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ORIGINAL ARTICLE

Study on Expired and Unexpired Drugs on Different Pathogenic Bacteria

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ABSTRACT

There is a growing trend in the different antibiotics against microbes. However, studies were found that a microorganism interacts at molecular level in a unique way against a range of antibiotics. The present study was undertaken to analyze the effect of expired and unexpired drugs which are commonly used by Indians without doctor's prescription. Due to a change in chemical composition or a drop in potency, expired medical items may be less effective or dangerous. Expired drugs may harbor bacteria, and sub-potent medicines may even fail to treat infections, resulting in more serious diseases and antibiotic resistance. There is no assurance that the drug will be safe and effective after the expiry date. Sensitivity test and Spectrophotometry were performed for the selected expired and unexpired antibiotics and drugs - Ampicillin, Norflox-TZ, and Althorn. The growth pattern of microbes in antibiotics before and after their expiry was performed and then compared. It was evident that fresh forms of antibiotics have significantly inhibited the growth of microbes as compared to expired drugs. The expired forms of antibiotics lost their efficacy in expired drugs.

KEYWORDS | antibiotics, spectrophotometry, expired, unexpired drugs

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INTRODUCTION

Our health is regarded as the most valuable asset we own. Individuals, the government and commercial entities spend a lot of money on healthcare in today's medical system. So, it's critical to comprehend the economic concerns surrounding healthcare administration and medical therapy. Pharmaceutical drugs are chemicals that are used in medicines or drugs. Pharmaceutical drugs and medicines are available to all age groups via various channels (oral, topical, optic, ophthalmic) depending on the form of medicine (tablet, cream, ointment, ear drops, eye drops, etc.) for treatment of acute and serious illnesses caused by various microorganisms. They are used not only for illness treatment and diagnosis, but also for prevention of illnesses. However, even a small amount of contaminated medicines can be fatal to humans. The production of pharmaceutical drugs and medicines is a collaborative effort between pharmaceuticals and microbiologists. The process of drug development begins with the discovery of a drug molecule having therapeutic value in fighting, controlling, preventing, or curing illnesses. The creation and characterization of such molecules, known as "active pharmaceutical ingredients" (APIs), as well as their examination to provide preliminary safety and therapeutic

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effectiveness data, are prerequisites for drug development. Bacterial infection can cause degradation in a range of expired and unexpired durgs unfit for use. Pharmaceutical companies produce drugs and medicines to treat a variety of diseases, and they are labeled with an expiry date. The drugs are effective and safe for customers to ingest, but expired medicines are not. The expiry date printed on drugs and medicines indicates the day when the manufacturer guarantees full potency and safety of the drugs. Environmental conditions, microbial contamination, containers in which they are stored all contribute to the degradation of pharmaceuticals in three ways: physical, chemical, or microbial. Color and texture shift as a result of physical instability. Microbial proliferation leads to microbial deterioration, whereas chemical instability leads to oxidation, hydrolysis, and decarboxylation.

Expired Drugs: Medication lapse is the date after which a medication probably won't be effective or suitable for use by patient. Buyers can decide on the usability of a medicine by checking its expiry date printed on the bottle or packet. Medcines which are expired can be ineffective, inadequate or even dangerous.

Unexpired Drugs: Still valid or in use and effect but not terminated medicine that is not yet reached its expiration date. For the benefit of our immune system, sometimes we take antibiotic drug, which are chemicals, that go inside our body and attack the pathogenic bacteria so that it cannot live longer and multiply in our body. If the bacteria are susceptible to the antibiotic, then they stop growing and die simply.

Health: Wellbeing is seen as a source of happiness in our lives, or, to put it another way, health is regarded as a source of riches in our lives. Infections are treated and prevented by pharmaceutical products. These pharmaceutical items are designed to be safe during their development, storage, and use.

Pharmaceuticals are necessary for human health, but many of them include hazardous substances that can pollute the environment if they are not properly managed or disposed of. When pharmaceutical wastes are illegally disposed of, they can cause contamination in humans and animals, resulting in a wide range of toxicities. Many people keep unwanted, unused, or expired medications in their homes and then dispose them of into garbage cans or flush them down toilets.

