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# Comparison between chemical and natural disinfectant against *Escherichia coli* and *Streptococcus pyogenes*

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**Abstract :** *Disinfectants are used in our day today life for many purposes like cleaning floor, washing clothes, cleaning toilets, sinks, etc. So, its property is needed to be checked. In this work two categories of disinfectants were used; that are chemical (Harpic and Dettol) and natural disinfectants (Neem and Garlic) and their efficiency was checked against the two selected bacteria- Escherichia coli and Streptococcus pyogenes. Sensitivity test for bacteria against different disinfectants was checked one by one using 'Kirby-Bauer Disc Diffusion Sensitivity Testing. The bacterial suspension was spread on nutrient agar media and filter paper disc loaded with particular disinfectant was placed on it and incubated at 37° C for 24 hrs. Afterwards observation was taken for "zone of clearance i.e. inhibitory zone" and "Minimum Inhibitory Concentration (M.I.C)".The above protocol was followed thrice in triplicate for each disinfectant against each bacterium, so that exact and accurate results can be interpreted. It was found that chemical disinfectant were more efficient than natural disinfectant. Natural disinfectant besides being less efficient was proved to be effective against bacteria to a great extent. So, it can be said that besides being less efficient natural disinfectant can't be neglected and can be used in our day to day life. It also overcomes the disadvantages of chemical disinfectants like they are not allergic to humans, they are not hazardous, cost effective and easily available.*

***Key words :*** *Disinfectant, Escherichia coli, Streptococcus pyogenes, Zone of Inhibition, Minimum Inhibitory Concentration (M.I.C.).*

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## Introduction :

In this work, the main objective was to compare the disinfection ability of chemical and natural disinfectants on the viability of *Escherichia coli* and *Streptococcus pyogenes*, as well as to figure out the disinfectant efficiency on the two specified bacterium. Main focus was to check the

effectiveness of disinfectant, so that it can be applied in our day to day life.

Disinfectants are antimicrobial agents that are applied to non-living objects to destroy microorganisms that are living on the objects. Disinfection does not necessarily kill all microorganisms, especially resistant bacterial spores. It is less effective than sterilization techniques. Disinfectants are different from other antimicrobial agents such as antibiotics, which destroy microorganisms within the body and antiseptics, which destroy microorganisms on living tissue. Disinfectants work by destroying the cell wall of microbes or interfering with the metabolism.

Disinfectant can be of two kinds chemical as well as natural. Harpic and Dettol (synthetic organic disinfectant) were used as chemical disinfectant while Neem and Garlic as natural disinfectant. The selection of disinfectants (natural and chemical) was not at random rather it was chosen on the basis of chemical components present in them that are very much reliable as disinfectants. Some other reason behind choosing them were the day to day use of those disinfectant, in our household activity since long as well as trust of large number of population on these disinfectants that they kill germs for sure.

Harpic (disinfectant) is not just a solution of hydrochloric acid and water. The function of surfactants present is to reduce the surface tension of water and allow the aqueous solution on which Harpic is based i.e. to wet the surface of the toilet effectively. This allows product to spread over the surface rather than form droplets. Ammonium chloride is used in Harpic as it acts as a thickening agent so that the product will cling to the sides of the toilet pan and remain effective through many flushes.

Dettol is effective as antibacterial agent due to presence of Chloroxylenol. Other ingredients of Dettol are Terpinol, denatured absolute alcohol and Caramel for its brown color. It is effective against most of the common bacteria that cause infections (Mahmood et. al., 2008).

*Azadirachta indica*, is scientific name for Neem, belonging to the Meliaceae family. Salimuzzaman Siddiqui was the first scientist who studied about the various antimicrobial like antifungal, antibacterial and antiviral constituents of the Neem tree. In 1942, he extracted three bitter compounds from neem oil, which he named as nimbin, nimbinin, and nimbidin respectively (Zillur Rahman and Jairajpuri, 1993). These compounds are stable and found in substantial quantities in the Neem. There are almost 25 compounds of this category found in neem and are commonly called azadiracchin together. These compounds have great antibacterial efficiency (Biswas et. al., 2002).

