aureus* were separately inoculated in the prepared nutrient broth and incubated in water bath shaker (Stuart, UK) at 37 °C for 24 hours.

2.4. Antibacterial Assessment

15.0 ml of Muller Hinton agar (Critrion, USA) was poured into sterilized petri plates and was allowed to solidify at room temperature. 500 µl of bacterial nutrient broth was drawn and spread evenly on to the Muller Hinton agar plate. Sets of four antibiotic-coated gauze samples were taken and checked the antibiotic activity by using agar diffusion method and a sterilized pure gauze sample was used as the control.

3. RESULTS AND DISCUSSION

Antimicrobial Studies of Cotton Gauzes Coated with Antibiotics Aged for One Week

The cotton gauzes containing, separately, the different antibiotics aged for one week were placed, separately, in petri dishes containing Agar Broth inoculated with respective bacteria. Figure 1 (a) to (h) show the cotton gauzes coated with different antibiotics placed in petri dishes in which respective bacteria were grown. The same for blank cotton gauzes without any antibiotic attachment are shown in Figure 2 (a) and (b). The inhibition zones are clearly visible in all the samples that contain antibiotics but the samples that do not contain any antibiotic do not show any inhibition.

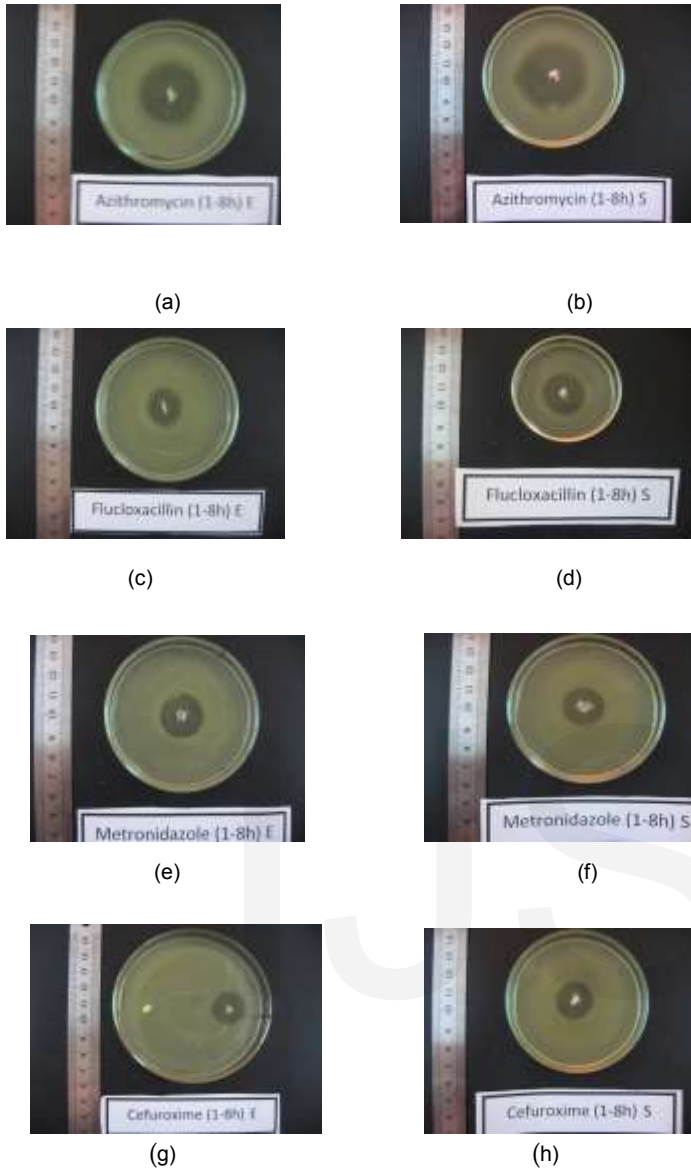


Fig.1. A week-aged cotton gauze coated with (a) Azithromycin placed in *Escherichia coli*, (b) Azithromycin placed in *Staphylococcus aureus*, (c) Flucloxacillin placed in *Escherichia coli*, (d) Flucloxacillin placed in *Staphylococcus aureus*, (e) Metronidazole placed in *Escherichia coli*, (f) Metronidazole placed in *Staphylococcus aureus*, (g) Cefuroxime placed in *Escherichia coli* and (h) Cefuroxime placed in *Staphylococcus aureus* showing the respective inhibition zones.

