Research Article

In vitro Evaluating Antimicrobial activity of Two Marketed Brands (Zomax and Zetron) in Kingdom of Saudi Arabia

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ABSTRACT

Oral administration of pharmaceuticals is one of the most popular methods of drug delivery. The drug Azithromycin acid is widely used in prescriptions in the Kingdom of Saudi Arabia. This research aimed to study the effect of two marketed brands, Zomax and Zetron, and compare them with standard pure drug. This study as post market monitoring of these drugs in community pharmacies. The microbiological sensitivity test was done against both gram positive and gram negative in *Escherichia coli*, *Salmonella typhi*, *Salmonella para typhi* and *Staphylococcus aureus*. Both drugs were given clear inhibition zone with more effect for Zetron, this might be due to high distribution of active ingredient in zetron than Zomax in formulation process or might be due to distribution of additives.

Keywords: Zetron, microorganisms, Zomax, Bacteria, Sensitivity Test, Inhibition Zone.

INTRODUCTION

Azithromycin is an antibiotic useful for the treatment of a number of bacterial infections[1], this include middle ear infections, strep throat, pneumonia, traveler's diarrhea, and certain other intestinal infections. It may also be used for a number of sexually transmitted infections including chlamydia and gonorrhea infections. Along with other medications, it may also be used for malaria. It can be taken by mouth or intravenously with doses once per day Common side effects include nausea, vomiting, diarrhea and upset stomach. An allergic reaction or a type of diarrhea caused by Clostridium difficile is possible. No harm has been found with use during pregnancy[1]. Its safety during breastfeeding is not confirmed, but it is likely safe.[2] Azithromycin is an azalide, a type of macrolide antibiotic. It works by decreasing the production of protein, thus stopping bacterial growth Azithromycin was first made in 1980[2]. It is on the World Health Organization's List of Essential Medicines. the most important medications needed in a basic health system[3].

It is available as a generic medication and is sold under many trade names worldwide[4]. The wholesale cost in the developing world is about 0.18 to 2.98 USD per dose. In the United States it is about 33 USD for a course of treatment[5].

Erythromycin, the first macrolide antibiotic discovered, has been used since the early 1950s for the treatment of upper respiratory tract and skin and soft tissue infections caused by susceptible organisms, especially in the penicillin-allergic patient.

Additionally, erythromycin is effective for the treatment of infections caused by some intracellular pathogens, including species of Legionella, Mycoplasma, and Chlamydia. Several drawbacks, however, have limited the use of erythromycin, including frequent gastrointestinal intolerance and a short serum half-life[6].

Chemistry

Erythromycin is a macrolide antibiotic whose structure consists of a macrocyclic 14-membered lactone ring attached to two sugar moieties (a neutral sugar, cladinose, and an amino sugar, desosamine). In the acidic environment of the stomach, it is rapidly degraded to the 8,9-anhydro-6,9-hemiketal and then to the 6,9,9,12-spiroketal form. The hemiketal intermediate may be responsible for the gastrointestinal adverse effects associated with erythromycin[7].

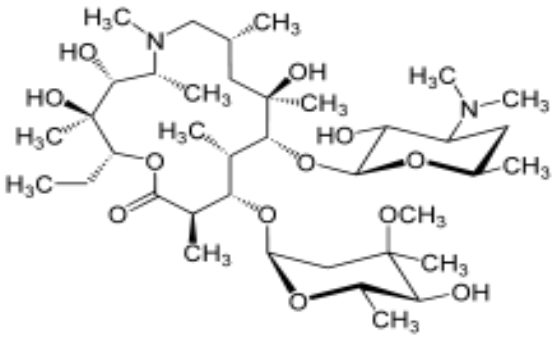

Figure 1: N – Methyl – 11 – aza -10 - deoxo -10-dihydroerythromycin A

Azithromycin (9-deoxo-9a-aza-9a-methyl-9a-homoerythromycin) is formed by inserting a methyl-substituted nitrogen in place of the carbonyl group at the 9a position of the aglycone ring. The resulting dibasic 15-membered ring macrolide derivative is more appropriately referred to as an ''azalide''. This structural change makes the compound more stable in acid, significantly increases the serum half-life and tissue penetration, and results in increased activity against gram-negative organisms and decreased activity against

some gram-positive organisms when compared with erythromycin[9]. Azithromycin is available as 250-, 500-, or 600-mg tablets; oral suspension (100–200 mg per 5 mL), and intravenous preparation (lyophilized 500 mg per10 mL vial).