Bacteria can infect a variety of expired and unexpired pharmaceutical items, resulting in corrosion of items that are not fit for use. Pharma companies produce medicines to treat a variety of diseases and label them with an expiry date. Due to a change in chemical composition or a drop in potency, expired medical items may be less effective or even dangerous. Expired drugs may harbor bacteria, and sub-potent medicines may even fail to treat infections, resulting in more serious diseases. There is no assurance that the drug will be safe and effective after the expiration date. As a result, the goal of this study was to assess the efficacy of expired medicines and determine how effective or ineffective they are in comparison to unexpired medicines. We employed mostly antibiotics in the experiment and conducted an antimicrobial investigation to determine the efficiency of widely used expired and unexpired medications against various harmful microorganisms.

OBJECTIVE

- Maintenance of Pathogenic Bacteria on nutrient agar media
- Morphological characterization of Bacterial strains.
- Study the effect of expired and unexpired drugs on bacterial growth by Agar Well Diffusion and Spectrophotometry.
- Study the effect of expired and unexpired drugs on bacterial growth by optical density and growth using Spectrophotometry.
- Potency is a measure of drug activity stated in terms of the amount necessary to generate an effect of a specific intensity in the discipline of pharmacology. At low doses, a very powerful medication elicits a bigger reaction, whereas a medication of lower strength elicits a tiny reaction. Affinity and effectiveness are proportionate. [Lipstich, 2012]
- A drug's shelf life is the period of time during which it keeps its true impact or effectiveness. It is the time span between the production date and the expiry date, starting from the day of manufacture.

- A drug's expiration date is the last day on which the manufacturer guarantees the drugs complete potency and safety. Most drug labels, including prescription, over-the-counter (OTC), and dietary (herbal) supplements, include an expiration date. [Marshall et al 2009]
- For legal and liability concerns, many pharmaceutical manufacturers are required by law to provide expiration dates on prescription goods prior to marketing.
- Manufacturers will not offer recommendations on the stability of pharmaceuticals after the original expiration date.
- Drugs that have passed their expiration date may not be harmful, but their strength or efficacy may have deteriorated. Intake of such treatments may not be as effective as it should be, and infection-causing microorganisms may become resistant to the medicine. There will be little or no impact if the same medicine is given again in the future. The circumstances in which the medicine is stored are also a determining element in the drug's shelf life. The medication should be kept in a cool, dry location away from extremes of temperature, humidity, or light. Long-term exposure to light can cause chemical reactions, causing the drug's chemical properties to change and eventually degrade.
- Antimicrobial resistance can develop when a drug's potency is reduced. The latter is also the outcome of inappropriate medication administration. Antimicrobial resistance has now evolved into a major worldwide issue. To battle bacterial and fungal diseases, it's crucial to take the right antibiotic with the right potency at the right time.
- In many tropical nations, poor quality medications represent a critical yet underappreciated public health issue. In therapeutic research, policy choices and the implementation of high quality medications are critical. There are obstacles in the fight against low quality medications, particularly counterfeits.
- Third World countries have been increasingly threatened by counterfeit and substandard medications and pharmaceuticals that have lost their efficacy as a result of deteriorating economic situations and lax implementation of current pharmaceutical and customs rules. The distribution of low-quality products. Medicines are a critical clinical and public health problem in the poor nations.
- Problems include under or over concentration of ingredients, poor quality ingredients, poor stability, inadequate packaging and a decline in potency. [Alghasham et al 2018]
- Antimicrobial agents are most often tested against bacteria in the log phase of multiplication to produce the maximum bactericidal effect. In an infection, bacteria may multiply less optimally. Alekshun and Levy, 2007.

