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# EVALUATION OF THE ANTIMICROBIAL EFFECT OF EXPIRED ORAL ANTIBIOTICS IN-VITRO.

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## ABSTRACT

**Background:** In spite of significant risks, as well as non-clinical importance due to loss of potency, stiff penalties against administration of expired antibiotics are still not appropriately enforced by health policy makers in many developing countries, possibly because of little evidence to support that expired medications are hazardous. **Objective:** To investigate the effect of expiration dates on in vitro bacteriostatic potentials of different oral antibiotics. **Materials and methodology:** The potency of five antibiotics, both expired and unexpired, Azithromycin, Clarithromycin, Levofloxacin and Cloxicillin and tetracycline were investigated, based on the antimicrobial potentials. The investigation was done by challenging local isolates of different concentrations of *S. aureus* bacteria; using Disc diffusion technique. **Result and disscution:** Although the expired antibiotics potency was less than that of the control ones, it was found that the microorganisms challenged with these antibiotics were evaluated as susceptible (According to NCCLS), as the diameter of inhibition zone lies within the susceptible range; Shown the comparison of effectiveness between expired and unexpired Azithromycin against Different bacteria Srcina sp., bacillus sp., staphylococci, salmonella sp and M.phelei, The difference between the expired and control antibiotic is not significant with P value = 0.570.

**KEYWORDS:** Expired oral antibiotics, potency, tetracycline.

**ABSTRACT**

In spite of significant risks, as well as non-clinical importance due to loss of potency, stiff penalties against administration of expired medications are still not appropriately enforced by health policy makers in many developing countries, possibly because of little evidence to support that expired medications are hazardous.[1]

Drug expiration date, which is mostly two to three years after the drug is manufactured, except in some cases, is the specific date until which the medication will be effective, if stored under proper conditions of light, temperature and moisture but if not stored under suggested conditions, the medications may lose potency even before the expiration dates. Whereas, in spite of warnings that medications that have passed their expiration can pose significant risk, and that it is extremely important to properly dispose of expire medications to avoid serious injuries to older adults and children.[2]

Not all scientists still agree that expired medications are unsafe. It has even been reported that many drugs stored under reasonable conditions retain 90% of their potency for at least five years, and sometimes much longer, after the expiration date on the label, while some further claimed that expiration date is a very conservative marketing ploy by drug manufacturers to keep consumers in restocking their medicine cabinets.[3]

Some studies provided preliminary microbiological results on the appreciable reduction in vitro bacteriostatic potentials as well as higher resistance and multiple antibiotic resistance rahc among expired oral antibiotics apanit from less-efficacy, administration of expired antibiotics can lead to increased antibiotic resistance and clinical faultier as well as adverse drug reaction.

**Objective**

The purpose of this study, is to investigate the effect of expiration dates on in vitro potency of oral antibiotic.

**INTRODUCTION**

An antimicrobial is an agent that kills microorganism or inhibits their growth. Antimicrobial can be grouped according to the microorganisms they act against. Antibacterial (commonly known as antibiotics) are used to combat bacteria and are classified according to their function. Antimicrobials that kill microbes are called microcidal and those that merely inhibit

their growth are called microstatic. Antibiotics can also be classified as bacteriostatic and bactericidal.[1]

Since the introduction of antibiotics 6 decades ago, clinicians have taken comfort in the relative safety and exceptional efficacy of these drugs. Patients initially looked on antibiotics as "wonder drugs," primarily because of their life-saving benefits in treating serious wounds, postsurgical infections, pneumonia, meningitis, endocarditis, and sepsis, as well as their dramatic effect on sexually transmitted infections and infections of the urinary tract. Antibiotics are available for use in the community with oral formulations, as well as in the hospital with parenteral preparations.[2]

Over the past several decades, antibiotics have been used enthusiastically by physicians, and patients have learned to expect them for the treatment of many ailments, even those that are known not to be caused by bacteria. It is widely believed that more than half of the community use of antibiotics is for the treatment of respiratory infections in children, well more than half of which are caused by viruses.[1]

