Antibacterial and antibiofilm effect of nano zinc-oxide and propolis nanoemulsion against strong biofilm producer coliform species isolated from chickens

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ABSTRACT
A total of 150 samples collected from freshly dead chickens were examined for Coliform bacteria. The results revealed isolation of 141 isolates (84 E. coli (56%), 6 Klebsiella pneumoniae (4%), 10 Klebsiella oxytoca (6.6%), 17 Klebsiella ozaeae (11.3%), and 24 Citrobacter freundii (16%)). Strong biofilms on Congo red agar were produced by 37 E. coli isolates (44%), 3 Klebsiella pneumoniae (50%), 5 Klebsiella oxytoca (50%), 7 Klebsiella ozaeae (41%) and 10 Citrobacter freundii (41%). Antimicrobial susceptibility test on strong biofilm producer isolates revealed that all isolates exhibited high resistance to Amoxicillin, Oxytetracycline and Erythromycin, Trimethoprim Sulfamethoxazole, while their sensitivity to gentamicin was high. Strong biofilm E. coli isolates were serotyped to O91:H21, O17:H18, O78, O114:K90, O26:K60, O121:H7, O128:H2, O113:H4, O159, O44/K74 and O55:K99. Klebsiella pneumoniae serotypes were K1 and K2. Zinc oxide nanoparticles were 45.86 ± 45.71 nm in size, 0.223 ± 0.149 PDI and zeta potential -14.2 ± 1.68 mv, while Propolis-NPs had particle size 291.6 ± 23.32 nm, 0.351 ± 0.026 PDI and zeta potential -9.88 ± 2.53 mv. SRB cytotoxicity assay showed that the highest concentration of propolis-NPs (75%) caused 88.88% cell viability, while ZnO-NPs showed cell viability more than 95% until 17.5 µg/mL. ZNO-NPs MIC was 35µg/ml for all coliform isolates. Its antibiofilm effect was observed at concentrations 8.75 and 17.5µg/ml for both E.coli and Citrobacter freundii, while Klebsiella species biofilm formation was inhibited at 17.5 µg/ml ZNO-NPs. Moreover Propolis-NPs MIC against all tested isolates was 37.5% while antibiofilm activity was detected at 18.75%.

1. INTRODUCTION

Coliform bacteria are present in the environment and the digestive tracts of animals, including humans, and are found in their wastes. It has the ability to ferment lactose with production of acid and gas. Its detection in food indicates unhygienic conditions. It includes Escherichia coli, Klebsiella, Enterobacter and Citrobacter (Markey et al., 2013). The most pathogenic member of coliform is E. coli that causes many diseases such as colisepticemia, coligranuloma, swollen head syndrome, meningitis, air sacculitis, and pericarditis in both humans and animals (Fairbrother et al., 2005).Bacteria attached each other forming biofilm which protect it from antimicrobial agents causing high economic losses (Chakraborty et al., 2018). Genus Klebsiella has many species as K. pneumoniae, K. oxytoca, K. ozaeae and K. rhinoscleromatis. They are opportunistic pathogens causing nosocomial infections, septicaemia, pneumonia, rhinoscleroma, ozena, chronic granulomatous disease and hemorrhagic colitis(Tantawy et al., 2018). E. coli are serotyped on the basis of their O (somatic), H (flagellar), and K (capsular) surface antigens(Tajbaksh et al., 2016) while Klebsiella species are sero-grouped by their capsular (K) antigens. Citrobacter causes severe diarrhea, urinary tract infections, pneumonia, neonatal meningitis and brain abscesses (Murray et al., 2010). The main citrobacter species are Citrobacter diversus and Citrobacter freundii. Citrobacter freundii has many virulence factors as toxins, proteolysis, hemolysis and biofilm formation (Fakrudin et al., 2014). Excessive use of antimicrobial drugs leads to multi-drug resistant coliform bacteria that cause multiple untreatable diseases and high mortality. Nanotechnology has become alternative method for finding a treatment of pathogenic bacteria (Siddiqui and Rahman, 2018). Nanoparticles with small size have more antimicrobial activity than that of large size (Chwalibog et al., 2010). Zinc oxide nanoparticle is an inorganic material that acts as antimicrobial agent against wide range of pathogenic bacteria (Chitra and Annadurai,2013).There have been very few reports of the nano-propolis because of lower size of it, the body absorbs nano-propolis more readily so antibacterial activity of nano-propolis may be more effective than propolis (AbdelMageed et al., 2019). Propolis is a resinous bee product that possesses several biological properties including antiviral, antibacterial, antifungal, anticancer, anti-oxidant and anti-inflammatory activities (Hegazi and Hady, 2001). So this study was aimed to evaluate the antibacterial and antibiofilm effect of nano zinc oxide and propolis nanoemulsion against multi-drug resistance coliform species isolated from freshly dead chickens.