  The researchers studied the impact of a variety of antimicrobial drugs on gram-positive and gram-negative bacteria during non-growing and slow-growing stages. Only ciprofloxacin and ofloxacin were bactericidal (3-order-of-magnitude killing) against nongrowing gram-negative bacteria, and no medications were bactericidal against Staphylococcus aureus. Gentamicin (an aminoglycoside), imipenem (a carbapenem), meropenem (a carbapenem), ciprofloxacin (a fluoroquinolone), and ofloxacin (a fluoroquinolone) showed up to 5.7 orders of magnitude greater killing than piperacillin or cefotaxime against the extremely slowly growing gram-negative bacteria examined. This is in contrast to ideally grown bacteria, which were killed by a wide range of antibiotic classes with a 99.9% success rate. Antibiotic king was highly dependent on the growth rate of the gram-positive and gram-negative bacteria we studied. Slow death by chemotherapeutic drugs has uncertain clinical implications for established bacterial infections and infections involving foreign substances.
- Barbara Tourette prosser *et al* (1987) Antibiotics are often ineffective against organisms in exopolysaccharide biofilms, according to research. The effect of antibiotics on bacteria in formed biofilms is studied using a simple approach. Escherichia coli ATCC 25922 cells were suspended in buffer and dispersed on 0.5-cm 2 catheter disks after

being cultured overnight at 37°C on Mueller-Hinton agar. The disks were rinsed, moved to petri plates containing 20 ml of broth, and incubated for 20 to 22 hours at 37°C, during which time thick biofilms were formed. Disks were cleaned, put in broth or antibiotic broth, and incubated for 4 hours at 37°C. With 400,Ig of amdinocillin or cefamandole per ml, viable bacterial counts fell from 103 to 104 CFU/cm2 in 24 hours. In 24 hours, a combination of 400 micrograms of each antibiotic per ml reduced viable counts to undetectable levels (100 CFU/cm2). This method was used to test a variety of drugs and microbes.

MATERIALS & METHODS

Nutrient-Agar Media:

Requirement: Flask, Petriplates, cotton plugs, foil, peptone, beef extract, NaCl, agar, D/W, autoclave, laminar air flow chamber, weighing balance.

Composition of Nutrient Agar Media

Sodium Chloride =8gm		Sodium Chloride=0.8 gm	
Peptone	= 5 gm	Peptone	0.5 gm
Beef extract	= 3 gm	Beef Extract	0.3 gm
Agar	= 20 gm	Agar	2 gm
Distilled water	1000ml	Distilled water	100ml
pH	6.8	pH	6.8

Preparation of Nutrient Agar Slant/Petriplate

- Weigh each and every ingredients and transfer them to a conical flask containing D/W.
- After addition of every component, make up the final volume 1000 ml by adding D/W and adjust pH. Heat it a little to dissolve the components.
- Cotton plug the flask and cover its mouth with al foil and autoclave it.
- Along with the media, autoclave empty wide mouthed cotton plugged test tubes and petriplate at 121°C for 20 min at 15 partial pressure.
- After autoclaving transfer the material in laminar air flow cabinets & aseptically dispense 7-8ml of the medium into each sterilized test tube or 20-25 ml into the petriplates.
- Plug the tubes and also close the petriplates.
- For slant preparation place a 10 ml pipette or any wooden stick on the bench top and lean the tubes of melted agar. Do this properly so that the slanted surface of the medium extends to the bottom of the tube.
- Leave the tubes and petriplates until it solidifies. Slant used for storage of pure culture and petriplates used for streaking and isolating colonies.

Culturing of Microorganism By Streaking Method:

Pathogenic microbes were taken from lab and culture on nutrient agar plate by streaking method under sterile condition.

Morphological Characterization of Microbes by Gram Staining

Gram Staining is a method of separating bacteria into two main groups via differential staining (gram positive and gram negative). The nucleic acids of bacteria and background tissues are stained with the cationic dye crystal violet. Iodine is used to launder the crystal violet staining, resulting in a purple complex. Due to the impermeability of their cell walls, certain bacteria resist differentiation. The tissue background and some kinds of bacteria lost their staining when an appropriate differentiator (eg, alcohol or acetone) was used, but they took up a cationic dye (saffranine) of contrasting hue (typically red/pink) that was afterwards applied. The purple staining microorganisms are termed as "Gram positive", whereas the microorganisms that take up the counter stain (red/pink) are termed as "Gram negative".

