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A review of the literature on antimicrobial preservatives: Definition, properties, classification, safety, side effects and antimicrobial effectiveness testing

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ABSTRACT

Antimicrobial preservatives are excipients that are utilized in both sterile & non-sterile pharmaceutical products that are given in several doses. Antimicrobial preservatives are mainly utilized to prevent the growth of germs that may arise throughout the course of a product's uses. It is necessary to determine the efficiency of antimicrobial preservatives in the case of certain excipients. It is discussed in this article the most important aspects to consider when selecting preservatives, the principles of the preservative effectiveness test, and the relevance of regulatory requirements for preservative effectiveness testing in the practice of medicine. Antimicrobial effectiveness testing is required to establish the efficacy of preservative systems in multidose products that are carried out by the manufacturer.

KEYWORDS

Antimicrobial preservatives; microbes; excipients

1. DEFINITION

The pharmaceutical products which contain an aqueous vehicle (e.g. syrups, powders for oral suspensions & external products) need safeguarding against microbial contamination, which can disturb product stability or infect the customers. Therefore, antimicrobial agents are utilized in these product formulations [1-4].

Additionally, antimicrobial preservatives are employed to prevent non-sterile dosage forms against microbiological development or from bacteria introduced inadvertently while the manufacturing process. Preservatives, which are utilized as additives in the manufacturing of cosmetics

and pharmaceuticals, are often found in these goods. In the packaging of sterile goods packaged in multiple-dose containers, antimicrobial preservatives are utilized, in order to prevent the formation of bacteria as a result of the repeated withdrawal of individual doses from the container [2,3].

The antimicrobial agents are very harmful compounds that could be avoided. For maximum patient safety, the preservative concentration that has been demonstrated to be active in the final packaged product would be at a level that is not harmful to individuals. If the active ingredients in the product formulation have inherent antibacterial properties, the antimicrobial preservative concentration might be at minimum. Whether antimicrobial efficacy is inherent in the product composition or results as a consequence of the usage of an antimicrobial preservative, it could be successful [1].

2. PROPERTIES OF THE IDEAL PRESERVATIVE [5]

A. It is effective against a broad range of species even at low concentrations, which is a plus.
B. Chemical and physical stability under typical usage settings throughout a broad pH and temperature range (it could sustain activity while the product manufacturing, shelf life, and usage) are important considerations.
C. At the concentrations necessary, it is soluble in water.
D. It is compatible with a broad range of pharmaceuticals & excipients.
E. Does not have an odor, taste, color, or stinging sensation.
F. Toxic & sensitizing properties are not present at the recommended dose.
G. It could not be a source of irritation.
H. A substance that has no reaction with the container or the closure (i.e., it does not adsorb, penetrate, or interact with the container or the closure).
I. Reasonable pricing.

3. USES OF PRESERVATIVES

The usage of preservatives in product preparations could be considered whenever the chance of microbiological contamination and growth exists, whether during the preparation process or the patient's usage of the product [6,7].

Antimicrobial agents are required for the administration of the following dosage forms: [8]

1. *Aqueous emulsions* (including semisolid or ointment-type formulations): Because the aqueous phase is conducive to the development of microbes, all aqueous emulsions need an antimicrobial ingredient.

2. Suspensions: In order to guard against bacteria, yeast, & mold contamination, suspensions "intended for any route of administration" could include an antimicrobial agent that is appropriate for the application.

3. Oral Solutions: It is also common for antimicrobial compounds to be added in order to inhibit the development of bacteria, yeast, & molds.

4. Ophthalmic solutions: Each solution must include an appropriate material or combination of substances that will inhibit the development of microorganisms mistakenly introduced when the container is opened while usage, or that will kill microorganisms that are accidentally introduced when the container is opened while usage. Antibacterial agents cannot be utilized in ophthalmic solutions when they are intended for usage in surgical procedures, despite the fact that they could be sterile. Antibacterial agents may be potentially irritating to the eyes.

5. Ophthalmic Ointments: A proper substance or a mixture of substances could be present in ophthalmic ointments in order to prevents bacteria that are accidentally introduced while in uses, unless otherwise indicated in the specific monograph or the formula itself is bacteriostatic to inhibit the development of microorganisms.