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2. MATERIAL AND METHODS

2.1. Ethical Approval

This research was approved by Institutional Animals Care and Use Committee of faculty of veterinary medicine, Benha university (approved number BUVTM) 21-04-23

2.2. Collection of samples

A total of 150 samples were collected from freshly dead chickens (spleen, liver, gizzard, intestine, heart and lung), from different farms and shops at Qaluobia governorate for isolation of coliform bacteria.

2.3. Isolation and Identification of coliform (Markey et al., 2013)

A loopful from each sample was inoculated in MacConkey broth (oxoid) and incubated at 37°C for 24hrs, then subcultured on MacConkey agar. Pink colonies were picked and identified by their cultivation on differentiated media as Brillant green media, Xylose-lysine-deoxychylolate (XLD) agar, Eosin methylene blue agar medium (EMB) and biochemical tests with reference strains (E.coli ATCC25922, Citrobacter freundii ATCC8090, K. pneumoniae ATCC700603).

2.4. Biofilm formation by isolated strains (Subramanian et al., 2012)

The biofilm production was detected on Congo Red Agar (CRA) medium. Black colonies with a dry crystalline consistency indicated strong biofilm production. Weak biofilm remained pink while darkening of the colonies with absence of dry crystalline colonies indicated intermediate result. The experiment was performed in triplicate. Edwardsiella tarda MW362141 was used as control positive.

2.5. Antibiotic sensitivity assay (Markey et al., 2013)

The strong biofilm producer isolates were subjected to the disk diffusion test against 5 antimicrobial discs (Bio analyse); Amoxicillin (25µg), Oxytetracycline (30µg), gentamicin (10µg), Erythromycin (15µg), Trimethoprim Sulfamethoxazole (25µg). The inhibition zones were interpreted according to CLSI (2008)

2.6. Serological identification (Markey et al., 2013)

Strong biofilm E. coli isolates were serotyped using E. coli polyvalent and monovalent O antisera, H and K sera (SEIKEN supplied from MAST ASSURE™). K.pneumoniae specific antiserum (Statens Serum Institute, Copenhagen, Denmark) was used for serotyping of K.pneumoniae

2.7. Characterization of ZnO-NPs and propolis-NPs

ZnO-NPs and propolis-NPs (75%) were obtained from Animal Health Research Institute in Dokki. They were characterized by determination of their particle size, size distribution and zeta potential by photon correlation spectroscopy using particle size analyzer Dynamic Light Scattering (DLS) (Zetasizer Nano ZN, Malvern Panalytical Ltd, United Kingdom). Moreover, the cytotoxicity of different concentrations of ZnO-NPs (2.18µg/ml-35µg/ml) and propolis-NPs (4.68%-75%) were determined by sulforhodamine B (SRB) assay, using Green monkey kidney cells (Nawah Scientific Inc., Mokatam, Cairo, Egypt), according to (Skehan et al., 1990)

2.8. Antibacterial and antibiofilm effect of ZNO-NPs and Propolis-NPs

Minimum inhibitory concentrations (MICs) and antibiofilm effect of ZNO-NPs and Propolis-NPs were determined against strong biofilm producer coliform isolates according to (Basumati et al., 2021). Different concentrations of propolis-NPs(75, 37.5, 18.75, 9.3 and 4.68 %) and ZnO-NPs (35, 17.5, 8.75, 4.3 and 2.18µg/ml) were used

3. RESULTS

3.1. Prevalence of coliform bacteria

Coliform species were isolated from 141/150 samples. They were identified as E. coli (84, 56%), Klebsiella pneumoniae (6, 4%), Klebsiella oxytoca (10, 6.6%), Klebsiella ozanae (17, 11.3%), and Citrobacter freundii (24, 16%).