*Allium sativum*, commonly known as garlic, is a species in the onion genus, *Allium*. Among the members of the onion family, garlic has by far the highest concentrations of initial reaction products, making garlic much more potent than onion, shallot, or leeks. It contains alliin and allicin that are responsible for its antibacterial properties. They are sulphurous compounds that are recovered when garlic bulb is crushed. They enter bacterial cell and causes its lysis by hindrance in its metabolism (Hamel et. al., 1975).

Selection of *E. coli* and *S. pyogenes* as bacteria to test on was based on the fact that they are highly pathogenic and cause of many hazardous disease. It was hypothesized, if the selected disinfectants could inhibit these two selected bacterium which are so pathogenic, then they would defiantly inhibit and kill wide range of infectious germs.

*Escherichia coli* is a Gram-negative, facultative anaerobic and rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of warm-blooded organisms (P. Singleton, 1999). Most of the *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in their hosts, and are occasionally responsible for product recalls due to food contamination.

*Streptococcus pyogenes* is a spherical, Gram-positive bacterium. *S. pyogenes* displays streptococcal group A antigen on its cell wall and typically produces large zones of beta-haemolysis (the complete disruption of erythrocytes and the release of haemoglobin) when cultured on blood agar plates, and is therefore also called group A (beta-haemolytic) Streptococcus (GABHS or GAS).*S. pyogenes* is the cause of many important human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases (Jim Dwyer, 2012).

Sensitivity test for disinfectants was performed by commonly used agar diffusion method which is designed to determine the smallest concentration of test compound (any chemical) needed to inhibit the growth of a microorganism. This technique is also called as Kirby-Bauer disc diffusion testing which is used to test strength of any compound which is inhibitory to specific microorganisms (Mohanty, 2010).

**Materials and Methods :**

**Source of test bacteria:** The selected test bacteria were *E. coli* and *S. pyogens*. Pure broth culture of both bacteria was taken from two different places. *E. coli* pure culture was taken from **"**Aman Hospital and Research Center, Anishabad Patna" and another bacterium i.e. *S. pyogens* was taken from "Bhagwat pathological laboratory, Moti Chawk Khagaul Patna".

**Source of disinfectants:** Dettol (60 ml) was purchased from "National Medical's located at New Punaichak, Patna 23". Harpic (200 ml) was purchased from "Big Bazar located at Patliputra, Patna". Neem leaves were plucked from a Neem tree in "Patna Women's College campus". Garlic was purchased from "Bari Badalpura, Khagaul Patna local market".

**Preparation of different concentration of disinfectants:**

**(i) Chemical disinfectant :** Six different concentrations i.e. 0%, 10%, 25%, 50%, 75%, and 100% of Harpic and Dettol were prepared in autoclaved distilled water respectively as done by Saha *et. al.* in his work, just difference was that in this work preferred dilutions were changed.

**(ii) Natural disinfectant :** Surface sterilization of Neem leaves and Garlic bulb was done to avoid contamination that may alter the most probable result. It was then air dried in aseptic condition, later ground in motar pestle. Then it was filtered through sterilized muslin cloth. The obtained filtrate was considered as 100% concentrated as used by Saba Irshad *et.al*. (2011) Subsequently, different concentrations (0% to 75% by dilution) of respective natural disinfectants were prepared, using autoclaved distilled water.

**Preparation of media:** Nutrient agar media (1500 ml) was prepared. The component of media i.e. peptone, beef extract, NaCl and agar was weighed and dissolved in distilled water. pH was maintained at 7±0.2. Prepared media was autoclaved.

**Preparation of disinfectant disc:** Sterilized punching machine and filter paper was used to cut the disc out of filter paper. The cut disc was reautoclaved. The disc was loaded with different concentration of respective disinfectants. The loaded disc was placed on sterile blotting paper to remove excess of disinfectants loaded on it.

**Pouring of media and spreading of culture**: The prepared Nutrient agar media was poured on autoclaved sterile petri plates (9.5 cm).Then it was left to solidify under aseptic condition. 0.1 ml of the broth of respective bacterial culture was used to inoculate the media, and then spread uniformly.

