Research Article

Evaluation the efficacy of two categories of disinfectants, traditional and Nano types on *Brucella* microorganism in different conditions.

Khalafallah, S.S.[1], Zaki, H.M.[2], Seada A.S.[3]

1Hygiene and Animal Management Department, Faculty of Veterinary Medicine, Benha University.
2 Brucella Department, Animal Health Research Institute, Cairo, Egypt.
3Tanta Laboratory Branch, Animal Health Research Institute, Egypt.

ABSTRACT

Brucellosis is a zoonotic disease that transmitted by many ways between animals and humans. Disinfection of environments surrounded animals and good removal of infection from animal house has a very important role in the prevention and control of brucellosis. In our study we used some types of disinfectants against Brucella melitensis to evaluate its efficacy and if it is effective against Brucella melitensis in different environmental conditions. Our study included some traditional types of disinfectants and antiseptics (Virkon® S, Cidex, Sodium hypochlorite, Betadine and Dettol) and three types of Nano-disinfectants (Dettol with Silver-NPs, Glutaraldehyde with silver-NPs and Calcium oxide-NPs). Reduction rate was used for estimation the efficacy of different types of used disinfectants. The results showed that the bactericidal effects of the used disinfectants were influenced by increasing of their concentration and more exposure time specially Vircon S, however presences of dirty conditions and low temperature significantly decrease the efficacy of disinfectants specially Dettol. In other side Nano-disinfectants had better effect than ordinal types specially Glutaraldehyde with silver NPs. Our study suggested that Brucella melitensis is affected by commonly used disinfectants. However, the bactericidal efficacy was decreased with presence of dirty conditions and low temperatures. Nano-disinfectants had superior effect on Brucella.

Keywords: Brucella, Nano, disinfectant, traditional, control.

1. INTRODUCTION

Brucella spp. is a Gram-negative bacterium spread widely among different hosts through many means of transmission (Corbel 2006). *Brucella* infection causes abortion of pregnant animals at late stage of gestation also causes orchitis in males in both animals and human (Alton *et al*., 1988). *Brucella microbe* is discharged in milk, discharges from uterus or vagina after abortion or parturition, fetal membranes and into urine of infected animals. *Brucella* can stay alive in environment for long time, that depending on environmental conditions such as low temperature, pH and humidity (Al-Majali *et al*., 2009).

Brucella could survive in many materials as dust, drinking water or manure and slurry. Also aborted fetuses, soil, meat and dairy products may keep the microbe inside it for considerable periods of time depending on suitable condition (WHO, 2006). Infection with brucellosis occur due to direct or indirect contact with infected animals or contaminated environment (Foster *et al*., 2007). *Brucella* although it can remain alive in dirty environment but it is known to be susceptible to heat treatment, disinfection, and direct sunlight (Pappas *et al*., 2005).

Disinfection is a very important element of brucellosis control program as well as other efforts so choice of the type of disinfectant should be after good evaluation (OIE, 2004). Each used disinfectant has advantages, side effects and suitable application method. For example, gluteraldehyde is very strong disinfectant, it used for disinfection of metals and material which is sensitive to heat but it is very corrosive to skin. Chlorine is an intermediate level disinfectant that used for disinfection of biological material, equipment, medical supplies and environmental surface. It is of low cost, fast acting, but it has corrosive effect on metals and irritant to skin (Rutala, 1996). Many researches indicated that *Brucella* is sensitive to most available disinfectants as halogens, ethanol, phenol and formaldehyde but every type needs to be evaluated to decide the proper mean of application (Corbel, 2006).

Nano-based disinfectant can be used to reduce the bacterial burden in environment and can be effective against resistant organisms as *E-coli*, *salmonella* spp. and *Martha*, so using of new types of nano disinfectants with silver-NPs and Calcium oxide NPs as showed in table would be helpful for control of many types of infectious bacteria (Saengkiettiyut *et al*., 2008; Rai *et al.*, 2012).

Silver nano particles had a good efficacy against bacteria. Its Killing effect possibly occurred due to bacteriostatic effect of silver. Although silver was so effective for killing the pathogenic bacteria, the formation of toxic product inside bacterial cells may have some irritable reaction to skin at the site of application (Sökmen *et al.*, 2001). Inorganic nano-metal oxides as (MgO, ZnO and CaO nanoparticles) can be used as anti-microorganism agents for pathogen control. It have oxidative effect against microorganism cells. It has good penetration power and good stability under environmental condition (Cha *et al*., 2012). Silver known as a strong antibiotic and has wide range of industrial applications in healthcare and external medicine, also silver nano particles had bactericidal effect against wide sector of bacteria and increase the efficacy of other antibacterial agents if combined with it (Hossain *et al.*, 2014). Nanoparticles of Silver (Ag-NPs) represent an important nano medicine-based advance in the fight against poly-resistant bacteria. In laboratory the antibacterial activities of kanamycin, erythromycin, chloramphenicol and ampicillin were increased in the presence of Ag-NPs against tested bacterial strains, so it is recommended to adding of Ag-NPs to anti-bacterial agents to enhance its efficacy (Fayaz *et al*., 2010).