Mechanism of Action and Resistance

The macrolide antimicrobials exert their antibacterial effects by reversibly binding to the 50s subunit of the bacterial ribosome. This interaction inhibits RNA-dependent protein synthesis by preventing transpeptidation and translocation reactions[8]. The macrolides bind to domain V of the 23S ribosomal RNA (rRNA)[10].

Medical Uses

Azithromycin is used to treat many different infections, including acute otitis media, nonstreptococcal bacterial pharyngitis, gastrointestinal infections such as traveler's diarrhea, respiratory tract infections such as pneumonia, cellulitis, babesiosis, *Bartonella* infection, chancroid, cholera, donovanosis, leptospirosis, Lyme disease, malaria, *Mycobacterium avium* complex disease, *Neisseria* meningitis, pelvic inflammatory disease, pertussis, scrub typhus, toxoplasmosis, and salmonellosis. It is used to prevent bacterial endocarditis and some sexually transmitted infections. It is also effective against localized dental infections, uncomplicated skin and skin structure infections, urethritis and cervicitis and also genital ulcer disease.

Azithromycin is used as a second line treatment for strep throat and for those allergic to penicillin. It has a similar antimicrobial spectrum to erythromycin, but is more effective against certain Gram-negative bacteria, in particular, *Haemophilus influenza* (although it would not be the first choice of treatment in this infection).

Azithromycin resistance has been described and is endemic in many areas. Long-term use in treating *Staphylococcus aureus* infections with azithromycin may increase bacterial resistance to this and other macrolide antibiotics. Azithromycin has been shown to be effective against malaria when used in combination with artesunate or quinine however the optimal dose for this is not yet known.

Dosage and Administration

Adults

For respiratory tract infections, otitis media and skin & soft tissue infections: 500 mg once daily for 3 days or an alternative to this as 500 mg once on day 1, followed by 250 mg once daily for next 4 days.

For sexually transmitted diseases like genital ulcer, non-gonococcal urethritis and cervicitis due to Chlamydia trachomatis: a single 1 gm (1000 mg) dose. For the treatment of urethritis and cervicitis due to Neisseria gonorrhoeae: a single 2 gm (2000 mg) dose. In typhoid, 500 mg once daily for 7 days. In Cholera, a single 1 gm (1000 mg) dose. In Shigellosis, 500 mg once on day 1, followed by 250 mg once daily for next 4 days.

Children

Age/Body weight	Daily Dose	Duration
From 1 month	10 mg/kg	3 days
15-25 kg	200 mg	3 days
26-35 kg	300 mg	3 days
36-45 kg	400 mg	3 days

Drug Interactions

Aluminum-and magnesium-containing antacids may reduce the peak serum levels but not the AUC of azithromycin. Carbamazepine, hexobarbital, and phenytoin: Serum concentrations of these agents have been elevated by azithromycin, increasing the pharmacologic effects and risk of adverse reactions. Monitor serum concentrations of these agents and observe the patient for adverse reactions. Adjust the dose as needed. Cyclosporine, theophyllines Levels may be elevated by azithromycin, increasing the risk of toxicity. Monitor drug levels and adjust the dose as needed. Digoxin Monitor digoxin levels and observe the patient for signs of digoxin toxicity Ergot derivatives (eg, dihydroergotamine, ergotamine) Acute ergotism manifested as peripheral ischemia has been reported. Closely monitor for ergotism[11-13].

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. In this review, we focused on the use of antimicrobial testing methods for the *in vitro* investigation of extracts and pure drugs as potential antimicrobial agents. After the revolution in the "golden era", when almost all groups of important antibiotics (tetracyclines, cephalosporins, aminoglycosides and macrolides) were discovered and the main problems of chemotherapy were solved in the 1960s, the history repeats itself nowadays and these exciting compounds are in danger of losing their efficacy because of the increase in microbial resistance[15]. Currently, its impact is considerable with treatment failures associated with multidrug-resistant bacteria and it has become a global concern to public health[16,17].

For this reason, discovery of new antibiotics is an exclusively important objective. Natural products are still one of the major sources of new drug molecules today.

They are derived from prokaryotic bacteria, eukaryotic microorganisms, plants and various animal organisms.

Microbial and plant products occupy the major part of the antimicrobial compounds discovered until now[18]. Plants and other natural sources can provide a huge range of complex and structurally diverse compounds.

Recently, many researchers have focused on the investigation of plant and microbial extracts, essential oils, pure secondary metabolites and new synthetized

molecules as potential antimicrobial agents.

However, when we reviewed the published articles on the antimicrobial effect of these natural products, the comparison between results is often difficult, because of the use of different non-standardized approaches inoculum preparation techniques, inoculum size, growth medium, incubation conditions and endpoints determination.

