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Original Article

Comparative Evaluation of the Effects of Binzalkonium Chloride, Iodine, Gluteraldehyde and Hydrogen Peroxide Disinfectants against Avian Salmonellae Focusing on Genotypic Resistance Pattern of the Salmonellae Serotypes toward Benzalkonium Chloride

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ABSTRACT

Using disinfectants in poultry houses is a common practice to ban the zoonotic pathogens like *Salmonella*. A major concern in using disinfectants is the emergence of bacteria strains that resist some disinfectants. This phenomenon is manifested in the resistance of some *Salmonella* serotypes against quaternary ammonium compounds. Such resistance is attributed to *qacEΔ1* gene which may be possessed by some *Salmonella* serotypes. This work aimed to evaluate the resistance of *Salmonella* serotypes (*S.* Typhimurium, *S.* Infantis and *S.* Entiridis) against different disinfectants (benzalkonium chloride, iodine, gluteraldehyde and hydrogen peroxide). The effect of the disinfectants were evaluated by treatment of the bacteria with different concentrations (1:100, 200 and 400) at different temperatures and periods. Bacterial count was performed before and after the treatment. PCR for presence of *qacEΔ1* gene was also performed before and after the treatment. The biocidal effect of the disinfectants found to be dependent on concentration, temperature and treatment period in addition to the type of the disinfectant. Hydrogen peroxide proved to be the most active agent followed by gluteradehyde, iodine and benzalkonium chloride. A link between the resistance against benzalkonium chloride and the existence of *qacEΔ1* gene was proven in *S.* Typhimurium, whether treated or not treated with benzalkonium chloride.

INTRODUCTION

Throughout recent decades, *Salmonellosis* importance increased substantially considering the poultry industry around the world. Salmonellosis is seen as the causative agent in charge of different pathogenic courses in humans and animals with more than a million of infectious cases among people annually (CDC 2011). Salmonella revealed itself as the most identified microorganism in broiler, turkey and pig fresh meats (European Food Safety 2010). Other studies conducted in Turkey and in other countries uncovered high incidences of *Salmonella* spp. of checked chicken meat samples (Minami *et al*., 2010; TA *et al*., 2012; Kim *et al*., 2016). *Salmonella* is introduced to human food from the contamination of poultry carcasses with infected feces or eggs. Many isolates of *Salmonella* serovars were detected from contaminated poultry but an estimation of the precise number of serovars is still difficult as more than 3000serovars are known (Sing, 2015; El-Sharkawy *et al*., 2017). Serovars of *Salmonella* may be hosted in different animal species and cause different diseases. The majority of the serovars have slight specificity toward their host species (Bäumler *et al*.,1998; Sheela *et al*., 2003). Roughly, *Salmonella* genus is classified to three groups. The invasive and highly adapted-host Group 1. The invasive and non-host adapted as Group 2 that comprises 10-20