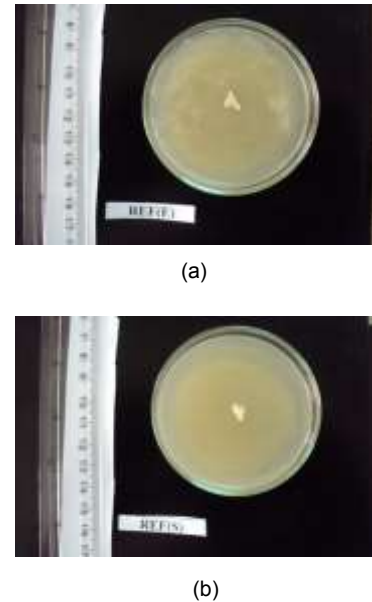


Fig.2. Sterilized cotton gauze placed in (a) *Escherichia coli* and (b) *Staphylococcus aureus*. The respective inhibition zones calculated from these data are shown in Table 1

TABLE 1

INHIBITION ZONES OF DIFFERENT ANTIBIOTICS ATTACHED ON TO COTTON SURFACES IN DIFFERENT BACTERIA CULTURES.

Antibiotic/ Antimicrobial Agent	Bacteria type	Diameter of the Inhibition Zone in mm
Azithromycin	<i>Escherichia coli</i>	32
Azithromycin	<i>Staphylococcus aureus</i>	37
Flucloxacillin	<i>Escherichia coli</i>	20
Flucloxacillin	<i>Staphylococcus aureus</i>	21
Metronidazole	<i>Escherichia coli</i>	21
Metronidazole	<i>Staphylococcus aureus</i>	20
Cefuroxime	<i>Escherichia coli</i>	17
Cefuroxime	<i>Staphylococcus aureus</i>	21
None	<i>Escherichia coli</i>	0
None	<i>Staphylococcus aureus</i>	0

It is interesting to note that, from the data given in Table 1, Azithromycin is effective against both *Escherichia coli* and *Staphylococcus aureus* with the highest inhibition zones which are over 10 mm larger than the diameters of the inhibition zones of other antibiotic attached cotton gauzes. Azithromycin has relatively broad but shallow antibacterial activity. It inhibits some Gram-positive bacteria, some Gram-negative bacteria, and many atypical bacteria. It is, therefore, possible that Azithromycin is effective against both *Escherichia coli* and *Staphylococcus aureus*. It is also apparent that Azithromycin has larger inhibition zone for

Staphylococcus aureus than that for *Escherichia coli* despite the fact that the cell wall of the former Gram-positive bacteria is thicker than that of the latter Gram-negative bacteria. As shown in Figure 3(a), the chemical structure of Azithromycin is large and the compound is relatively nonpolar. Hence, it is difficult for Azithromycin molecules to readily surpass the outer membrane of Gram-negative bacteria. However, although the cell wall of Gram-positive bacteria is larger than that of Gram-negative bacteria Azithromycin seems to cross the thicker cell wall of Gram-positive bacteria more readily than the thinner cell wall of Gram-negative bacteria since the latter has an outer membrane that restricting the penetration of Azithromycin molecules towards the inner environment of the cell. According to the mechanism of action Azithromycin molecules should reach the inner environment of the cell to inhibit RNA-dependent protein synthesis in bacterial cells by binding to the 50S subunit of the 70S bacterial ribosomes. This explains why Azithromycin is giving a large inhibition zone for Gram-positive *S. aureus* bacteria when compared to the inhibition zone of Gram-negative *E.coli* which is 5 mm less than that of the former one.