Coliform isolates

![Fig1. Prevalence of different cloiform isolates](image)

Table (1): Biofilm formation by coliform isolates

<table>
<thead>
<tr>
<th>Strong</th>
<th>Intermediate</th>
<th>Weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli(84)</td>
<td>37</td>
<td>44</td>
</tr>
<tr>
<td>K. pneumoniae(6)</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>K. oxytoca(10)</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>K. ozanae(17)</td>
<td>7</td>
<td>41.2</td>
</tr>
<tr>
<td>C. freundii(24)</td>
<td>10</td>
<td>41.7</td>
</tr>
</tbody>
</table>

3.2. Biofilm formation

Strong biofilms were produced by 37 E. coli isolates (44%), 3 Klebsiella pneumoniae (50%), 5 Klebsiella oxytoca (50%), 7 Klebsiella ozanae (41%) and 10 Citrobacter freundii (41%) (Table 1)

3.3. Antimicrobial sensitivity test

Coliform isolates showed resistance to more than one antibiotic. More than 81.08% of E. coli and 70% of Citrobacter freundii isolates were resistant to Amoxicillin, Oxytetracycline, Erythromycin and Trimethoprim, Sulfamethoxazole, while Klebsiella spp isolates showed complete resistance (100%) to them. Lowest degree of resistance was detected to Gentamicin (Table 2)

3.4. Serotyping of strong biofilm producer coliform isolates

Serotyping of E. coli revealed 11 E. coli serotypes, O114;K90 was the predominant one (Table 3), while Klebsiella pneumoniae isolates were serotyped into K1 (2/3) and K2 (1/3)

3.5. Characterization of ZnO-NPs

ZnO-NPs showed particle size of 45.86±45.71 nm, 0.223±0.149 PDI, Zeta potential of -14.2 ± 1.68 mv, while propolis-NPs had particle size of 291.6 ± 23.32 nm, 0.351 ± 0.026 PDI, Zeta potential of -9.88 ± 2.53 mv. SRB cytotoxicity assay proved that all propolis-NPs concentrations were safe on Vero green monkey kidney cells; their viability at highest concentration (75%) was 88.88%. Also ZnO-NPs showed cell viability more than
Table 2: Antimicrobial sensitivity tests for strong biofilm coliform isolates

<table>
<thead>
<tr>
<th></th>
<th>Amoxicillin</th>
<th>Oxytetracycline</th>
<th>Gentamicin</th>
<th>Erythromycin</th>
<th>Trimethoprim</th>
<th>sulfamethoxazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (37)</td>
<td>R: 35/94</td>
<td>R: 2/6</td>
<td>R: 33/90</td>
<td>R: 4/10</td>
<td>R: 7/19</td>
<td>R: 30/81</td>
</tr>
<tr>
<td>K. pneumoniae(3)</td>
<td>R: 3/100</td>
<td>R: 0/0</td>
<td>R: 3/100</td>
<td>R: 0/0</td>
<td>R: 0/0</td>
<td>R: 3/100</td>
</tr>
<tr>
<td>K. oxytoca (5)</td>
<td>R: 7/100</td>
<td>R: 0/0</td>
<td>R: 7/100</td>
<td>R: 0/0</td>
<td>R: 1/14</td>
<td>R: 7/100</td>
</tr>
</tbody>
</table>

R: resistant  S: sensitive

Table 3: Serotyping of E. coli isolates (n=37)