Materials Required: Grams iodine dye, Saffranin dye, crystal violet, glass slides, inoculation loop, culture, distilled water, bacterial culture burner, and microscope.

- Take clean slides and add a drop of water on it.
- Use the loop to pick a culture in the inoculation loop and make a smear on the slide.
- Heat fix the slide.
- Flood the slide with crystal violet for 1 minute and wash away the excess stain in water.
- Flood the slide with Grams Iodine for 1 minute and wash under water.
- Decolorize with acetone and immediately wash under water.
- Counter stain with Saffranin for 45sec.
- Air dry the slides and observed under

microscope.

Collection of Different Drugs: Both expired and unexpired tablets of Ampicillin (500 mg), Norflox- TZ (1000 mg), Althrocin (250 mg) were collected from medical shops.

Preparation of Drugs: These capsules and tablets were dissolved in 10ml sterile water used for the experiments.

To Test the Effect of Expired and Non-Expired Drug On Bacteria By Agar Well-Diffusion Method:

The Agar Well-Diffusion test or the Kirby-Bauer Disk-Diffusion method is a means of determining the effect of an antimicrobial agent against different bacteria and fungus grown in culture.

Method:

- Nutrient agar media was prepared and plated under aseptic conditions.
- Wells were created at equal distance in the agar media by punching holes in the solidified agar with the help of a sterilized pipette tip.
- A drop of the melted agar was dropped into the well to seal the bottom of the well
- 200µl of the pathogenic bacteria inoculated in nutrient broth was spreaded nutrient agar plate
- 200µl of the expired drugs sample and 200µl of the non expired drugs sample was added in well 1 and 2 respectively.
- The plates were incubated at 37°C for 24 hours in incubator.

The antibiotics diffuses out from the wells into the agar in a gradient, so the agar closest to the well has the highest concentration and the concentration of the antibiotic decreases as antibiotic move further away from the well. The zone of inhibition was observed and further it was measured. This test was performed in order to test for the appropriate antibiotic concentration to be used. The four different antibiotics tested were Ampicillin, Combiflam, Norflox-TZ, Althrocin.

Effect of Drugs on Bacterial growth by Optical Density- Preparation of Nutrient Broth

Nutrient Broth is used to grow a wide variety of non-fastidious microorganisms. It is one of the nonselective medium used in ordinary microbial growth. Due to the presence of peptone and beef extract, this very basic formulation promotes the development of non-fastidious bacteria.**Materials Required:**

Composition of Nutrient Agar Media

Sodium Chloride =8gm		Sodium Chloride=0.8 gm	
Peptone	= 5 gm	Peptone	0.5 gm
Beef extract	= 3 gm	Beef Extract	0.3 gm
Distilled water	1000ml	Distilled water	100ml
pH	7	pH	7

Method:

1. Weigh the chemical ingredients of the respective media and transfer to a beaker/ flask containing 500ml of distilled water.
2. Gently heat the contents with slight agitation to dissolve the ingredients properly.
3. Pour more distilled water to make the final volume to 1 liter.
4. Measure the pH of the medium and adjust it to the required pH using HCl or NaOH depending on the PH
5. Cap the mouths of the flask using cotton plug and tightly cover with aluminum foil.
6. Autoclave the flasks with the ingredients at 121°c for 30 minutes.
7. After autoclaving nutrient broth media was transferred in test tube all work was done under laminar air flow
8. All pathogenic bacteria taken from lab and maintained on nutrient agar plate was inoculated in nutrient broth media poured in test tube by using the inoculated loops in laminar air flow
9. And 200 µl of all selected antibiotics were added into the bacterial culture separately and incubated for 24 hrs and O.D. was taken at 0 hrs and then strains were kept in incubator at 37°C for 24hrs and again O.D. was observed on the next day at 600nm.

RESULTS AND DISCUSSIONS

In the present study microbes provided by the lab were inoculated on nutrient agar plate. For this purpose, Nutrient agar media was prepared.