serovars which are able to infect poultry and sometimes humans. Among these groups are *Salmonella* Enteritidis*, Salmonella* Typhimurium, and *Salmonella* Infantis. Group 3 (non-host adapted & non-invasive serovars)which included the major serovars of *Salmonella* and mostly are harmless to animals and humans (Barrow & Neto, 2011; Coble et al., 2013; Sing, 2015). The pathway of transmission and blowout of *Salmonella* may take place vertically or/and horizontally. Reservoirs of *Salmonella* included different living organisms such as farm animals, pigeons, humans, wild birds and waterfowl. Other potential sources comprised rodents, pets and insects that may act as vehicles for passing infection between birds and from house to house. Studies showed that feeding is regarded as the most common rout for cross spread (Sing, 2015; El-Sharkawy *et al*., 2017). Control of the disease is maintained by precise biosecurity level including periodical serological assessment and quarantine of infected birds (Bouzoubaa, Lemainguer, & Bell, 1992). Poultry *Salmonellosis* which resulted in disease and death leading to variable reduction in productivity, caused huge losses in the economy of this industry (Kaura *et al*., 1990; Swayne, 1998).To achieve the target of minimizing infection, routine disinfection programs are performed in poultry houses throughout production stages. Many types of chemical disinfection agents used, still need for better evaluation (Rumi*et al*., 2012). Commonly used disinfectants that are applied on livestock houses have known chemical features and familiar modes of action. These disinfectants were reviewed and investigated by many researchers (Denyer & Stewart, 1998; Lambert, 2008). The effect of glutaraldehyde and formaldehyde attributed to their ability to add alkyl group and generate cross-links between protein causing an addition to binding to peptidoglycans of the cell wall. Similarly, Formaldehyde was found to form crosslinking between DNA and proteins. The stability of glutaraldehyde was found to be better at acidic pH but its bactericidal effect was more at alkaline conditions (Gorman *et al*., 1980). Gluteraldehyde damage activity is faster than formaldehyde. Aldehydes, particularly formaldehyde, not readily posed to inhibition by organic stuffs (Gorman *et al*., 1980). Halogen-liberating chemicals express their disinfection by releasing active forms (chlorines or iodine). The presence of chlorines as hypochlorous acid or hypochlorite anions act as an oxidizing agent that destroys both the bacterial membrane and the DNA. Halogen releasing compounds have disadvantage related to their relative fast suppression by organic rubbles (Bessems, 1998). Other types of oxidizing agents are pyroxegens where peracetic acid is commonly used as a disinfectant. They performed their effects using OH radical, which disrupt vital cellular components (McDonnell & Russell, 1999). Considerable part of pyroxegens activity lost owing to reactions with the organic matters (Chapman, 2003; Russell, 2004). Disinfection by hydrogen peroxide is an excellent option. Using hydrogen peroxide to disinfect *E. coli* was found to be dependent on its concentration (Labas *et al*, 2008). Hydrogen peroxide vapor (HPV) showed excellent disinfection ability against *Salmonella* Typhimurium, *E.* coli and *Listeria monocytogenes* with dependency on concentration and time of exposure (Back, 2014).The cationic surfactants, like quaternary ammonium compounds, exert their activity by disrupting the bacterial membranes but some indicators pointed effects against the cytoplasmic components (Chapman, 2003), particularly when using high concentrations (Lambert, 2008). *Salmonella* Enteritidis isolates Quaternary ammonium was effective against *Salmonella* Enteritidis isolates during all contact times (Cardoso *et al*., 2008). Formulations of disinfectants containing quaternary ammonium compounds (QACs) including benzalkonium chloride have poor effects against gram-negative bacteria, coliforms, and spores (Wales *et al*., 2006). A combination of benzalkonium chloride and glutaraldehyde eliminated the risk of *Salmonella* Enteritidis and prevent the invasion of the microbe to the edible part of the eggs (Aksu *et al*., 2006). Preparations composed from formaldehyde, glutaraldehyde and quaternary ammonium compound (QAC) showed better performance against *Salmonella* than oxidizing agents in Turkey houses (Mueller-Doblies *et al*., 2010). *Salmonella* and in general all microbial contaminants even individuals from the same species showed dissimilarities in their susceptibility toward different frequently used disinfectants. At the same time, similar disinfectants with different chemical formulations may show variations in the effectiveness against microbial contaminants (Gehan *et al*., 2009). Davison *et al*. (2003) evaluated five groups of disinfectants against *Salmonella* Enteritidis. They concluded that failure of some compounds to defeat the microbe in some poultry houses attributed to the reinfection of the houses by microbe carriers like rodents or other sources that reintroduce the infection (Davison *et al*., 2003).Using identical control parameters for different animal houses showed different outputs in struggling against *Salmonella*. This phenomenon cleared when certain premises showed *Salmonella*