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>O91:H21</td>
<td>3</td>
<td>8.1</td>
</tr>
<tr>
<td>O17:H18</td>
<td>1</td>
<td>2.7</td>
</tr>
<tr>
<td>O78:4</td>
<td>4</td>
<td>10.8</td>
</tr>
<tr>
<td>O121:H7</td>
<td>1</td>
<td>2.7</td>
</tr>
<tr>
<td>O128:H2</td>
<td>3</td>
<td>8.1</td>
</tr>
<tr>
<td>O113:H4</td>
<td>1</td>
<td>2.7</td>
</tr>
<tr>
<td>O159:1</td>
<td>1</td>
<td>2.7</td>
</tr>
<tr>
<td>O44:K74</td>
<td>3</td>
<td>8.1</td>
</tr>
<tr>
<td>O55:K9</td>
<td>3</td>
<td>8.1</td>
</tr>
<tr>
<td>O114:K90</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>O26:K60</td>
<td>7</td>
<td>18.9</td>
</tr>
</tbody>
</table>

%: According to number of E. coli isolates

Table 5: Antibacterial and antibiofilm effect of nanozinc oxide on isolated coliform

<table>
<thead>
<tr>
<th>Nano zinc conc</th>
<th>E. coli O14:K90 and O26:K60</th>
<th>Klebsiella Pneumoniae (K1 and K2)</th>
<th>Klebsiella oxytoca</th>
<th>Klebsiella ozanae</th>
<th>Citrobacter freundii</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 µg/ml</td>
<td>-ve (MIC)</td>
<td>-ve (MIC)</td>
<td>-ve (MIC)</td>
<td>-ve (MIC)</td>
<td>-ve (MIC)</td>
</tr>
<tr>
<td>17.5 µg/ml</td>
<td>Very low (Antibiofilm)</td>
<td>Very Low (Antibiofilm)</td>
<td>Low (Antibiofilm)</td>
<td>Low (Antibiofilm)</td>
<td>Low (Antibiofilm)</td>
</tr>
<tr>
<td>8.75 µg/ml</td>
<td>Low (Antibiofilm)</td>
<td>High</td>
<td>High</td>
<td>Low (Antibiofilm)</td>
<td>Low (Antibiofilm)</td>
</tr>
<tr>
<td>4.3 µg/ml</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>Low (Antibiofilm)</td>
<td>High</td>
</tr>
<tr>
<td>2.18 µg/ml</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
</tbody>
</table>

High, low and -ve refer to bacterial growth

Table 6: Antibacterial and antibiofilm effect of propolis-NPs on isolated coliform

<table>
<thead>
<tr>
<th>Propolis-NPs conc</th>
<th>E. coli O14:K90 and O26:K60</th>
<th>Klebsiella Pneumoniae (K1 and K2)</th>
<th>Klebsiella oxytoca</th>
<th>Klebsiella ozanae</th>
<th>Citrobacter freundii</th>
</tr>
</thead>
<tbody>
<tr>
<td>75 %</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>37.5 %</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>18.75 %</td>
<td>Low (Antibiofilm)</td>
<td>Low (Antibiofilm)</td>
<td>Low (Antibiofilm)</td>
<td>Low (Antibiofilm)</td>
<td>Low (Antibiofilm)</td>
</tr>
<tr>
<td>9.3 %</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>4.68 %</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
</tbody>
</table>

High, low and -ve refer to bacterial growth.

3.6. Antibacterial and antibiofilm effect of ZNO-NPs and Propolis-NPs on strong biofilm producer coliform isolates

The MIC of nano-zinc-oxide against E. coli, Klebsiella pneumoniae, Klebsiella oxytoca, Klebsiella ozanae and Citrobacter freundii was 35µg/ml. Antibiofilm was observed at ZNO-NPs concentrations 8.75 and 17.5 µg/ml for both E. coli and Citrobacter freundii, while Klebsiella species biofilm was inhibited at 17.5 µg/ml (Table 5). Propolis-NPs MIC was 37.5 % for E. coli, Klebsiella pneumoniae, Klebsiella oxytoca and Klebsiella ozanae and Citrobacter freundii, while it inhibited biofilm formation by all of them at concentration 18.75 % (Table 6)