The fact that a plant extract exhibits antimicrobial activity is of interest, but this preliminary part of data should be trustworthy and allow researchers to compare results, avoiding work in which researchers use the antimicrobial activity investigation only as a complement to a phytochemical study.

A variety of laboratory methods can be used to evaluate or screen the *in vitro* antimicrobial activity of an extract or a pure compound.

The most known and basic methods are the disk-diffusion and broth or agar dilution methods.

Other methods are used especially for antifungal testing, such as poisoned food technique.

To further study the antimicrobial effect of an agent in depth, time-kill test and flow cytofluorometric methods are recommended, which provide information on the nature of the inhibitory effect (bactericidal or bacteriostatic) (time-dependent or concentration-dependent) and the cell damage inflicted to the test microorganism.

Owing to the new attraction to the properties of new antimicrobial products like combating multidrug-resistant bacteria, it is important to develop a better understanding of the current methods available for screening and/or quantifying the antimicrobial effect of an extract or a pure compound for its applications in human health, agriculture and environment.

Therefore, in this review, the techniques for evaluating the *in vitro* antimicrobial activity were discussed in detail[19-21].

Post-Marketing Surveillance (PMS)

(Also P M S) is the practice of monitoring the safety of a pharmaceutical drug or medical device after it has been released on the market and is an important part of the science of pharmacovigilance.

Since drugs and medical devices are approved on the basis of clinical trials, which involve relatively small numbers of people who have been selected for this purpose - meaning that they normally do not have other medical conditions which may exist in the general population - postmarketing surveillance can further refine, or confirm or deny, the safety of a drug or device after it is used in the general population by large numbers of people who have a wide variety of medical conditions[14].

MATERIALS AND METHODS

Materials and Culture Media

Azithromycin Powder	Used as standard (from India)
Mueller Hinton Agar	(Oxoid Ltd, England)
MacConkey Agar	(Oxoid Ltd, England)
Nutrient Agar	(Oxoid, Ltd) England

Microorganisms

Isolated (*Salmonella Typhi, Salmonella para Typhi, Staphylo coccus aureus, Escherechia coli).*

Microbiological Test

Microbiological test was carried out for new formula in four isolated laboratory species to inhibit and ensure the effectiveness of the antibiotics. And those species are *Salmonella typhi, Salmonella para, Staph. Aureus and Escherichia coli*[22].

Azithromycin Sensitivity Test using Disc Diffusion Kirby-Bauer

For each test and standard 1mg is taken and dissolved in 10 ml distilled water then 1ml was taken from it and dissolved in other10 ml distilled water.[22]

Antibiotic Disc Preparation

Filter paper was cut into small disks of about 4 mm in diameter then it enclosed in a sealed container and sterilized in oven.

Half number of the disks impregnated with Azithromycin test suspension and the others with standard suspension then the disks are dried in oven at 60°C for 20 minutes (serial dilution was made to obtain concentration 10µg/ml as follow: 1mg was dissolve in 10ml and then 1ml was taken and dissolve in another 10 ml).

$$Dilution\ factor = \frac{(R * V)}{O}$$

Where:

R is required concentration,

V is required volume

O is origin concentration

Inoculums was prepared from each bacterium under test

- *Staphylococcus aureus*
- *E. coli*
- *Salmonella Typhi*
- *Salmonella Para Typhi*

Inoculums preparation is the most important step in any susceptibility test. Inocula are prepared directly by inoculating colonies grown overnight on an agar plate, into broth media. Then the numbers of bacteria tested was standardized using McFarland turbidity standards[15].

McFarland turbidity standards: The McFarland 0.5 standard is used, which contains 99.5 ml of 1% sulfuric acid and 0.5 ml of 1.175% barium chloride, this solution is dispensed into tubes comparable to those used for inoculums preparation.

The McFarland 0.5 standard provides turbidity comparable to that of a bacterial suspension containing 1.5×10^8CFU/ml[22].

Inoculation and incubation

After preparation of standard inoculums suspension, a sterile cotton swab is dipped into the suspension, pressed to remove excess liquid, and then swabbed evenly across the surface of a Mueller Hinton agar plate (plates of 9mm are used). (Each inoculum suspension was inoculated into three media labeled test (T), standard (S) and control(C)).

- Within 15 minutes of inoculation, the individual Azithromycin disks (one disc per plate) are applied to the agar media with a forceps and gently pressed to ensure contact with the agar.

- The Azithromycin Test disks are applied in the plates labeled (T)

- The Azithromycin acid Standard disks are applied in the plates labeled(S). While other plate's labeled (c) without antibiotic disks were used to control growth.