negative results after decontamination program while other premises still showed positive for *Salmonella* even after repetitive cleaning and disinfection processes (Davison *et al*., 2003).These notifications proposed variations in the resistance response of *Salmonella* toward disinfection agents that are attributed to molecular basis related to specific genes (Paulsen *et al*., 1993). Prolonged exposure to disinfectant and repeated use may induce resistance ability among *Salmonella* species and reduction in its effectiveness (Sander, 2002; Randall*et al*., 2004). Benzalkonium chloride is a cationic quaternary ammonium chloride derivative, which exerts surface activity. This compound is frequently used as a disinfectant in poultry houses. Resistance against benzalkonium chloride (BKC) are reported among different types of bacteria like *Listeria monocytogenes* (Mereghetti *et al*., 2000) and *Staphylococcus* spp. (Taheri *et al*., 2016). Gaining of resistance genes, *qacE* and *qacEΔ1* seen as one of the main mechanisms resulted in resistance ability against quaternary ammonium chloride derivatives (McDonnell & Russell, 1999). *qacEΔ1* emerged as a mutant of *qacE* gene. It seems partially to function as a multidrug transporter (Kazama *et al*., 1999). *qacE* gene is considered a common gene among gram-negative bacteria as it is located in the conserved region of integrons class 1 at the 3′ side (Paulsen *et al*., 1993). Class 1 integrons regarded as main donors to multidrug resistance among gram-negative bacteria (Fluit & Schmitz, 2004). The different mobile regions containing *qacE* gene cassettes on class 1 integrons described as mobile elements that typically coding resistance against antibiotics (Gaze *et al*., 2005; Chuanchuen, 2007). Horizontal gene transfer is a fundamental process for transferring genes between bacteria including antimicrobial resistance genes, which appeared among pathogenic bacteria (Wright, 2012). Resistance against antimicrobial agents identified in *Salmonella enterica* strains (Ángel *et al*., 2014; Bengtsson-Palme *et al*., 2014). *qac*genes is considered an important player in adapting to different cationic compounds. Gradual adapting to double increase in quaternary ammonium compounds (QAC) concentration was reported recently in *Staphylococcus aureus* having *qac* genes (Smith*et al*., 2007). In spite of low QAC resistance among staphylococci strains which carry-*qac* genes, adapting exposure to QAC induced appearance of resistant strains (Sundheim *et al*., 1998). Exposing of *qac* positive staphylococci to sublethal doses of benzalkonium chloride (BKC) induced adaptation ability toward BKC by these bacteria (Heir, 1998). Resistance to chlorhexidine reported to increase in *qac*-positivegenes after gradual exposure of *Staphylococcus aureus* to the disinfectant (Vali *et al*., 2008). Other reports described resistance against BKC and chlorhexidine and some disinfectants in *qac*-negative staphylococci (Heir *et al*., 1998; Vali *et al*., 2008). Increased resistance toward the disinfectant may also be attributed to changes in structure of the cell wall (McDonnell & Russell, 1999).Reports indicated that bacteria showing amplified resistance ability toward disinfectants were more resistance to antibiotics. These contradicted reports made discussion about mechanism of resistance against cationic compounds controlled by *qac* genes or other mechanism still opened (Jaglic & Cervinkova, 2012).Other reports supported presence of close interface between antibiotic resistance and presence of *qac* genes that correlated to resistance against quaternary ammonium compounds (De Marco *et al*., 2007; Theis *et al*., 2007; Ángel *et al*., 2014). *Qac* genes of the gram-negative Enterobacteriaceae reported in linkage with plasmid-mediated class 1 integrons which docking different genes coding resistance to antibiotic (Espedido *et al*., 2008; Zhao *et al*., 2012). Comparable outcomes also reported in *Pseudomonas aeruginosa* (Jeong *et al*., 2009; Colinon *et al*., 2010). Reports concerning connection between antibiotics resistance and resistance against disinfectants showed that exposure to different types of cationic disinfectants may accused with selection of antibiotics resistant bacterial strains (Hegstad *et al*., 2010; Jaglic & Cervinkova, 2012).

MATERIALS AND METHODS

Microorganisms

Salmonella Infantis, *Salmonella* Typhimurium and *Salmonella* Enteritidis serotypes, were provided by the Department of Microbiology, Faculty of Veterinary, University of Ankara. The bacteria was previously isolated from a poultry house then the serotypes characteristics were validated by serological tests. Trypticase Soy Broth (TSB, Oxoid) was supplemented with 20% Glycerin (Merck) used to preserve the isolates and the serotyped strains(ARDA, 2006).