4. DISCUSSION

The results of the current study revealed isolation of 141/150 coliform bacterial isolates (94%), from visceral organs of freshly dead chickens of different ages and sexes. E. coli was isolated from 84 samples (56%), (figure 1), which nearly agreed with Hussein et al., (2022) who recorded 59.3% E. coli recovery from septicemia broiler chickens and Gharieb et al., (2023) who stated 55% E. coli isolation rate from visceral organs. On the other hand, higher isolation rate (86%) and lower one (30 %) were detected by Halfaou et al., (2017) and Abd EL-Tawab et al., (2017), respectively. Moreover, 3 Klebsiella species were isolated: K. pneumoniae (4%), K. oxytoca (6.6%), K. ozanae (11.3%), (Figure 1). Those species were also isolated by previous authors; Li et al., (2022) isolated K. pneumoniae (4.6%), El-Tawab et al., (2022) isolated K. pneumoniae (75%) and K. oxytoca (25%), El-Tawab et al.,(2018) isolated K. oxytoca (1.9%), Fielding et al., (2012) isolated K. ozanae 94.1 %, Younis et al.,(2016) isolated K. pneumoniae (73.33 %) and K. oxytoca(26.67 %), Mi et al.,(2019) isolated K. pneumoniae (14.4%). Among the isolated coliforms spp., there were 24Citrobacter freundii isolates (16%), (Figure 1). That was strictly consistent with El-Tawab et al.,(2018) who isolated Citrobacter freundii (9.6%) from visceral organs of dead chickens. Biofilms are micro-communities formed by combination of bacterial cells together within a matrix of extracellular polymeric substances. They are important virulence factor which comes up with the protection of the microorganism not only from altered pH, osmolarity, nutrients scarcity, mechanical and shear forces.
but also decreases their susceptibility to the antibiotics and host’s immune cells (Sharma et al., 2019). The ability of the isolated coliform spp. to form biofilms was evaluated by Congo red agar method (qualitative assay). The results varied among all of them were biofilm producer but showed varying degrees (Table 1). E. coli exhibited strong biofilm (44%), intermediate (34.5%) and weak biofilm (21.4%) and also Tajbaksh et al., (2016) found that 80 E. coli isolates were able to produce biofilm; strong biofilm (18.75%), intermediate biofilm (25%) and weak biofilm (56.25 %). Within the isolated Klebsiella spp., strong biofilms were formed by 50 % of K. pneumoniae and K. oxytoca and 41.2% of K. ozanae. Unlike, Abebe et al., (2020) who illustrated biofilm formation by Klebsiella oxytoca isolates; 74%, 16% and 5% were strong, moderate, weak biofilm producers, respectively. In addition to Klebsiella pneumoniae isolates that were strong (60%) and moderate (40%) biofilm producers, respectively. While Lamey et al., (2023) revealed that 92.5% of E. coli isolates were strong biofilm producers and 7.4% were medium while 23 Klebsiella spp isolates showed strong biofilm (91.3%) and medium biofilm (8.6%). Citrobacter freundii isolates in the recent study also formed strong (41.7), intermediate (37.5%) and weak biofilm (20.8%). while other study was conducted by Bunyan et al., (2020) who revealed strong biofilm (71.4%), moderate biofilm (14.3%), and weak biofilm (14.3%) isolates from patients. Another study detected formation of biofilm in clinical samples from patients (Zogaj et al., 2003). Strong biofilm producer Coliform isolates were tested to their antibiotic susceptibility against 5 antibiotics using disc diffusion method. They were resistance to more than one antibiotic (Table 2). More than 81.08% of E. coli and 70% of Citrobacter freundii isolates were resistant to Amoxicillin, Oxytetracycline, Erythromycin and Trimethoprim Sulfamethoxazole, while Klebsiella spp isolates showed complete resistance (100%) to them. All tested isolates exhibited lowest degree of resistance to Gentamicin (14-30%). Similarly, Gharieb et al., (2023) reported that E. coli showed resistance to Oxytetracycline (74.54%) and sensitive to Gentamicin (56.36%); in contrast, sensitive to Trimethoprim Sulfamethoxazole (60%). Another study conducted