Antibiotics have been used for decades in the treatment of acne vulgaris and many other common skin conditions and have been used adjunctive to surgical drainage for a large number of deeper cutaneous and facial abscesses and carbuncles. That antibiotics are frequently prescribed in response to patient requests for these miracle drugs to treat trivial or nonbacterial infections and that most physicians are very comfortable prescribing these agents by telephone for presumed but not proven bacterial infections are understatements.[1]

Initially, sulfonamides and penicillins dominated the field, but cephalosporins and penicillinase-resistant semisynthetic penicillins soon followed. Macrolides and tetracyclines were also introduced relatively early during the history of antibiotics as broad-spectrum agents for a variety of indications. The development of macrolides included the introduction of clarithromycin and the azalide azithromycin, both of which had improved tolerability and activity.[1]

Tetracyclines were widely accepted as oral agents for the treatment of respiratory, urinary, and skin infections and intravenous preparations also were used. Some concern about micro vesicular fat deposits in hepatic cells was raised with high-dose intravenous tetracyclines, especially during pregnancy, and parenteral tetracyclines eventually were replaced with other

agents. Chloramphenicol was introduced relatively early on as a broad-spectrum antibacterial agent, but its use has been seriously limited by its hematologic side effects.[1]

Trimethoprim-sulfamethoxazole eventually replaced sulfonamides for most of their indications. Cephalosporin were developed first for the treatment of penicillin-resistant staphylococcal infections, as well as for the treatment of simple urinary tract infections. These agents, along with the penems, represent the mainstays of parenteral antibacterial treatment. b-lactamase inhibitors were introduced in such combinations as ticarcillin-clavulanate, amoxicillin-clavulanate, ampicillin-sulbactam and piperacillin-tazobactam and they have provided temporary relief from resistance in some infections. These drugs are still in common use today.

Colistin and polymyxin B were initially the only drugs available to treat pseudomonal infections and after almost disappearing for 3 decades, these agents are reentering the clinical arena.[1]

Aminoglycosides were introduced first as adjunctive treatment in tuberculosis and then for the treatment of serious gram-negative rod infections and bacteremia. These drugs are noted for their rapidly bactericidal activity.

The fluoroquinolones were initially introduced as urinary tract agents and for the treatment of gonorrhea, but these rapidly bactericidal agents have been further developed in the past decade with the introduction of several agents used for oral or parenteral treatment of a wide variety of infections, including urinary tract infections, intra-abdominal sepsis, and infections of the respiratory tract.

Glycopeptides were introduced 130 years ago and are still in common use for the treatment of gram-positive coccal infections, especially those due to methicillin-resistant strains of *Staphylococcus aureus*. Rifamycins were introduced early for the treatment of tuberculosis and are now used as adjunctive agents in the treatment of a variety of bacterial diseases.

A streptogramin combination, dalfopristin-quinupristin, was introduced a few years ago to treat methicillin-resistant *S. aureus* and resistant enterococcus infections.

Linezolid was recently introduced as a parenteral and oral agent for the treatment of grampositive coccal infections, with special activity against methicillin-resistant *S. aureus*

and vancomycin-resistant enterococci. Most recently, telithromycin, the first ketolide, was introduced for the treatment of respiratory infections, especially those due to macrolide- and penicillin-resistant pneumococci. Anaerobic infections became treatable 13 decades ago with the introduction of clindamycin and metronidzaole, and these drugs are still in frequent use today. As a result of the rather profligate use of antibiotics over the past half-century, it is no wonder that we are now facing the prospect of losing the battle against many bacterial diseases. About 10 years ago, Marc Lipsitch, in the New York Times.[1]

Opined rather presciently that "the evolution of resistant strains may soon outpace the development of new drugs, leaving doctors powerless to treat infections that were once considered routine.