**Kirby-Bauer disc diffusion method:** The prepared disinfectant disc was placed on respective labelled Petri plates under aseptic condition as done by Saha et.al. (2009) in his work. Then it was incubated at 37°C in incubator for 24 hrs. After 24 hrs, observation was taken and diameters of inhibition zones were noted down.

**Results and Discussion :**

**A. Chemical Disinfectant**

**(a) Harpic:** It was an efficient disinfectant as it showed zone of inhibition even at lowest concentration i.e. 10% by dilution (Table 1). At highest concentration i.e. 100% by dilution, it was most effective on both the bacterium. However its effect on *S. pyogenes* was more as compared to *E. coli*, as the inhibition zone obtained on *E. coli* (Table 2) plates were smaller than those of *S. pyogenes*. M.I.C. of Harpic for *E. coli* and *S. pyogenes* is 10% by dilution.

**Table 1. Zone of inhibition (diameter) shown by Harpic on *E. coli***

| Sl. No. | Different concentration of disinfectants (%) | Zone of inhibition shown (cm) | | | |
|---|---|---|---|---|---|
| | | I | II | III | Average |
| 1. | 0% | 0.0 | 0.0 | 0.0 | 0.0 |
| 2. | 10% | 0.8 | 1.0 | 1.0 | 0.934 |
| 3. | 25% | 1.2 | 1.6 | 1.4 | 1.4 |
| 4. | 50% | 2.0 | 1.6 | 1.6 | 1.734 |
| 5. | 75% | 2.3 | 1.8 | 1.9 | 2.0 |
| 6. | 100% | 2.9 | 3.0 | 3.0 | 2.6 |

**Table 2. Zone of inhibition (diameter) shown by Harpic on *S. pyogenes***

| Sl. No. | Different concentration of disinfectants (%) | Zone of inhibition shown (cm) | | | |
|---|---|---|---|---|---|
| | | I | II | III | Average |
| 1. | 0% | 0.0 | 0.0 | 0.0 | 0.0 |
| 2. | 10% | 0.6 | 0.5 | 0.7 | 0.6 |
| 3. | 25% | 2.4 | 1.4 | 2.2 | 2.0 |
| 4. | 50% | 2.0 | 3.0 | 2.8 | 2.6 |
| 5. | 75% | 2.4 | 3.0 | 2.7 | 2.7 |
| 6. | 100% | 3.0 | 3.7 | 3.4 | 3.36 |

**(b) Dettol:** It was effective on *E. coli* as it was able to show inhibition even at lowest concentration i.e. 10% by dilution (Table 3). But it was less effective on *S. pyogenes* as it was totally unable to inhibit growth of *S. pyogenes* (Table 4). At 25% by dilution very less effect was seen and even at 100% dilution the inhibition zone was not satisfactory. M.I.C. of Dettol for *E. coli* and *S. pyogenes* is 10% by dilution and 25% by dilution.

**Table 3. Zone of inhibition (diameter) shown by Dettol *E. coli***

| Sl. No. | Different concentration of disinfectants (%) | Zone of inhibition shown (cm) | | | |
|---|---|---|---|---|---|
| | | I | II | III | Average |
| 1. | 0% | 0.0 | 0.0 | 0.0 | 0.0 |
| 2. | 10% | 0.5 | 0.4 | 0.6 | 0.5 |
| 3. | 25% | 0.6 | 0.6 | 0.5 | 0.56 |
| 4. | 50% | 0.6 | 0.7 | 0.6 | 0.63 |
| 5. | 75% | 1.0 | 0.9 | 1.1 | 1.0 |
| 6. | 100% | 1.3 | 1.2 | 1.5 | 1.33 |

**Table 4. Zone of inhibition (diameter) shown by Dettol *S. pyogenes***

| Sl. No. | Different concentration of disinfectants (%) | Zone of inhibition shown (cm) | | | |
|---|---|---|---|---|---|
| | | I | II | III | Average |
| 1. | 0% | 0.0 | 0.0 | 0.0 | 0.0 |
| 2. | 10% | 0.0 | 0.0 | 0.0 | 0.0 |
| 3. | 25% | 0.1 | 0.0 | 0.0 | 0.0333 |
| 4. | 50% | 0.5 | 0.5 | 0.6 | 0.53 |
| 5. | 75% | 0.7 | 0.6 | 0.7 | 0.6 |
| 6. | 100% | 0.8 | 1.0 | 0.9 | 0.9 |