6. Parenteral Products [9]: When using injectable preparations for multiple-dose administration, a suitable material or combination of chemicals could be added to limit the growth and development of germs. This is true regardless of whether the preparation is sterilized by heat, cold, or any other means.

Preservatives are utilized to: 1- Prevent the product from being attacked by microorganisms. 2- Improve the activity and effectiveness of the medicine 3- Extend the shelf life of the substance. 4- Improve the stability of the product by preventing change and destruction by microbes while it is being stored.

It is not necessary to add a preservative in the following cases: [10]

1- As soon as the product is received, it will be put to usage. The product is created using appropriate procedures that minimize the possibility of contamination throughout the preparation and administration processes.

2- There is no water to be found. Because microorganisms need water for development, preparations that do not include water, as an example tablets, powders and hydrocarbon ointments, do not serve as growth media, except for ocular and non-aqueous injections, which do serve as growth media.

3- The pH of the medium is either neutral or slightly alkaline. < 3, or > 9. Note: Despite the fact that this pH range for suppression of growth is true for the majority of microorganisms, several resistant molds have been proven to flourish in medium with higher pH levels with pH < 3.

4- Antimicrobial characteristics are already present in the formulation due to the presence of one or more antimicrobial ingredients.

Preservatives are contraindicated in the following situations: 1. Newborns & infants. 2. Ophthalmic solutions designed for usage in the eyes while eye surgery, using non-intact ophthalmic solutions, corneal transplantation or intraocular injection. 3. Parenteral products in large quantities > 30 ml.

4. CLASSIFICATION OF PRESERVATIVES

Preservatives are classified based on a variety of factors, including the following: mechanism of action, chemical class, and source.

4.1. Classification based on mechanism of action [11]

1-Antioxidants: These substances work by preventing the oxidation of the active pharmaceutical ingredients (and/or other excipients) in the formulation. They are self-reducing agents that oxidize themselves while also prevent oxidation from occurring in the surrounding environment. Consequently, components that are prone to oxygen oxidation are affected. They can be relied upon as a good protection against degeneration, which takes into account the two most important factors: oxygen & sunshine. Vitamin E, Vitamin C, butylatedhydroxylanisole (BHA), and butylatedhydroxytoluene are examples of such compounds (BHT, propyl gallate, and so on).

2-Antimicrobial agents' antibiotics: These antimicrobial medicines are effective against both gram positive & gram negative microorganisms; induces the decomposition of pharmaceutical formulation (e.g. benzoates; include sodium benzoate & benzyl benzoate).

3-Sorbates chelating agents [12]: These agents combine with the medicinal component to produce a compound that prevents degradation. Pertaining to the pharmaceutical formulation, Disodium ethylenediamine tetraacetic acid (EDTA), polyphosphates, and citric acid, are examples of such substances.

4.2. Classification based on chemical class [13-15]

1-Alcohols & glycols:

a) Ethyl alcohol: It is common to find pharmaceuticals & cosmetics that include varied amounts of ethanol or aqueous ethanol. The bactericidal & antibacterial preservative properties of ethanol extend beyond its traditional role as a solvent. As a solvent, disinfectant, stabilizer, & water-miscible co-solvent, propylene glycol is utilized in a wide range of medicinal & cosmetic products. It is comparable to ethanol as an antiseptic, and it is just slightly less efficient against molds than ethanol.

b) Glycerin [13,15]: Glycerin is incorporated in various medicinal formulations comprising oral, otic, ophthalmic, topical, and parenteral medicines. It is utilized as an antimicrobial preservative, co-solvent, emollient, humectant, plasticizer; sweetening agent, tonicity agent; solvent.

c) Benzyl Alcohol [13,16]: Benzyl alcohol is an antimicrobial preservative that may be found in a range of products including cosmetics, foods, and a variety of pharmaceuticals, among other things. In addition to its bacteriostatic properties, benzyl alcohol is an antimicrobial preservative that is effective against Gram-positive bacteria, as well as molds, fungi, and yeasts, among other things. Benzyl alcohol has a weak antibacterial impact on the vast majority of Gram-positive organisms. Although select Gram-positive bacteria are very sensitive to the substance's antibacterial properties. In general, benzyl alcohol has a lower potency against Gram-negative organisms than it does against Gram-positive organisms, according to the literature. In spite of the antibacterial characteristics of benzyl alcohol, molds and yeasts have developed resistance to it.