Materials and method:

Bacterial suspension of *Brucella melitensis*. (Wang *et al.*, 2015).

Brucella melitensis biovar 3 is an endemic strain in Egypt. It was isolated from slaughtered serologically positive animals and its isolation and typing took place at *Brucella* department of -Animal Health Research Institute, Cairo, Egypt. It was reactivated and cultured 3 days before its using. It was plated onto tryptone soya agar (TSA, Oxoid) and incubated at 37°C with 10% (vol/vol) CO_2. A bacterial suspension at OD600=1.0 (equivalent to about 10^9 cfu /mL was diluted with physiological saline and kept until the test.

Disinfectants suspension preparation. (Park and Chen, 2011).

Five types of traditional disinfectants including Virkon® S, Glutaraldehyde (Cidex), Sodium hypochlorite (Bleach), Betadine and Dettol. Three types of nano disinfectants including Dettol (Chloroxylenol) with silver-NPs, Glutaraldehyde (1). All disinfectants were freshly prepared according to the manufacturer's instructions prior to test.

Table (1) types of used disinfectants and its ingredients.

Commercial name	Active ingredient	Recommended concentration	Application
Virkon® S	Potassium peroxy monosulfate and sodium chloride	1%	Animal house and equipments.
Cidex	Glutaraldehyde	2.4%	Equipments.
Bleach	Sodium hypochlorite	2g/L	iological material smooth surfaces
Betadine	Povidone iodine	1%	Skin and mucous membranes.
Dettol	Chloroxylenol (phenol)	1%	kin of workers and skin of animals
ettol with Silver- NP	Chloroxylenol & Ag-NPs	100 ppm	Animal house and equipments.
ilutaraldehyde with silver-NPs	(C5H8O2) &Ag-NPs	100ppm	Animal house and equipments.
alcium oxide NPs	Cao nanopartical	100 ppm	Animal house and equipments.

Determination of the MBC of different types of disinfectants.

Each disinfectant was diluted by a two-fold serial dilution method using sterile distilled water in test tubes, every tube have 1.9 ml of disinfectant. 100 µL of bacterial suspension (10^9 cfu/mL) was added to test tubes containing the different concentrations of examined disinfectant, vortexed and incubated for 20 min. Sterile distilled water used as a control. After the exposure time, 100 µL of the bacterial suspension from all concentrations of each disinfectant was spread on the TSA plates. The growth was examined after incubating for 72 hours at 37°C, and the minimal inhibitory concentration (MIC) values were recorded as showed in table(2). The lowest concentration at which the bacteria could not survive was recorded as MIC. Then, 0.5 mL MIC bacterial suspensions were sub-cultured in 4.5 mL liquid media without chemicals at 37°C to detect any bacterial survival activity. After 72 h, 100 µL of the mixed culture were spread over a TSA plate, and the MBCs of the tested disinfectants were determined. Testing of every disinfectant dilution was performed in triplicate manner.

Table(2) MIC of each type of disinfectants.

disinfectants	Vircon S	Cidex	bleach	Betadin	Dettol	Dettol & Silver-NPs	Glutaraldeh & silver-NP	Calcium oxide NPs
MIC at 37°	0.0750%	0.0125%	0.01%	0.63%	0.250%	0.065%	0.030%	0.045%

The bactericidal effect of disinfectants under different environmental conditions. (Randall *et al*., 2004).

To evaluate the efficacy of disinfectants under different environmental conditions, we used the MBC of each disinfectant with saline, soil and fecal matter. Then, 20% suspensions of soil and fetal matter which collected from animal house and sterilized by autoclaving were prepared and stored till examination. An amount of 1.9 mL of MBC of each disinfectant was added to each test tube then 100 μL of bacterial suspension (109cfu/mL) were added to the test tubes. Then, 2 mL from saline, sterile soil and sterile fecal suspension added to each tube. Sterile distilled water was used as a negative control, after that, all tubes kept at room temperature for different time intervals (1 min, 5 min and 10 min). Ten-fold dilution was used for every test tube and the contents plated onto TSA media to estimate the viable bacteria counts. Three plates used for each sample and the bactericidal activates were estimated by calculation of the reduction factor of viable organisms as following;

Reduction factor (RF) = Log10 cfu (negative control) − Log10 cfu (disinfection group).

The bactericidal effect of chemical disinfectants at low temperatures. (Suller and Russell, 1999).