**B. Natural Disinfectant**

**(a) Neem (*Azadirchata indica*):** It was effective on both the bacteria at higher concentration i.e. 25% to 100% by dilution. Also, it was more effective against *S.pyogenes* as compared to *E. coli* (Tables 5 and 6). However at lowest concentration i.e. 10% it was not effective against any bacterium. M.I.C. of Neem for *E. coli* and *S. pyogenes* is 25% by dilution for both.

**Table 5. Zone of inhibition (diameter) shown by Neem *E. coli***

| Sl. No. | Different concentration of disinfectants (%) | Zone of inhibition shown (cm) | | | |
|---|---|---|---|---|---|
| | | I | II | III | Average |
| 1. | 0% | 0.0 | 0.0 | 0.0 | 0.0 |
| 2. | 10% | 0.0 | 0.0 | 0.0 | 0.0 |
| 3. | 25% | 0.2 | 0.1 | 0.1 | 0.2 |
| 4. | 50% | 0.6 | 0.6 | 0.5 | 0.567 |
| 5. | 75% | 0.8 | 0.7 | 0.6 | 0.7 |
| 6. | 100% | 1.0 | 1.0 | 0.8 | 0.933 |

**Table 6. Zone of inhibition (diameter) shown by Neem *S. pyogenes***

| Sl. No. | Different concentration of disinfectants (%) | Zone of inhibition shown (cm) | | | |
|---|---|---|---|---|---|
| | | I | II | III | Average |
| 1. | 0% | 0.0 | 0.0 | 0.0 | 0.0 |
| 2. | 10% | 0.0 | 0.0 | 0.0 | 0.0 |
| 3. | 25% | 0.5 | 0.4 | 0.4 | 0.433 |
| 4. | 50% | 0.7 | 0.9 | 0.6 | 0.733 |
| 5. | 75% | 1.0 | 0.8 | 0.7 | 0.833 |
| 6. | 100% | 1.0 | 0.9 | 1.2 | 1.03 |

**(b) Garlic (*Allium sativum*):** Garlic also proved to be a good disinfectant. It showed inhibition zone on both the selected bacteria. However, it is more effective on *S. pyogenes* than *E. Coli* (Tables 7 and 8). At 10% by dilution *E. coli* showed no effect but at higher concentration i.e. from 25% to 100% by dilution, the zone of inhibition was observed. Its effect on *S. pyogenes* was remarkable as it showed its efficiency even at lower concentration. M.I.C. of Garlic for *E. coli* and *S. pyogenes* is 25% by dilution and 10% by dilution, respectively.

**Table 7. Zone of inhibition (diameter) shown by Garlic *E.coli***

| Sl. No. | Different concentration of disinfectants (%) | Zone of inhibition shown (cm) | | | |
|---|---|---|---|---|---|
| | | I | II | III | Average |
| 1. | 0% | 0.0 | 0.0 | 0.0 | 0.0 |
| 2. | 10% | 0.0 | 0.0 | 0.0 | 0.0 |
| 3. | 25% | 0.0 | 0.1 | 0.0 | 0.033 |
| 4. | 50% | 0.3 | 0.4 | 0.2 | 0.3 |
| 5. | 75% | 0.5 | 0.3 | 0.3 | 0.367 |
| 6. | 100% | 0.5 | 0.5 | 0.6 | 0.53 |

**Table 8. Zone of inhibition (diameter) shown by Garlic *S. pyogenes***

| Sl. No. | Different concentration of disinfectants (%) | Zone of inhibition shown (cm) | | | |
|---|---|---|---|---|---|
| | | I | II | III | Average |
| 1. | 0% | 0.0 | 0.0 | 0.0 | 0.0 |
| 2. | 10% | 0.3 | 0.4 | 0.6 | 0.43 |
| 3. | 25% | 0.8 | 0.9 | 0.6 | 0.767 |
| 4. | 50% | 1.5 | 1.8 | 1.7 | 1.667 |
| 5. | 75% | 1.9 | 2.1 | 1.8 | 1.933 |
| 6. | 100% | 2.3 | 2.4 | 2.1 | 2.267 |

The combined results are shown in Fig 1 and 2.