**Antibiotic resistance**

Many well-documented examples associate antibiotic resistance with increased use of these agents. It's familiar with methicillin-resistant *S. aureus* in hospitalized patients, and we are getting more used to seeing serious cutaneous, pulmonary, and other infections caused by methicillin-resistant *S.aureus* in patients who have had no exposure to hospitals or other medical care facilities.[1]

The incursion of nosocomial (or hospital acquired) and community-associated methicillin-resistant *S.aureus* has reached the stage of a serious public health crisis These organisms are variably susceptible to fluoroquinolones. Vancomycin-resistant Enterococcus faecium and Enterococcus faecalis have achieved prominence in the last decade.[1]

Primarily as gastrointestinal colonizers but also as causes of serious intra-abdominal infections and endocarditis. Excessive use of third-generation cephalosporins, as well as glycopeptides, has been implicated in the emergence of vancomycin-resistant enterococci. *S. aureus* strains with intermediate resistance or heteroresistance that require increased vancomycin MICs have been reported, and these glycopeptidenon susceptible staphylococci are more likely to cause failure of glycopeptide therapy.[1]

We are still fortunate that only a very few strains of vancomycin-resistant *S.aureus* have been reported around the world.[1]

Macrolide-resistant group A b-hemolytic streptococci or Streptococcus pyogenes have been reported from many countries, and resistance rates may be as high as 40%, although dramatic

reduction in resistance may be seen with reduced consumption.[1] Penicillin- and macrolide-resistant Streptococcus pneumonia have been reported from all countries with increasing incidence and reports of these infections have filled several pages of Clinical Infectious Diseases. Pneumococci also have shown increasing resistance to some fluoroquinolones, although the overall rates are low.[1]

B-lactamase-producing Homophiles influenza and Moraxella catarrhal is are well known and ampicillin and amoxicillin are no longer effective for the treatment of infections caused by these organisms.[2]

Resistant gram-negative rods have also emerged over the past 3 decades.[2]

Many common enteric organisms produce potent b-lactamases that mediate resistance to the penicillin's and cephalosporin.[2]

Multiply resistant gram-negative rods that produce extended-spectrum b-lactamases are causing particular problems in hospitals and these organisms may be resistant to most b-lactam drugs, as well as quinolones. The emergence of these resistant strains has been associated with excessive use of third-generation cephalosporin.[2]

Other b-lactamases, such as the metallo-enzymes, which confer additional resistance to carbapenems, are also increasingly being identified. Fluoroquinolone-resistant Escherichia coli are increasing, especially in centers that use fluoroquinolone prophylaxis for their patients with neutropenia, even in the face of reduced mortality associated with these drugs.[1]

Aminoglycoside- and carbapenem-resistant pseudomonads and other gram-negative organisms have been reported around the world, and clindamycin-resistant Bacteroides fragilis are well known.[2]

Antibiotic resistance is driven by the density of antibiotic use, combined with the level of compliance with infection control measures to prevent spread of resistant bacteria. At the population level, several studies have shown a correlation between outpatient use of an antibiotic class and the percentage of bacterial isolates resistant to this class (Albrich et al., 2004; van de Sande-Bruinsma et al., 2008).

This relationship between antibiotic use and resistance has also been demonstrated at the hospital level, for example for the carbapenems(Lepper et al., 2002; Lopez-Lozano et al., 2000).

Herrmann developed a dynamic, bio-economic model to better understand the pricing policy of a company which holds the monopoly for an antibacterial compound (Herrmann, 2010). Thismodel revealed three phases: (i) under patent protection when themonopolist endogenously manages the level of antibiotic efficacy(quality) and the infected population (market size); (ii) approaching the end of patent protection when the monopolist behaves more and more short-sightedly, leading to a continuous decrease in the price of the antibiotic; and (iii) after patent expiration when the monopolist behaves competitively in a generic industry, which results in a discontinuous fall of price of the antibacterial(Herrmann, 2010).

In the field of pharmacology, potency is a measure of drug activity expressed in terms of the amount required to produce an effect of given intensity. A highly potent drug evokes a larger response at low concentrations, while a drug of lower potency evokes a small response at low concentrations. It is proportional to affinity and efficacy.[1]

The shelf life of a drug is the duration of time for which the drug retains its real effect or potency. It begins from it's date of manufacturing and it is the time period between the manufacturing date and the expiry date.[1]

The expiration date of a drug is the final day that the manufacturer stipulates the full potency and safety of the drug. Drug expiration dates exist on most medication labels, including prescription, over-the-counter (OTC) and dietary (herbal) supplements.[1]

It is required by law that many pharmaceutical manufacturers insert expiration dates on prescription products, prior to marketing.[1]

