**Materials and Methods:**

**2. 1. Preparation of isolated bacterial strains:*****according to* (Sutton *et al.,* 2002).**

*E.coli and Salmonella* were isolated from poultry hatcheries and then were identified serologically after that were prepared by Seed-Lots systems (seed-lot culture maintenance techniques)to obtain two working suspensions of concentration 3x106CFU / 0.1 mL suspension through:

**2.1.1.** The tested strains grown on Lauryl Sulphate broth at 35°C for 24 hours.

**2.1.2.** Sterile Phosphate buffer with pH 7.2 was used for making heavy test suspensions via inoculating it in harvested microbial suspensions (with sterile swab or loop).

**2.1.3.** The suspensions were measured via making serial dilutions then plate counts were done using Eosin Methylene Blue agar plates and Xylose Lysine Desoxycholate agar plates (XLD) which are suitable for each microorganism and choose suspensions of concentration 3×106 CFU/ 0.1 mL as working suspensions.

**2.2. Preparation of tested Disinfectants: *according to* (Linton et al., 1987)**

**2.2.1. Tested disinfectants:**

**Aquavinol®5%**

Manufactured by: Aqua chemicals Egypt.

Composition: 6 % phenolic crystals and 40% coal tar oils.

**Presept®2.5%**

Marketed by: Advanced sterilization products (a Johnson & Johnson company).

Composition: Tablets each contain 2.5 gram sodium dichloroisocyanurate (NaDCC).

**poviment**®

Manufactured by: SFT (Sabsabi for trading).

Composition: each 1000 ml contain PVP iodine 30000 mg and Ment. **MM8**®

Manufactured by: SFT (Sabsabi for trading).

Composition: contain per ml 125 mg Quaternary ammonium compound, 50 mg glutaraldehyde, 130 mg isopropanol and 3 mg pine oil.

Tested Disinfectants (Aquavinol®5%, Presept®2.5%, Poviment® and MM8®) were prepared to obtain the final dilutions by using USP purified water at pH 5-7 from sterilized tap water.

**2.2.2. Antibacterial Effectiveness Test:**

The tested disinfectants were diluted to 90% of used concentrations with known volumes of previously settled bacterial suspensions during the test acts as a matter of challenge for accounting the dilution error and difference during the actual situation of bactericidal agents preparation. Finally, Four commercially available disinfectants were applied in vitro at various concentrations used were 1%, 1.5% and 2% within various contact times of 30, 60, 90 and 120 minutes on contaminated area by *E.coli* **(**O91: H21 strain) and S. *Typhimurium* at a titer of 3×106 / cm2 to evaluate the effectiveness of used disinfectants against these pathogens.

***2.3.* The Surface Challenge Test was performed in vitro*:*** This test was applied according to **(Clontz, 2008)**.

Accurately, large squares (20cm×20cm) of the surfaces area were used for application of these disinfectants at various dilutions at temperature of 30°C. Each large square was divided into small squares of 4 cm x 4 cm and were artificially contaminated with the cultured broth for 24 hours of the tested microorganisms and acts as the initial bacterial counts of the tested pathogens and were counted before disinfectants application. after application of each prepared disinfectant at intervals of 30, 60, 90 and 120 minutes, using sterile swabs for picking up the viable microorganisms from previously contaminated small squares. Whole swabs were directly transferred into sterile cotton plugged test tubes that contain 10 ml nutrient broth and 1 ml of the neutralizer of the applied preparation was added and then incubated at 37°C for 24 hours. Any detectable bacterial growth was confirmed by culturing on specific agar plates. The bacterial count for each dilution should be read then multiplied its average by the reciprocal of the same dilution level.