Inoculation of Bacteria on Nutrient Agar Plates: Two Bacterial cultures were taken from the lab and the streaked onto nutrient agar plates. The plates were kept in an incubator at 37°C for 24 hrs. Growth was observed.

Figure 1 Nutrient Agar Media

Figure 2 Strain 1

Figure 3 Strain 2

Morphological Identification of Bacteria

Gram Staining: It is done to distinguish between Gram positive and Gram negative bacteria.

Bacteria	Gram +/-	Shape
E.coli (Strain1)	Negative	Rod
Bacillus cereus (strain2)	Positive	Rod

Table 1 Result of Gram Staining

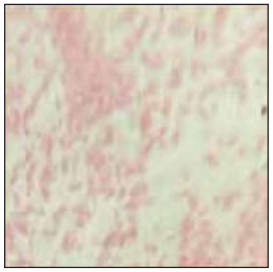

Figure 4 Microscopic result of E.coli and Bacillus subtilis

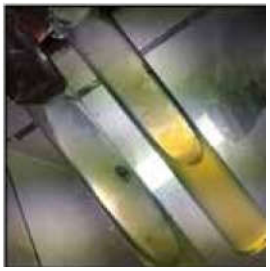

Figure 5 Maintained slants of both strains

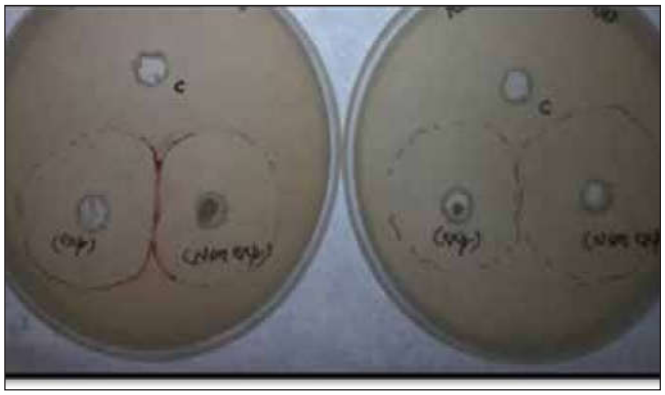

Figure 6 Well Diffusion result of expired and unexpired Ampicilin on E.coli and Bacillus cereus

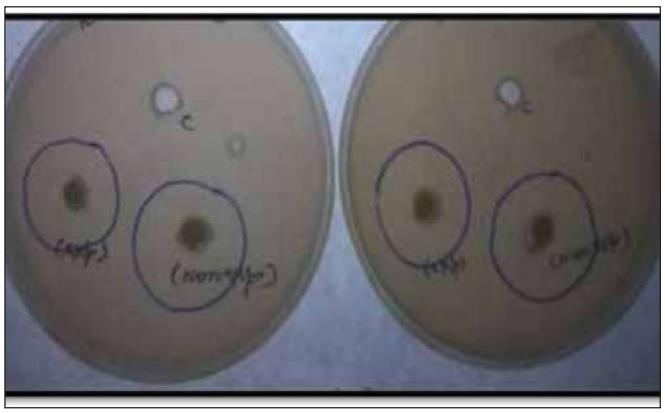

Figure 7 Well Diffusion result of expired and unexpired Norflox-TZ on E.coli and Bacillus cereu

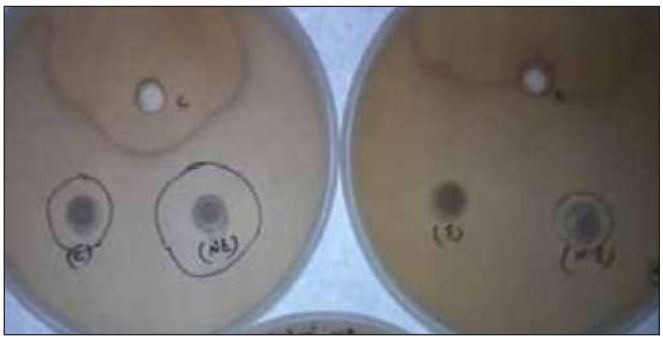

Figure 8 Well diffusion result of expired and unexpired Althrocin on E.coli and Bacillus cereus