Manufacturers will not make recommendations about the stability of drugs past the original expiration date for legal and liability reasons.[1]

Drugs that are past their expiry date may not be toxic, but may have lost their potency or effect. Intake of such drugs might not be as effective as it is supposed to be and may result in infection-causing microbes immune to the drug. If the same drug is administered again in future, there will be little or no effect. The environment conditions in which the medicine is

stored, is also a determinant factor in the shelf life of a drug. The drug should be stored in a cool and dry place where it is not exposed to high temperature, high humidity, or strong light. Prolonged exposure to light may induce chemical reactions, altering the chemical property of the drug, leading to degradation.[1]

A decrease in the potency of a drug can lead to antimicrobial resistance. The latter also results from the incorrect administration of the drug. Antimicrobial resistance has now become a serious global problem. Thus, it's important to take the precise drug with the required potency at the appropriate time of storage to combat bacterial and fungal infections.[1]

Poor-quality drugs are a vital but is a neglected public health problem in many tropical countries. Policy decisions and implementation of good-quality medicines are important in therapeutic research**.** There are challenges related to the fight against poor quality drugs, and counterfeits in particular.[1]

Due to a decline in economic conditions and poor enforcement of existing pharmaceutical and customs regulations, third world countries have been faced with a growing threat from counterfeit substandard medicines and drugs that have lost their potency. The distribution of substandard medicines in the developing world is a serious clinical and public health concern. Problems include under or over concentration of ingredients, contamination, poor quality ingredients, poor stability, inadequate packaging and a decline in potency.[1]

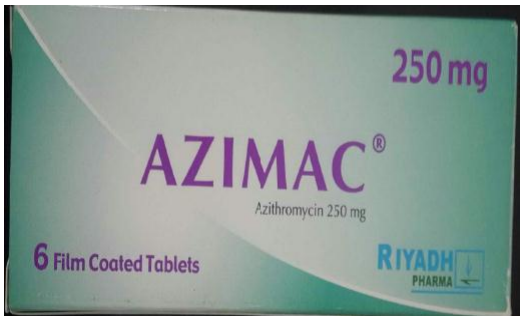
There have been reports of treatment failures with commercially available sulphadoxine/pyrimethamine (SP) and amodiaquine (AQ) brand marketed by wholesale pharmacies.[1]

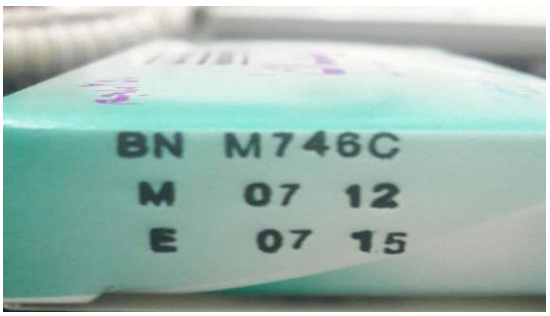
## MATERIALS AND METHODOLOGY

Six different antibacterial agent [Azithromycin, Clarithromycin, levofloxacin, cloxacillin, Tetracycline, Doxycycline] both control [un expired] and investigated [expired] antibiotic were compered based on the antimicrobial potentials by challenging local isolates of different concentration of *S. aureus* using agar diffusion technique, which is quantitative method

Measuring the diameter of inhibition zone and correlate it to the potency of the antibiotic as well as the broth dilution technique.

The culture media used is Muller Hinton agar adjusted at pH 7 and incubation period 24 hours under aerobic conditions.[4]




**RESULTS AND DISCUSSION**

Although the expired antibiotics potency was less the that of the control one, it was found that the microorganisms challenged with these antibiotics were evaluated as susceptible (according to NCCLS) as the diameter of inhibition zone lies within the susceptible range**.**

| Antibiotic | Reference SusciptibleZone of Inhibition (mm) | Zone of Inhibition of Expired antibiotic (Mean ± SD) | Interpretation of Zone Diameter (R,S, I) |
|---|---|---|---|
| Azithromycin | ≥ 20 mm | 25.71mm ± 2.024 | Susceptible (S) |
| Clarithromycin | ≥ 18 mm | 22.43 mm ±2.955 | Susceptible (S) |
| Levofloxacin | ≥ 20 mm | 25.43 mm ±3 .599 | Susceptible (S) |
| Cloxacillin | ≥ 15 mm | 29.14 mm ± 4.78 | Susceptible (S) |
| Tetracycline | ≥ 20 mm | 24.86mm ± 3.81 | Susceptible |