by Abd El-Dayem et al., (2020) reported that E. coli was resistant to Amoxicillin, Sulfamethoxazole 75%, 100%, respectively. Additionally Abd El-Tawab et al., (2016) that found E. coli had resistance to Erythromycin (100%), Trimethoprim Sulfamethoxazole (93%) and Gentamycin (40%). Regarding Klebsiella spp, it has been reported by El-Tawab et al.,(2022) that they showed 100% resistant to Oxytetracycline and Trimethoprim Sulfamethoxazole.Furthermore, previously Klebsiella spp showed resistance to Oxytetracycline (100%), Erythromycin (90%), and Gentamycin (65%) in study carried by M. I. et al., (2019). Additionally, Younis et al., (2016) demonstrated 100% resistance to Amoxicillin by Klebsiella spp. Comparably Citrobacter freundii revealed high sensitivity to Gentamicin, and resistant to Oxytetracycline, Trimethoprim Sulfamethoxazole by El-Tawab et al., (2018). Thirty-seven strong biofilm producer E.coli isolates were identified serologically. The results identified 11 serotypes (Table 3). The most common serotype was O114:K90 (27%), followed by O26:K60 (18.9%), O78 (10.8), O91:H21, O128:H2, O55:K59 (8.1%, each) and O17:H18, O121:H7, O113:H4, O159 (2.7%, each). The obtained results came close to findings of Eid and Merfan et al., (2013) who serotyped E.coli isolates into O114:K90 (17.86%), O26:K60 (10.71%), O44:K74 (3.57%), O55:K59 (14.29%), O125:K70 (14.29%) and O111:K58 (10.71%). Also Hussein et al., (2022) identified E. coli O55 (4.5%) and O44 (16.6%), Rosario et al., (2004) found E. coli O78 (5%) and O91, While Abd El- Tawab et al., (2016) revealed identification of E. coliO44, O78, O114, O26 and O91. Three strong biofilm K. pneumoniae were identified serologically using hyper muco-viscosity capsular antigen into K2, K1 that agreed with Jian-Li et al., (2017) and Lamey et al., (2023). Emergency of antibiotics resistance phenomena was the motive for finding antibiotics alternatives. One of them is application of Nanotechnology ( Siddiqui and Zaman, 2018). In the current study, antibacterial and antibiofilm effect of Zinc oxide NPs and Propolis nanoemulsion were estimated against strong biofilm of E. coli, Klebsiella pneumoniae, Klebsiella oxtytoca, Klebsiella ozanae and Citrobacter freundii. MIC of Zinc oxide NPs was 35 µg/ml against Escherichia coli, Klebsiella spp and Citrobacter freundii (table 5), which was close to previously determined MIC (31.25 µg/ml) against E. coli by Alekshish et al., (2018). Propolis NPs MIC which determined by ten-fold dilution in-vitro was found to be 37.5% for E. coli, Klebsiella pneumoniae, Klebsiella oxtytoca, Klebsiella ozanae and Citrobacter freundii growth. For the authors own sake, there have been very few reports of the nano propolis but the antibacterial effect of propolis had been reported by many authors such as (Hegazi and Hady, 2001) which determined MIC ranged from 1800 to 3200 µg /ml for Escherichia coli. In contrast, E. coli showed resistance against propolis (Hegazi et al., 2014). The biofilm formation was initiated at concentrations 8.75 and 17.5µg/ml Zinc oxide NPs by both E. coli and Citrobacter freundii, while at 17.5 µg/ml for Klebsiella species (Table 5). While, Propolis nanoemulsion inhibited biofilm formation by all of them at concentration 18.75 % (Table 6). In our experience, it was the first report evaluated the antibiofilm effect of Zinc oxide NPs and Propolis nanoemulsion against coliform spp.

5. CONCLUSIONS
From the obtained results it could be concluded that coliform spp isolated from visceral organs of freshly dead chickens showed antibiotic resistance to commonly used antibiotics; Amoxicillin, Oxytetracycline, Erythromycin and Trimethoprim Sulfamethoxazole. Zinc oxide NPs yielded a safe antibacterial effect which may provide good antibiotics alternatives and using of natural nanoemulsion like propolis as antibiofilm and antibacterial has no residue and safe in cell till high concentration.

CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest for current data

6. REFERENCES