To evaluate the effect of low temperatures on the bactericidal effects of each disinfectant, suspension of bacteria with disinfectants and with saline, soil and fecal matter were prepared as described before and kept on ice for 1, 5 and 15 minutes. Then the activities of each disinfectant at different concentrations and in low temperatures were calculated by employing the reduction factors.

Statistical analysis. (Licht , 1995).

Statistical analysis was performed by ANOVA. Significant differences were accepted at $P < 0.05$.

Results:

All traditional disinfectants had good reduction rate when compared with saline and its efficacy increased with the increasing of exposure time. While with organic matters as (soil and feces) its reduction rate decreased specially Dettol and Cidex which had the lowest reduction rate with the presence of organic matters (Dettol; 75% and 73%. Cidex; 70% and 69% with soil and fecal matters respectively) as showed in Figure (1).

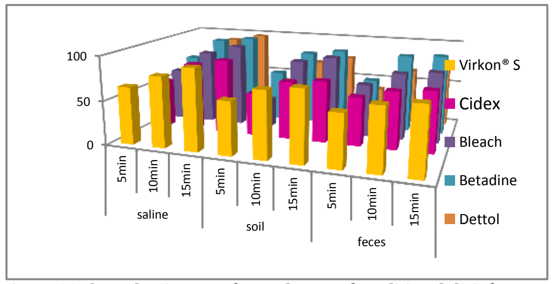

Figure (1) the reduction rate for each type of traditional disinfectants in different conditions with different times.

Nano disinfectants reduction rate had advance on traditional types, as the effect of Dettol (96%, 78%, 77% with saline, soil and feces respectively) and Glutaraldehyde (99%, 90%, 84% with saline, soil and feces respectively) and This was increased when combined with Silver-NPs while Calcium-NPs (90%, 70%, 75% with saline, soil and feces respectively). It was clear that all disinfectants had lower effect especially with presence of organic matters as showed in Figure (2).

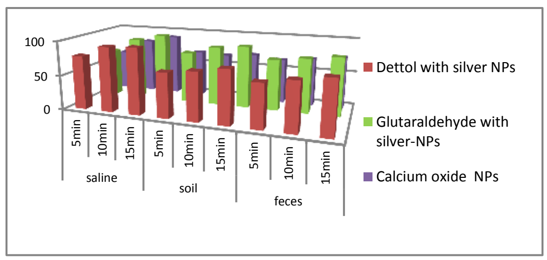

Figure (2) reduction rate of nano disinfectants in different condition and different times.

The reduction rate of all traditional disinfectants decreased in low temperature. It had a low reduction rate with saline while with presence of organic matters it dramatically decreased specially Vircon S (70%, 50%, 49% with saline, soil and feces respectively) and Cidex (53%, 46%, 43% with saline, soil and feces respectively) which had the lowest reduction rate while other types had acceptable reduction rate as showed in Figure (3).

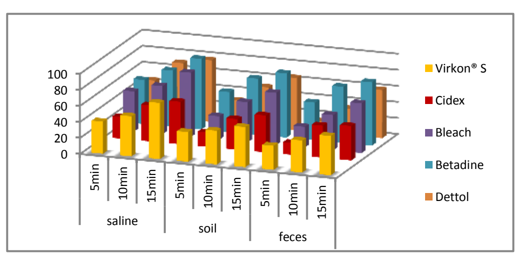

Figure (3) reduction percent of colony count for traditional disinfectants at low temperature.

Nano disinfectants had good reduction rates at low temperature even with presence of organic matters especially Glutaraldehyde with silver-NPs (90%, 78%, 88% with saline, soil and feces respectively) and Dettol with silver-NPs (84%, 80%, 80% with saline, soil and feces respectively) which had the highest reduction rate as showed in Figure (4).

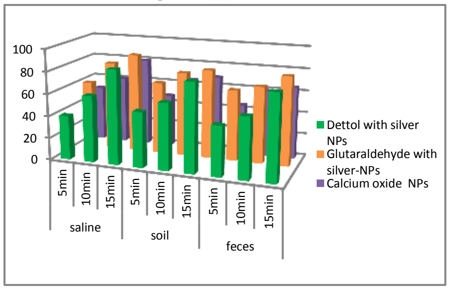

Figure (4) the reduction rate of colony count for Nano disinfectants at low temperature.