## Evaluation of the microbial susceptibility on expired antibiotics:

**Figure 1**

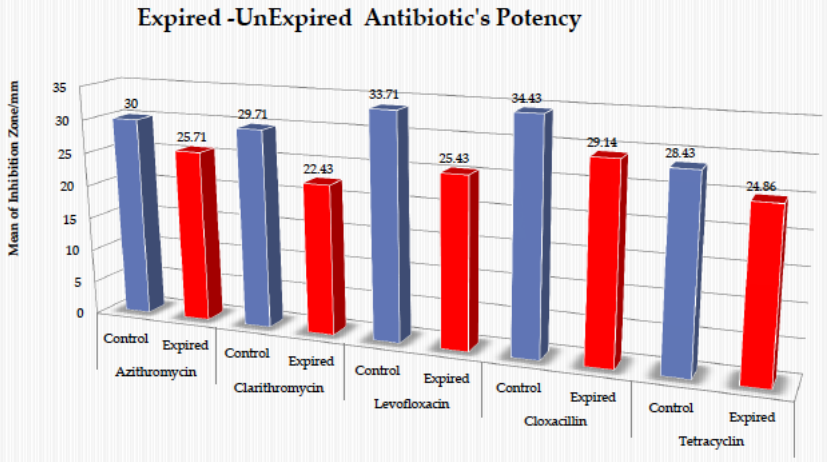
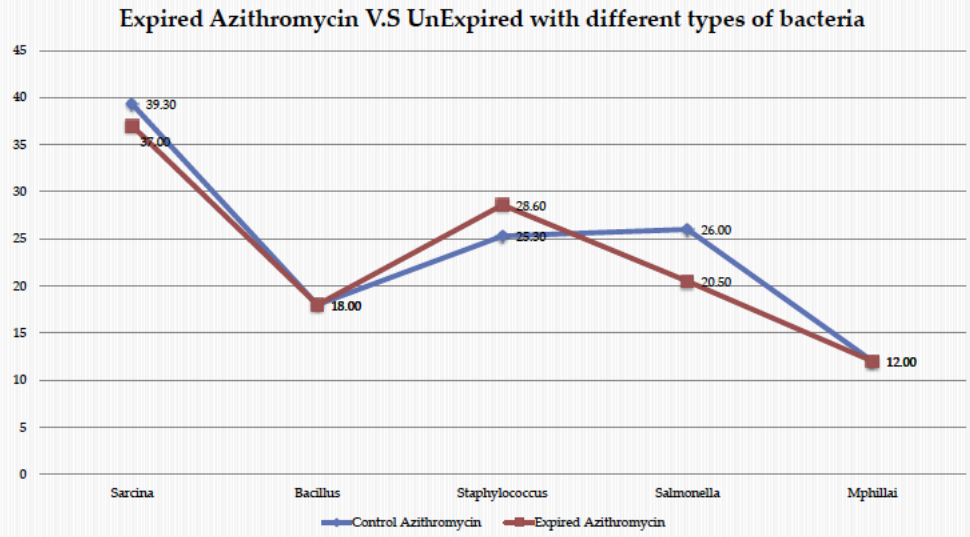



**Figure 2**




Shown the comparison of effectiveness between expired and unexpired Azithromycin against Different bacteria Srcina sp., bacillus sp., staphylococci, salmonella sp and M.phelei, The difference between the expired and control antibiotic is not significant with P value = 0.570

**Recommendations**

1. If the antibiotic is needed, and the patient is not able to replace the expired antibiotic, there is no evidence that it is unsafe to take the medication in most cases
2. Guidelines for storage of essential medicine should be followed with antibiotics

3. Public health policies based on national guidelines should include monitoring of the quality control of the expired antibiotic

**Suggestions for future researches**

1. More researches to be done on liquid dosage forms
2. Expired antibiotics with older expiration dates should be investigated

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