Micro organisms	**Expired Ampicillin** (Zone of Inhibition in cms)	**Unexpired Ampicillin** (Zone of Inhibition in cms)
E.coli	4.1	4.8
Bacillus cereus	3.5	3.7
	Expired Norflox-TZ (Zone of Inhibition in cms)	**Unexpired Norflox-TZ** (Zone of Inhibition in cms)
E.coli	4.3	4.7
Bacillus cereus	2.3	2.5
	Expired Althrocin (Zone of Inhibition in cms)	**Unexpired Althrocin** (Zone of Inhibition in cms)
Shigella dysenteriae E.coli	1.7	2.5
Bacillus cereus	1.0	1.4

Table 2 Antimicrobial Activity of Microorganisms against expired and unexpired drugs.

Micro organisms	**Expired Ampicillin** (Zone of Inhibition in cms)	**After 24-Hour Incubation** (Zone of Inhibition in cms)
E.coli	0.076	0.025
Bacillus cereus	0.018	0.013
	Unexpired Ampicillin (Zone of Inhibition in cms)	**After 24-Hour Incubation** (Zone of Inhibition in cms)
E.coli	0.212	0.258
Bacillus cereus	0.019	0.028
	Unexpired Norflox-TZ (Zone of Inhibition in cms)	**After 24-Hour Incubation** (Zone of Inhibition in cms)
E.coli	0.533	0.528
Bacillus cereus	0.416	0.400
	Expired Norflox-TZ (Zone of Inhibition in cms)	**After 24-Hour Incubation** (Zone of Inhibition in cms)
E.coli	0.388	0.475
Bacillus cereus	0.391	0.683
	Unexpired Althrocin (Zone of Inhibition in cms)	**After 24-Hour Incubation** (Zone of Inhibition in cms)
E.coli	0.240	0.146
Bacillus cereus	0.161	0.145
	Expired Althrocin (Zone of Inhibition in cms)	**After 24-Hour Incubation** (Zone of Inhibition in cms)
E.coli	0.155	0.159
Bacillus cereus	0.107	0.115

Table 3 Data represents OD of Microbial growth in antibiotics in 600 nm.

Well Diffusion Method:

Effect of expired and unexpired drugs on microorganisms was checked by Agar Well Diffusion method:

1 Effect of Ampicillin

2 Effect of Norflox-TZ

3 Effect of Althrocin

Effect of expired and unexpired drug Ampicillin, Norflox, Althrocin were studied against these two bacteriae - *E.coli* and *Bacillus cereus*, and zone of Inhibition was measured. It was clear that expired drug had less effective in comparison to unexpired drugs.

When expired and unexpired drug was added to check effect drug on bacterial growth, 24 hr grown culture of each strain was taken and growth was estimated by spectrophotometer, and used this O.D. as a control and drugs were added in each tube and incubated for 24 hr and again growth was checked and O.D. was compared from initial O.D. in both types of drugs. From O.D. it was clear that effect of expired drug showed lesser effect on bacteria so O.D. is higher in expired drugs, while O.D. was lesser in case of non-expired drugs.

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CONCLUSIONS

- The study shows the broad spectrum of the Forensic pharmacy as the name states to examine the illegal drugs.
- Most commonly illegal scope in the pharmacy is the re-stamping the unexpired drugs. So the study made to the harmful effects of taking the expired drugs.
- Present study concluded that unexpired drugs showed maximum zone of inhibition as compared to expired drugs.
- Unexpired drugs have significantly inhibited the growth of microbes as compared to expired treatment.
- The expired forms of antibiotics lose their efficacy drastically.
- Thus, expired medical products are less effective or dangerous due to a change in chemical composition or a decrease in strength/biological half-life.

The present study concludes that unexpired drugs showed maximum zone of inhibition as compared to expired drugs. Unexpired drugs have significantly inhibited the growth of microbes as compared to expired treatment.

The expired forms of antibiotics lose their efficacy drastically. Thus, expired medical products are less effective or dangerous due to a change in chemical composition or a decrease in strength. IJFMP

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