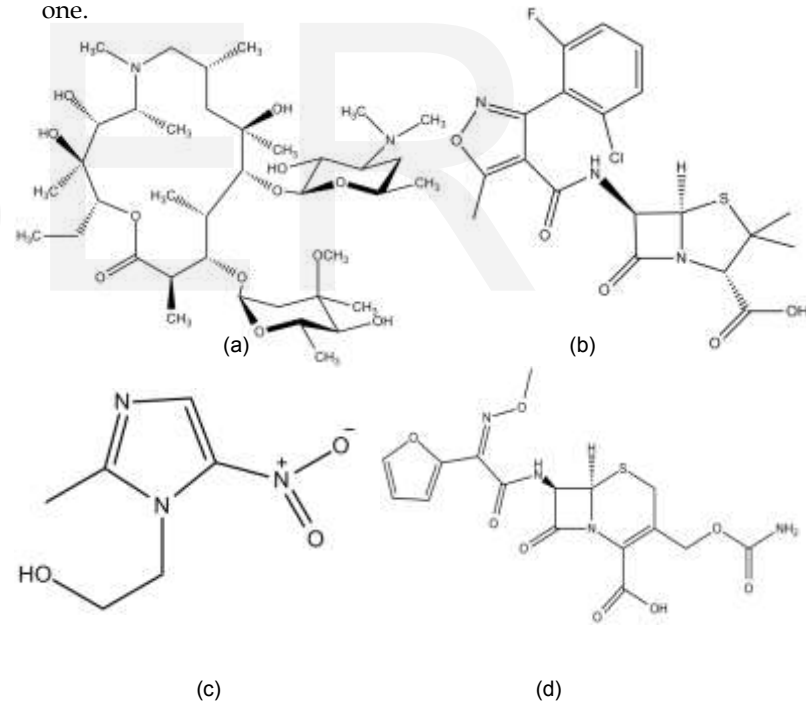


Fig.3. Chemical Structures of (a) Azithromycin, (b) Flucloxacillin, (c) Metronidazole and (d) Cefuroxime.

Although, Flucloxacillin, Metronidazole and Cefuroxime are structurally different when they are impregnated in cotton gauzes they seem to have approximately same inhibition zones for both types of bacteria chosen. Flucloxacillin is a penicillin-type antibiotic that can act against both Gram-positive and Gram-negative aerobic and anaerobic bacteria. The drug acts by inhibiting the cell wall synthesis mediated by binding to penicillin binding proteins. These penicillin-binding proteins are located inside the bacterial cell wall and hence Flucloxacillin inhibits the third and last stages of bacterial cell wall synthesis by inhibiting crosslinking of the linear peptidoglycan polymer chains. Flucloxacillin molecule has an isoxazolyl group on the side chain of the penicillin nucleus [Figure 3(b)] and this group facilitates β -lactamase resistance and hence Flucloxacillin can bind to penicillin-binding proteins and thereby inhibit peptidoglycan crosslinking. Cell lysis is then mediated by the bacterial cell wall autolytic enzymes such as autolysins. (17) These enzymes specifically hydrolyze mucopeptide polymers in the bacterial cell wall. As such, Flucloxacillin does not have to penetrate cell wall to inhibit cell wall synthesis and, therefore, its effect is independent of the cell wall thickness. Hence, it gives approximately the same inhibition zones for both types of bacteria (Inhibition zone of 20 mm for E.coli and 21 mm for S. Aureus). It is possible that the bacterium with thinner cell wall is better affected than that with the thicker cell wall. This difference in mode of action of the two drugs may explain why the inhibition zone of Flucloxacillin is less than that of Azithromycin.

Metronidazole belongs to the nitroimidazole class as can be seen from its structure given in Figure 3(c). It inhibits nucleic acid synthesis by disrupting the DNA of microbial cells. This function can only occur when metronidazole is partially reduced, and because this reduction usually happens only in anaerobic cells, it is effective against anaerobic bacteria. In the presence of oxygen, the reduction of metronidazole is not possible and hence it has a little effect on aerobic bacteria. S. aureus are a Gram-positive bacteria which is often hemolytic on blood agar. S. aureus is facultative anaerobe that grows by aerobic respiration or by fermentation that yields principally lactic acid. The bacteria are catalase-positive and oxidase-negative. E. coli is a Gram-negative, facultative anaerobic (that makes ATP by aerobic respiration if oxygen is present, but is capable of switching to fermentation or anaerobic respiration if oxygen is absent) and nonsporulating bacterium. Since both bacteria can function under anaerobic conditions it is likely that Metronidazole could be effective on both bacteria and, as evident from our results, the antibiotic gives approximately same inhibition zones for both bacteria.

Cefuroxime, whose chemical structure is shown in Figure 3(d), interferes with the peptidoglycan synthesis of the bacterial cell wall by inhibiting the final transpeptidation

needed for the cross-links (18). Therefore, the lower values of inhibition zones obtained for this antibiotic compared to those of Azithromycin is probably due to this reason. It is interesting to note that Cefuroxime has 4 mm longer inhibition zone to Gram-positive *Staphylococcus aureus* than that to Gram-negative E.coli. Since the mode of action of Cefuroxime is to damage cross-linking that is exhibited in Gram-positive bacterial cell walls this result of higher inhibition zone for Gram-positive bacteria matches well with the mode of action of the antibiotic.