d) Isopropyl Alcohol [13,17]: Isopropyl alcohol is widely used as solvent in topical treatments, and as a cosmetic element in both cosmetic and pharmaceutical products. Additionally, it is utilized as a disinfectant and an antimicrobial preservative, in addition to its other purposes. At concentrations more than this threshold, isopropyl alcohol at a concentration greater than 70% (vol/vol) is bactericidal. It is a more effective antibacterial preservative when compared to ethanol 95 percent by volume (v/v).

e) Phenol [13,18]: Phenol is primarily utilized as an antibacterial preservative in parenteral pharmaceutical products, as an example intravenous fluids. It has also been utilized in the formulation of topical medicinal treatments & cosmetic items. It has a wide range of applications as an antiseptic & disinfectant.

f) Chlorocresol [13,19]: When utilized as an antibacterial preservative in cosmetics & pharmaceutical compositions, chlorocresol is very effective. Chlorocresol has bactericidal action against both Gram-positive & Gram-negative organisms (including Pseudomonas aeruginosa),

spores, molds, & yeasts. It also has antifungal activity against molds & yeasts and antibacterial activity against bacteria.

2-Organic acids:

a) Benzoic Acid [13,20]: Benzoic acid is a preservative that is extensively utilized in cosmetics, foods, & medications to prevent the growth of bacteria. Benzoic acid is also utilized as an antifungal agent in topical medicinal preparations, as an example Whitfield's Ointment & other similar products. Only the undissociated acid exhibits antibacterial capabilities, & the activity of the acid is consequently dependent on the pH of the media in which it is utilized. When it comes to Gram-positive bacteria, benzoic acid has considerable bacteriostatic activity against the vast majority of species, with less action against Gram-negative bacteria in general. Molds & yeasts are also inhibited to a considerable extent by this compound.
b) Sodium Benzoate [13,21]: Sodium benzoate is largely employed as an antibacterial preservative in cosmetics, foods, & pharmaceutical formulations, except for pharmaceutical formulations. It is also used as a lubricant for tablet & capsule formulations. It is sometimes preferred over benzoic acid in particular situations because of its increased solubility, which is one of the reasons behind this. Sodium benzoate has antibacterial & antifungal effects, making it a useful ingredient in many products.
c) Potassium Benzoate [13,22]: Potassium benzoate is utilized as an antimicrobial preservative in a wide range of beverages, foods, and medicinal formulations, with the primary goal of preventing the development of germs. It is becoming more common to uses potassium benzoate in place of the more often utilized sodium benzoate in applications where a low sodium content is desirable, such as food preparation. Phosphoric acid (KOH) is a chemical substance that is extensively utilized in the production of food products and is generally regarded as non-toxic and non-irritant.
d) Sorbic Acid [13,23]: In medicines, foods, enteral preparations, & cosmetics, sorbic acid is an antimicrobial preservative having antibacterial & antifungal characteristics that is utilized as a preservative. It is generally employed as an antifungal agent, but it also has antibacterial qualities that make it useful in other applications. Given its low stability & efficacy against bacteria, sorbic acid is commonly utilized in conjunction with other antimicrobial preservatives or glycols when synergistic effects seem to be occurring in the product.
e) Potassium Sorbate [13,24]: Potassium sorbate is an antimicrobial preservative having antibacterial & antifungal activities that is utilized in medicines, foods, enteral preparations, & cosmetics.

Potassium sorbate is also utilized as a preservative in cosmetics. When potassium sorbate is taken in conjunction with other ingredients, its effectiveness is boosted.

Because of the synergistic effects of antimicrobial preservatives and glycols, they could be avoided. Potassium sorbate is a chemical compound. Because of this, sorbic acid is used in about twice as many medicinal formulations than citric acid, because of its higher solubility and stability in water.