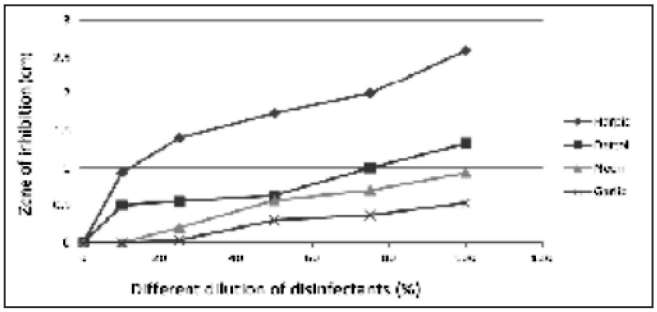

**Fig 1. Graph of Zone of inhibition (diameter) shown by *E. coli***

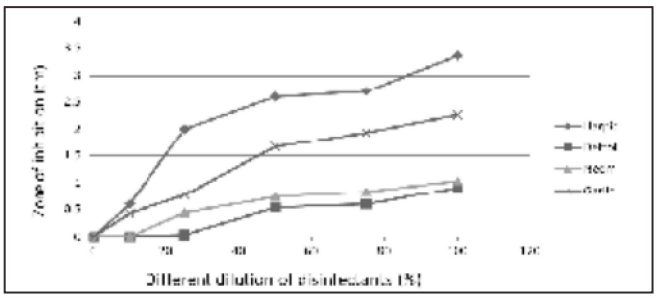

**Fig 2. Graph of Zone of inhibition (diameter) shown by *S. pyogenes.***

## Conclusion :

On the basis of our observation it is concluded, that chemical disinfectant is more efficient than natural disinfectants. It is also a point of concern that all chemical disinfectant cause allergic reaction; to some extent while handling and when ingested accidentally/ intentionally, it can be fatal too. To conclude it through, natural disinfectants are less effective as compared to Chemical disinfectants; still it can be preferred for household purposes due to hazards related with chemicals. However, use of chemical disinfectants can be promoted at industries, hospitals, laboratories and other working places.

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## *References :*

Biswas, Kausik, Ishita Chattopadhyay, Ranajit K.Banerjee and Uday Bandyopadhyay (2002) Biological activities and medicinal properties of Neem (*Azadirachta indica*). *Current Science*, 82(11): 1336-1345.

EL Mahmood, A. M. and Doughari, J. H. (2008). Effect of Dettol on viability of some microorganisms associated with nosocomial infections. *African Journal of Biotechnology* 7(10): 1554-1562.

Hamel, Paul B. and Chiltoskey, Mary U. (1975). *Cherokee Plants and Their Uses*. A 400 Year History. Herald Publishing, 35.

Jim Dwyer (2012). *An Infection, Unnoticed, Turns Unstoppable.* The New York Times.

Mohanty A. (2010). Physiochemical and Antimicrobial Study of polyherbal *Pharmacieglobal*, 4(04): 1-3.

Saba Irshad , Muneeba Butt and Hira Younus (2011). In-Vitro Antibacterial Activity of Two Medicinal Plants Neem (Azadirachta indica) and Peppermint.*International. Research Journal of Pharmaceutical*, 01:9-14.

Saha AK, Haque MF, Karmaker S and Mohanta MK. (2009). Antibacterial effects of some antiseptics and disinfectants. *Journal of Life Earth Science*, 3-4: 19-21.

Singleton P. (1999). *Bacteria in Biology*. Biotechnology and Medicine, 5: 444–454.

Zillur Rahman S and Shamim Jairajpuri M. (1993). *Neem in Unani Medicine*. Research and Development Society of Pesticide Science, India, New Delhi, 2(21): 208-219.