Discussion:

Brucellosis is very important zoonotic disease infect nearly all animal species and human it causes many loses in animal production and human health sectors. *Brucella* microorganism present in secretion of infected animals and polluted the surrounded environment so good hygienic measures including strict disinfection should be applied to reduce the prevalence of the disease. Brucellosis still endemic in Egypt especially Nile delta, it distributed between all types of domestic animals and it even isolated from catfish of Nile **(Tittarelli *et al*., 2005; Wareth *et al*., 2014).**

The efficacy of traditional disinfectants against

***Brucella melitensis*:**

In our study all traditional disinfectants had a good reduction rate when applied with saline and its efficacy increased with the increasing of exposure time. While with organic matters as (soil and feces) its reduction rates decreased specially when using Dettol and Cidex which had the lowest reduction rate when applied in the presence of organic matters even for longer time periods as showed in Figure(1). Our results agree with Park & Chen, (2011) they reported that povidine-iodine have a good effect on *Brucella* microorganism so can be used in brucellosis control program. Alkaline disinfectants as (quaternary ammonium compound, sodium dichloro isocyanurate, potassium monopersulphate/sodium dichloroisocyanurate) have excellent efficacy against *Brucella* even in presence of organic matters (Yoo 2009). Evaluation of commonly applied disinfectants and antiseptics in Veterinary field against *Brucella* organisms indicated that all commonly studied disinfectants had a good efficacy, but some types need more contact time or increasing of its concentration especially with organic matters **(**Adel *et al*., 2015).

The reduction rate of all disinfectants decreased in low temperature, it slightly decreased with saline while with presence of organic matters it dramatically decreased specially in using vircon S and cidex which had lowest reduction rate while other types had acceptable reduction rate. These agree with the results of McDonnell and Russell, (1999), they mentioned that the bactericidal action of disinfectants usually increases with the increase of contact time and increase of temperature, liquid disinfectants had less activity or be completely inactivated under dirty conditions or at cold conditions due to decreasing of its reaction or organic substances prevent the disinfectant to reach and contact with the bacterial cell.

Our result also agree with Wang *et al.*, (2015) who reported that the examination of the activity of six types of disinfectants including; QAC, aldehydes, halogens, phenol and alkaline compounds by using the MBCs of every type. Their results indicated that all previous compounds were active against *Brucella* specially when its concentration and the surrounded temperature increased but with organic substances or low degree of temperature its activity decreased except sodium hypochlorite and sodium hydroxide which were less affected. Sodium hypochlorite and sodium hydroxide are preferred with dirty conditions or at low temperatures. Actually, the two disinfectants are often selected due to its lower price and low toxicity.

The result of Nano disinfectants against *Brucella mellitensis*:

By trying of some types of nano disinfectants to evaluate its efficacy against *Brucella* the result was as following; the effect of Dettol and Glutaraldehyde was increased when combined with silver-NPs while calcium-NPs had lower effect especially with presence of organic matters as showed in Figure (2).

Nano disinfectants had good reduction rate at low temperature even with presence of organic matters specially Glutaraldehyde with silver-NPs and Dettol with silver-NPs which had the highest reduction rate as showed in Figure (4). That agree with the results of Hossain *et al*., (2014) who reported that some nano elements can be used as disinfectants because it have antimicrobial properties and low possibility of harmful effect of the byproducts of disinfection which produced during traditional disinfection process.

Our results also agree with these of Shin *et al*., (2007) who mentioned that silver-Nano particles have a good bactericidal effect and can be a good disinfectant against many types of bacteria. Various nano-materials like carbon nanotubes, Ag, Au, CaO, ZnO, TiO2, chitosan, cationic peptides, etc. possess antimicrobial activities and therefore have been used for the treatment of infectious diseases affects their cellular membrane integrity, metabolic processes and morphology. This antimicrobial activity is due to its unique chemical and physical properties as high surface/volume ratio and its ability to penetrate the cell wall of microorganism (Dizaj *et al*., 2015).

Calcium oxide nanoparticles and calcium hydroxide-NPs can be used as antibacterial agents as it prevent the growth of bacteria at surfaces that coated with it (Louwakul *et al*., 2017). Mono oxide ions as calcium oxide and magnesium oxide are very effective against large number of Gram positive bacteria and Gram negative bacteria as well as spores and it stay effective for long time and in different environmental conditions (Stoimenov *et al*., 2002).

However, the previous results of some researcher disagree with our opinion as nano particles can't be safely used for disinfection because it have some disadvantages as toxicity and suspected carcinogenicity to animals and human. It may also produce a new generation of more resistant bacteria to disinfectant (Sökmen *et al.*, 2001; Hajipour *et al*., 2012).

Conclusion:

All types of used disinfectant were effective against *Brucella*. The efficacy of disinfectants influenced with increasing the contact time, concentration and temperature, but the efficacy of disinfectant decreased with presence of organic matters and at low temperature. The Nano type of disinfectants had a good efficacy against *Brucella* and its efficacy decreases to a lesser extend with presence of organic matters and low temperature so it needs more evaluation to its efficacy and if it safe for application in dairy farms.

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