3- Esters of p-Hydroxybenzoic Acid (Parabens) [13,25,26]:

Parabens are especially effective against yeasts and molds because they are effective throughout a wide pH range and have a broad spectrum of antibacterial activity. In order to compensate for the low solubility of parabens, the paraben salts, most notably the sodium salt, are commonly used in cosmetic formulations to enhance their efficacy. To a higher extent than they do against bacteria, parabens contain antifungal and antibacterial properties as well. Furthermore, they are more effective against Gram-positive bacteria than they are against Gram-negative bacteria, which is a significant advantage. The synergistic actions of parabens make it feasible to increase activity by mixing them in various formulations and products. The activity of parabens increases as the length of the alkyl moiety of the molecule is increased; nevertheless, their solubility decreases as the length of the alkyl moiety of the molecule is increased. As a result, mixtures of methyl-, ethyl-, propyl-, & butylparabens are often employed in conjunction with one another. The parabens Methylparaben & Propylparaben are the most often used.

a) Methylparaben [13,27]: Methylparaben is a chemical preservative that is extensively used in cosmetics, culinary goods, & pharmaceutical formulations as an antibacterial. It could be used alone as well in combination with other parabens or antimicrobial compounds to treat a wide range of skin issues. However, while it is the least active of the parabens, the antibacterial activity increases in direct proportion to the length of the alkyl moiety's chain, making it the most active. Both methylparaben & propylparaben have been used to extend the shelf life of a variety of medicinal formulations throughout the years.
b) Methylparaben Sodium [13,27]: Because of its superior water solubility compared to methylparaben, methylparaben sodium has the potential to be replaced for methylparaben in several applications.
c) Propylparaben [13,28]: Propylparaben is a preservative that is frequently used in cosmetics, culinary goods, and pharmaceutical formulations because of its antibacterial properties. If used alone, or in combination with other paraben esters (or with other antimicrobial agents), it has the potential to be

quite effective. It is one of the most widely used preservatives in cosmetics, accounting for about a quarter of all applications.

d) Propylparaben Sodium [13,28]: Propylparaben sodium is an antibacterial or antifungal preservative that is included in many water-based cosmetics, as well as in oral medications & oral care products. This paraben ester is often used in conjunction with other paraben esters.

4- Organic mercurial derivatives:

a) Phenylmercuric salts [13,29]: Phenylmercuric salts are growth-inhibiting agents with a wide range of activity when employed at the doses typically utilized for the preservation of medicines. They have bactericidal & fungicidal action that is sluggish to develop. It is primarily utilized as an antibacterial preservative in ocular preparations; however, it is also found in cosmetics, pharmaceutical formulations for injection, & topical pharmaceutical formulations for application. When utilized against bacteria & fungus, phenylmercuric salts have a broad pH range of activity.

b) Phenylmercuric Acetate [13,30]: Cosmetics and medicines, with a concentration of less than 0.007 percent of mercury estimated as the metal, can use phenylmercuric acetate as an alternate antibacterial preservative. Given its superior solubility over phenylmercuric nitrate, it is preferred. There are several uses for Phenylmercuric Acetate. Preservative phenylmercuric acetate, like phenylmercuric nitrate, has broad-spectrum antibacterial action & delayed bactericidal & fungicidal activity.

c) Phenylmercuric Nitrate [13,31]: To preserve ophthalmic preparations, phenylmercuric salts are often employed as antimicrobial preservatives, but they are also used in cosmetics, parenteral pharmaceuticals, & topical pharmacies.

d) Phenylmercuric Borate [13,32]: In place of phenylmercuric acetate or phenylmercuric nitrate, phenylmercuric boreate is employed as an antibacterial preservative. Both phenylmercuric acetate & phenylmercuric nitrate have been shown to be more irritating than methylmercuric nitrate. When it comes to antimicrobial action, phenylmercuric borate is comparable to phenylmercuric nitrate in its gradual, but steady, bactericidal & fungal activity.

e) Thimerosal [13,33]: Preservative Thimerosal has been used in biological & pharmaceutical products since the 1930s. Parenteral & topical medicinal formulations both uses it as an antibacterial preservative. Bacteriostatic and fungal activity are both present in this preservative, making it a viable alternative to benzalkonium chloride. Concerns over thimerosal's uses in medications have risen in recent years due to an expanded understanding of mercury's toxicity

and that of other mercury compounds. It has been suggested that thimerosal could not be used as a preservative in eye drops or vaccinations because of a growing number of reports of adverse responses, especially hypersensitivity. Additionally, cosmetics & soft contact lens solutions include thimerosal, which is also used in these applications.