The differences in sizes of inhibition zones could also be due to the ability of antibiotic molecules that are trapped in cotton gauze to diffuse around the cotton gauze. The molecules with higher diffusion coefficients could move a longer distance thus inhibiting the bacterial growth over a wider range. To the best of our knowledge, such a study of molecular diffusion of antibiotics that are trapped in cotton gauzes has not been carried out so far. Even the efficacies of different antibiotics towards same bacterium have not been compared. As such, this study presents the first of such comparison of different antibiotic efficacies on both E. coli and S. Aureus.

Antimicrobial Studies of Cotton Gauzes Coated with antibiotics aged for 22 months

The cotton gauzes containing, separately, the different antibiotics were prepared and placed in separate, sterilized, plastic containers with closed lids and kept under ambient conditions for 22 months. They were then taken, after such a long time of aging, and placed in petri dishes containing Agar Broth inoculated with respective bacteria. Figure 4 (a) to (g) show the cotton gauzes coated with different antibiotics, aged for 22 months, placed in separate petri dishes containing S. aureus and E. coli. The inhibition zones are extracted into Table 2.

TABLE 2

INHIBITION ZONES OF 22 MONTH-OLD COTTON GAUZE CONTAINING DIFFERENT ANTIBIOTICS IN *S. AUREUS* OR *E. COLI* TOGETHER WITH BLANKS IN THE SAME BACTERIAL CULTURE MEDIA.

Antibiotic/ Antimicrobial Agent	Bacteria type	Diameter of the Inhibition Zone in mm
Azithromycin	<i>Escherichia coli</i>	18
Azithromycin	<i>Staphylococcus aureus</i>	24
Flucloxacillin	<i>Escherichia coli</i>	0
Flucloxacillin	<i>Staphylococcus aureus</i>	0
Metronidazole	<i>Escherichia coli</i>	14
Metronidazole	<i>Staphylococcus aureus</i>	10
Cefuroxime	<i>Escherichia coli</i>	Data not available
Cefuroxime	<i>Staphylococcus aureus</i>	15
None	<i>Escherichia coli</i>	0
None	<i>Staphylococcus aureus</i>	0