- Within 15 minutes of disks placement, plates are inverted and placed in a 37 °C for 18 hours.

- After incubation the plates were examined, to make certain the test organisms has grown satisfactory, the diameter of each inhibition zone is measured using ruler or calipers.

- Once zone measurements have been made, the millimeter reading for each brand and standard are compared with that specified in the interpretive tables of the NCCLS documents[22].

RESULTS AND DISCUSSION

Table 1: Comparison between the Two Types of Azithromycin Combination with standard on Different Species of Microorganisms

Brand	Diameter (mm)			Surface Area (mm^2)		
	E. Coli	*Staph aureus*	*Salmonella Species*	*E. Coli*	*Staph aureus*	*Salmonella Species*
Zetron	10	16	8	78.5	200.96	50.24
Zomax	9	14	7	63.59	153.86	38.47
Standard	11	18	10	94.99	254.34	78.50

In two types of antibiotic brands tablet (Zetron and Zomax) the zone of inhibition is slightly larger in Zetron than Zomax this might be due to good distribution of active ingredient and both of them is lower than standard (pure powder of Zomax).

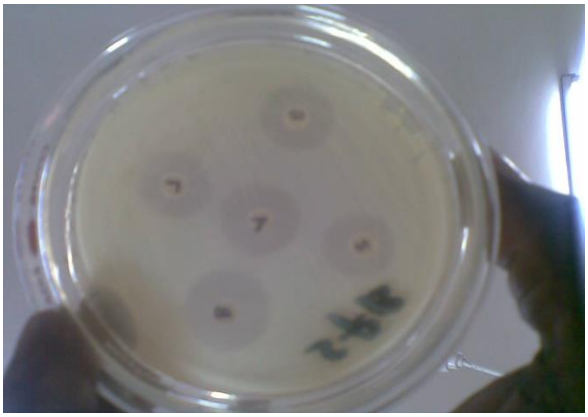

Figure 2: Inhibition Zone of *Staphylococcus aureus* with Zetron

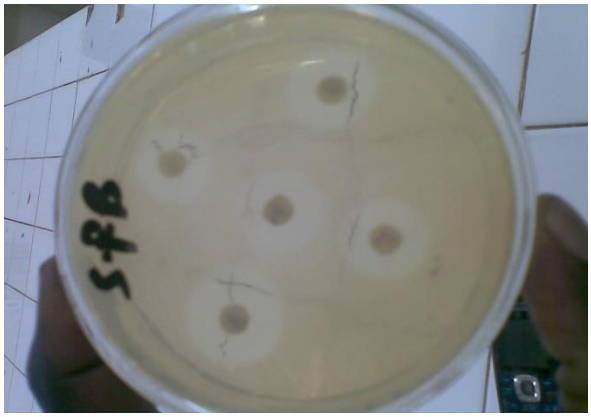

Figure 3: Inhibition Zone of *Staphylococcus aureus* with pure Standard antibiotic.

The disk was used in concentration 10µg according to (Oxoid Ltd, England) to give the require effect.

For Accuracy and precession of the result standard *Staphylococcus aureus* according to the national committee for clinical laboratory standards (NCCTS).[22]

The diameter of each inhibition zone was found after measuring, using a ruler and calibers for each brand as shown in Table 1 and Figure 2 and 3.

It is clear that arrangement of inhibition zone from microbiological results of zones of inhibition standard > Zetron > Zomax. All brands are active against selected

bacteria with more in *Staphylococcus aureus* this agreed with Kim[23].

The response of *Staphylococcus aureus* is more sensitive than *Salmonella typhi, Salmonella pra typhi* and *Escherichia coli* to Azithromycin, that clear in Table 1 and Figure 2, this due to the effect of macrolide group on gram positive specious rather than than gram negative specious like *Salmonella typhi, Salmonella pra typhi* and *Escherichia coli*[15], this result agreed with Susan[24] and Kim[23].

CONCLUSION

- The higher sensitivity of *Staphylococcus aureus* comparing to *Salmonell typhi* may be due to the genetic factor that make *Salmonella typhi* produce more polysaccharide which might be responsible for resistance.
- Macrolide group (especially Azithromycin) isn't prescribed for gram negative specious microorganisms due the high resistance of these microorganisms to macrolide group.
- The monitoring and quality control testing of medicines in pharmacies randomly to ensure the good storage conditions might ensure drug's effectiveness.
- The microbiological sensitivity test can be used as an indicative for variations of drugs activities in different formulae and give good indication for the efficacy of marketed drugs.
- The correlation can be made between microbiological sensitivity tests of different marketed drugs as an indication for its effectiveness *in vivo* studies.

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