5- Salts of quaternary ammonium base:

a) Benzalkonium Chloride [13,29]: In pharmaceutical formulations, it is used as an antimicrobial preservative in a similar way to that of other cationic surfactants such as cetrimide. It is a quaternary ammonium chemical that is used as an antimicrobial preservative in a similar way to that of other cationic surfactants such as cetrimide. It is one of the most widely used preservatives in ophthalmic preparations, accounting for about one-third of all applications. This preservative or excipient is used in conjunction with other preservatives or excipients, notably disodium edetate, to increase its antibacterial effectiveness against strains of the bacterium Pseudomonas. A broad variety of bacteria, yeasts, & fungi are susceptible to the activity of benzalkonium chloride solutions. Active against Gram-positive bacteria is more pronounced than against Gram-negative bacteria, & activity against bacterial endospores & acid-fast bacteria is low. When it comes to antibacterial action, benzalkonium chloride is highly influenced by the alkyl content of the chemical combination in which it is used. It is also employed in nasal & otic forms, and occasionally in conjunction with thimerosal to improve effectiveness. Benzalkonium chloride is also used as a preservative in small-volume parenteral preparations, where it is very effective.

b) Benzethonium Chloride [13,34]: It is an antibacterial preservative in pharmaceutical formulations that is a quaternary ammonium compound to protect against the growth of bacteria. A typical usage for this ingredient would be injectable, ophthalmic & oral formulations. The wetting & solubilizing agent benzethonium chloride, as well as a topical disinfectant, are further applications for this compound. Benzethonium chloride is a preservative that can be found in cosmetics (e.g.) deodorants. It can be used to kill bacteria. Traditionally, it has been used as a disinfectant & topical anti-infective agent in therapeutic settings. In these applications, however, it is no longer widely used due to the availability of more powerful antimicrobials, & it is now mostly reserved for usage as a stabilizer or preservative in only a small number of pharmaceutical & cosmetic formulations.

c) Cetylpyridium Chloride [13,35]: The antimicrobial preservative cetylpyridinium chloride is a quaternary ammonium cationic surfactant utilized in pharmaceuticals & cosmetic formulations. It is also known as cetylpyridinium chloride. As an antiseptic agent, it is used in a variety of therapeutic applications, including oral & throat care. It can be used alone or in conjunction with other

medications. It is also employed in nonparenteral formulations, as well as oral & inhalation preparations. It has been shown that mouthwashes containing cetylpyridinium chloride can reduce plaque development. It is bactericidal against Gram-positive bacteria, but only moderately effective against certain Gram-negative germs, according to the manufacturer. Cetylpyridinium chloride is also antimicrobial, and it has been shown to be effective against a variety of oral bacteria.

4.3. Classification based on source [11]

1- Natural Preservative: These agents are acquired from natural sources as an example plants, minerals, animals, and so on. These agents are mostly employed as food preservatives and include, for example, Neem oil, salt (sodium chloride), lemon, and honey (to name a few).

2- Preservatives synthesized in a laboratory: Chemically synthesized preservatives (e.g. benzoates, sodium benzoate, sorbates, propionets, nitrites) are used in the production of these products (Table 1).

Table 1. Some common preservatives used in pharmaceutical formulations

Preservatives	% Concentration in preparations			
	Oral Liquid	Ointments / Creams	Opthalmic / Nasal	Parenteral
Methylparaben	0.25	0.001-0.2	0.1	0.01-0.5
Ethylparaben	0.1- 0.25	0.001-0 .2	0.1	0.01-0.5
Propylparaben	0.5- 0.25	0.001- 0.2	0.1	0.005-0.02
Butylparaben	0.1- 0.4	0.001- 0.2	0.1	0.015
Benzyl Alcohol	3.0			0.5-10
Chlorobutanol	0.5	0.5	0.5	0.25-0.5
Phenol	0.1-0.5	0.25-0.5		0.065-0.02
Meta cresol	0.15-0.3	0.1-0.3		0.1-0.25
Chlorocresol	0.2	0.1-0.3		0.1-0.18
Benzoic acid	0.1-0.2			
Sorbic acid	0.1-0.2			
Thiomersal	0.1	0.01	0.01	0.01
Phenylmercuric nitrate	0.002-0.1	0.002	0.004	0.002
Propylene Glycol	15-30			
Benzylkonium Chloride	0.002-0.02	0.01	0.004-0.02	0.01
Benzethonium Chloride	0.01-0.02	0.01	0.004-0.01	0.01

5. SAFETY AND SIDE EFFECTS OF PRESERVATIVES [36-39]

In order to make an informed decision on a preservative system, it is important to evaluate the possibility of negative side effects. The low concentrations & low likelihood of harmful effects could be weighed against these concerns, which could be assessed against the risks of exposure.