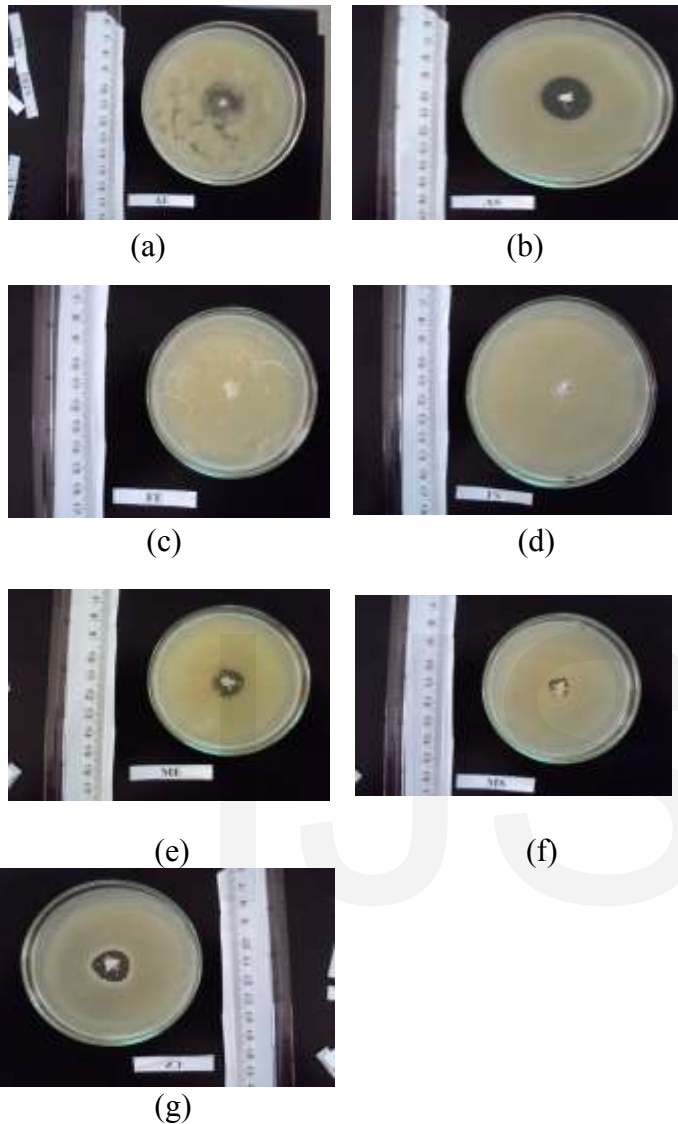


Fig.4. Twenty two-month old cotton gauzes impregnated with (a) Azithromycin in *Escherichia coli*, (b) Azithromycin in *Staphylococcus aureus*, (c) Flucloxacillin in *Escherichia coli*, (d) Flucloxacillin in *Staphylococcus aureus*, (e) Metronidazole in *Escherichia coli*, (f) Metronidazole in *Staphylococcus aureus* and (g) Cefuroxime in *Staphylococcus aureus*.

As evident from Tables 1 and 2, the same pattern is followed for both Azithromycin and Metronidazole in the two respective bacterial culture media: Azithromycin showing a larger inhibition zone in *Staphylococcus aureus* than that in *Escherichia coli* while Metronidazole following the reverse order as is the case for both antibiotics when aged for a week. Therefore, the explanations given above for the respective lengths of inhibition zones are justified with 22 month old samples also. It is intriguing to observe that at least these two antibiotics when impregnated on cotton gauzes have such high shelf-life of 22 months under ordinary ambient conditions. Flucloxacillin is not active when aged for 22 months probably due to its structural or conformational changes with time; the confirmation of which requires further study. However, there is a study of the stability of intravenous Flucloxacillin solutions used for hospital-in-the-home by To et al (19) and they found that unbuffered flucloxacillin 5% and 12% solutions lost up to 60% of flucloxacillin content when stored at 37 °C for 24 h. Adjustment of pH with phosphate buffer made the incubated solutions stable for at least 24 hours (except

flucloxacillin 12% in water for injection). However, we find that Flucloxacillin when impregnated in cotton gauzes remain stable for at least one week though not for 22 months.

Stability of Azithromycin in ophthalmic preparations under different conditions has been reported (20). It has demonstrated that the drug undergoes degradation when submitted to the ultraviolet light, germicide light, solar luminosity, acid solution, basic solution and hydrogen peroxide solution. Azithromycin for injection is known as ZITHROMAX which when diluted according to the instructions (1.0 mg/mL to 2.0 mg/mL) is stable for 24 hours at or below room temperature (30 °C or 86 °F) or for 7 days if stored under refrigeration (5 °C or 41 °F) [http://www.globalrph.com/azithromycin_dilution.htm].

There are studies concerned with the stability of metronidazole in solutions and suspensions (21). Interestingly, they found that Metronidazole tablets with a labelled expired date of April 1986 still showed 99.3% potency when assayed in January 1993. This may explain why Metronidazole is stable for at least 22 months when impregnated in cotton gauzes.

Regarding the stability of Cefuroxime, Galanti et al (22), have studied the long-term stability of cefuroxime and cefazolin sodium in intravenous infusions and found that, based on a shelf-life of 90% residual potency, the cefuroxime sodium concentration is stable for 13 days when stored at 4 °C, and the cefazolin sodium concentration is stable for at least 30 days at 4 °C. However, we find that Cefuroxime impregnated in cotton gauzes have antimicrobial activities even after 22 months of preparation (23). These stability tests of some antibiotic impregnated cotton gauzes may find their way in storing such gauzes in pharmacies as wound dressings for a long time. However, studying the chemical stability of these antibiotic molecules require detailed chemical analysis of the cotton gauzes impregnated with them through proper spectroscopic analyses and we are currently engaged in such chemical analyses.

4. CONCLUSIONS

Cefuroxime, Azithromycin, Metronidazole and Flucloxacillin separately impregnated cotton gauzes were prepared and their antimicrobial activities against both Gram-positive and Gram-negative bacteria were elucidated. Azithromycin shows the largest inhibition zones for both bacteria with better performance for *Staphylococcus aureus* in both cases. Metronidazole and Flucloxacillin show similar inhibition zones for both bacteria in week-old samples but Flucloxacillin does not show any activity when aged for 22 months. Cefuroxime shows the same pattern as does by Azithromycin. These findings are explained with the help of mechanisms of antimicrobial action of the antibiotics on bacterial species. This may be a viable method for topical

antibiotic treatment and to reduce bio-burden of antibiotics and to eliminate antimicrobial resistance.

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