It is preferable if a preservative is exclusively active against microorganisms & has minimal in which there are no adverse effects on mammalian cells. According to current practice, most preservatives have some effect on both microbial and mammalian cells, thereby extending the scope of this task. Alcohol is widely regarded as a harmless substance. The usage of benzyl alcohol in parenteral products, on the other hand, is not advised owing to the risk of lethal toxic syndrome in low-weight infants. The likelihood of developing sensitivity to benzyl alcohol in topical preparations is typically minimal. In a similar vein, the long chain alkyl alcohols cetyl & stearyl alcohol, which have a large chain length, are uncommon sensitizers.

Phenylethanol is a chemical that can cause minor irritation to the skin, eyes, & mucous membranes.

Carboxylic acids, as an example benzoic acid, can cause gastro-intestinal irritation as well as minor irritation of the skin, eyes, & mucous membranes. In nature, sensitivity is often thought to be of a moderate kind. As far as sensitivity to sorbic acid is concerned, it is believed to be rare. Allergic dermatitis & allergic conjunctivitis, on the other hand, are regarded to be rather uncommon conditions in the natural world. There have been no reports of systemic toxicity.

Because of their irritancy, parabens are deemed undesirable for usage in parenteral & ophthalmic products. There have been multiple cases of delayed hypersensitivity reactions to their topical usage, according to the FDA. These kind of responses, on the other hand, are rather rare.

Phenol-related adverse events are rather uncommon, which is likely due to the low inclusion levels that are normally utilized in the manufacturing process. In any ten-hour period, the total dose could not be more than 50mg. Despite the fact that chlorocresol is less hazardous than phenol, it potentially irritating to the skin, eyes, & mucous membranes. For intrathecal, intracisternal, & peridural injections, it is not permitted to be used. It has been observed that chloroxylenol can cause cross sensitivity. Chloroxylenol, on the other hand, is typically regarded to be less irritating than chlorocresol in most cases. The usage of hexachlorophene has decreased as a result of concerns about its neurotoxicity.

Despite the fact that sensitivity to quaternary ammonium compounds (QACs), such as benzalkonium chloride (BKC) is rare, it is a contact irritant that may exacerbate pre-existing dermatitis. BKC was ruled out for use in contact lens solutions due to the possibility that it might bind to soft

lenses and produce ocular pain as a consequence. The highest concentration of benzethonium chloride for ophthalmic and parenteral formulations is 0.02 percent, which is recommended as the maximum concentration. Some asthmatics can get bronchoconstriction as a result of BKC. Overdosing with solutions at concentrations greater than 0.03 percent can need immediate medical intervention.

The usage of organo-mercurial preservatives in topical & parenteral formulations has been widely documented. However, worries regarding the toxicity of mercurial compounds are often stated, resulting in usage restrictions, as an example the usage of mercurial compounds intra-vaginally. When utilized as eye drops, phenylmercuric salts have the potential to produce mercurialetis (a kind of mercuria). Although the incidence is modest & does not result in vision impairment, the medication is not recommended for long-term usage. Concentrations of 0.1 percent or higher in phenylmercuric salt are known to cause skin irritation. Thimerosal is a powerful sensitizer, & it is found in high concentrations in topical products. Users of soft contact lenses can have allergic reactions, with up to 10% of those who usage them potentially affected. Because of the possibility of hypersensitivity, it should not be used in either eye drops or vaccinations. Indeed, the European Medicines Agency & the Food & Drug Administration have advised that thimerosal be phased out of vaccinations in general.

EDTA is a preservation enhancer that is sometimes used. When it is employed in nebulizer solutions, it has been shown to cause dose-related bronchoconstriction, and its usage in these products is currently discouraged. When administered in people with renal impairment, EDTA salts might induce nephrotoxicity. Therefore, they should be avoided. EDTA has a high affinity for chelating calcium, which can result in calcium depletion. Although there have been cases of toxicity, EDTA is usually regarded to be safe.

6. ANTIMICROBIAL EFFECTIVENESS TESTING (AET) [40]

Despite the fact that chemical testing for preservative content is the feature that is often included in specifications, the efficiency of antimicrobial preservatives could be proved throughout development, while scale-up, and while the shelf-life. It is necessary to conduct an antimicrobial efficacy test in order to determine the efficiency of preservative systems in multi-dose dosage forms. Multi-dose products that do not include any extra preservatives must pass the test to prove that the product's inherent antibacterial powers are active. According to USP38, this test is carried out. An assessment of a product's preservative system's level of biological activity is what this test is designed to measure, according to the manufacturer. Product development employs the test to evaluate a

product's effectiveness, whereas stability uses the test to ensure that the preservative system is stable over time. Traditional quality control release tests are not meant to be used with this test. There is no way that this test can ever be a substitute for good production methods.

As part of the testing procedure, microorganisms are added to a predetermined amount of the product to assess its efficacy. Use of original containers is preferred if possible. Before using the containers, they must be incubated at room temperature for 28 days while being kept out of direct sunlight. As part of the compendial guideline materials, the mortality rate is computed & compared to accepted standards. There must be a thorough examination of the product's preservative system to ensure that it will continue to work properly over time without gathering harmful bacteria. In order to show that a product has been effective throughout the course of its entire life cycle, it is well-known that product testing must occur while the development phase as well as at the ultimate stability point.

Articles from the compendial collection have been grouped into four categories for the purpose of testing (Table 2). It is important to note that the method of administration has an impact on the antimicrobial efficacy criterion for these drugs. It is assumed that formulations including preservatives would fulfill basic efficacy criteria, regardless of whether they are packaged in multi-dosage containers or unit dose containers. Tables 3 & 4 include information on the microorganisms and medium employed, as well as the criteria for tested microorganisms. The test is not intended to be used as a quality control release test in the traditional sense. In no case the test should be considered a replacement for appropriate production procedures. The test includes inoculating a known quantity of microorganisms into a known amount of product in order to determine the effectiveness of the product. When at all practicable, the original containers are utilized for the assay. The containers are kept out of direct sunlight & incubated at room temperature for 28 days before being utilized. While a 28-day period, the mortality rate is calculated and compared to the acceptance criteria provided in the compendial guideline materials.

Table 2. Compendial product categories

Category	Product Description
1	Administered through intramuscular injection or other parenteral routes, as an example intravenously or intravenously via the skin
2	Nonsterile nasal products, emulsions, and other topically utilized aqueous-based products, as well as those applied to mucosal membranes
3	Non-antacid oral products, as an example those prepared using water-based bases or vehicles.
4	Aminoglycosides that are based on water.

Table 3. Microorganisms and media used

Bacteria	*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*	Tryptic Soy Agar (TSA)
Yeast	*Candida albicans*	Sabouraud Dextrose Agar (SDA)
Mold	*Aspergillus niger*	Sabouraud Dextrose Agar (SDA)

Table 4. Criteria for tested microorganisms

For Category 1 Products	
Bacteria	A 1.0-log decrease at 7 days was followed by a 3.0-log decrease at 14 days & a 0 log gain at 28 days relative to the baseline count.
Yeast & mold	No increase in day count from the original calculation at 7, 14, or 28 days
For Category 2 Products	
Bacteria	As of day 28, there was a 2.0 log decrease from the 14-day count & no rise from the 14-day count.
Yeast & mold	At 14 & 28 days, there was no change from the baseline total.
For Category 3 Products	
Bacteria	A 1.0 log decrease from the initial 14-day count and no increase in the 14-day count at the end of the 28-day period.
Yeast & mold	At 14 & 28 days, there was no change from the baseline total.
For Category 4 Products	
Bacteria, Yeast, & Molds	At 14 & 28 days, there was no change from the baseline total.

7. CONCLUSIONS

Adding antimicrobial preservatives reduces the risk of producing contamination from microorganisms, whether the product is being used or stored. It is critical to show that the agents work as intended and are safe for human consumption if at all feasible. For products that have antimicrobial qualities, they must also be tested for their efficiency in killing bacteria. All multi-dose products need proofs of antimicrobial efficacy before they are potentially sold. Preservative selection is critical. Preservative selection is influenced by a number of variables, including pH, dose form, and any additional excipients that can interact with the preservative.

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CONFLICTS OF INTEREST

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