Disinfectants

The disinfectants used were supplied as raw commercial materials from a local company. Disinfectants were 100% concentrations including iodine, hydrogen peroxide, glutaraldehyde and benzalkonium chloride (derivative of quaternary ammonium compounds).

Primers

In this study, the NCBI-BLAST [http://www.ncbi.nlm.nih.gov/] and Fast-PCR [http://primerdigital.com/fastpcr.html] programs were used to investigate genotypic profiles of resistance to benzalkonium chloride (quaternary ammonium compound) by using Integron class 1 which contain the gene responsible for resistance to quaternary ammonium compounds (*qacE*Δ1). The *qacE*Δ1 gene contains the target gene regions that researchers have identified(Chuanchuen *et al*., 2007). After obtaining the intron primer, DNA extraction of the bacteria (*Salmonella* Infantis, *Salmonella* Typhimurium and *Salmonella* Enteritidis strains) carried out. The primer base sequence used, the gene region of interest, product length and source information are shown in Table 1 below.

Table 1 – Primer base sequence.

Primer	Sequence	Target gene	Product length	Reference
qacEΔ1F	5'- ATCGCAATAGTTGGCGAAGT -3'			The present study
qacEΔ1 R	5'- CAAGCTTTTGCCCATGAAGC -3'	*qacEΔ1*	363 bp	

Bacterial counts

Bacterial counts were carried out during the study to estimate the concentration of the bacteria in the tested inoculum and to evaluate the effectiveness of the examined disinfectants. A modified (Miles & Misra1938) procedure was used to carry out the bacterial count. For the estimation of the bacterial concentration in the inoculum,1 ml of bacterial suspension at logarithmic growth phase was taken and diluted serially 10 folds in physiological saline and0.1 mL of dilutions (10^{-4}, 10^{-5}, and 10^{-6}) seeded onto the nutrient agar in 3 serial spreading patterns. The estimation of the bacterial number after using the disinfectants were determined by repeating the preceding steps after exposing the bacterial inoculum to the disinfectant. All sowing media incubate for 24 hours at 37 ° C and the average number of bacteria in 1 ml of the original suspension calculated according to the method reported by (ARDA, 2006).

Evaluation of the biocidal activity of the disinfectants

Disinfectant dilutions prepared from the stock solutions to give the ratios, 1: 100, 1: 200 and 1: 400 v/v of distilled water. To examine the biocidal activity of the disinfectants,8 ml of diluted solutions was added to one ml of organic material (skim milk) and mixed with one ml of bacterial serial dilutions prepared previously and then mixed homogeneously. The mixtures allowed standing for different periods of exposure time (5 minutes, 30 minutes, 60 minutes, 6 hours and 24 hours). 0.1 ml of the mixture was inoculated by spreading over nutrient agar and incubated for 24 hours at 4 ° C, 20 ° C and 37 ° C. The number of colonies were counted after 24 hours. The evaluation criteria for the antimicrobial activity of the disinfectants were assessed in relation to the contact time for each dilution of the disinfectants.

Evaluation of benzalkonium chloride (quaternary ammonium compound) genotypic resistance profile

In order to examine the genotypic resistance profiles of the benzalkonium chloride (quaternary ammonium compound), DNA extraction, amplification by PCR, electrophoresis and imaging performed for the class 1 integron in the investigated bacteria (*Salmonella* Typhimurium, *Salmonella* Infantis & *Salmonella* Enteritidis) without and after treatment with the disinfectant. The determination of the genotypic profile of the investigated bacteria without treatment with the disinfectant was carried out by the activation of the bacteria on nutrient agar and one colony inserted into each 1.5 ml eppendorf tubes and DNA extraction was performed (reference).

To examine the genotypic resistance profile of the bacteria related to quaternary ammonium compound (benzalkonium chloride), a disinfectant dilution (1:400 v/v of distilled water) was prepared and 8 ml of diluted benzalkonium chloride added to one ml of organic material (skim milk) and mixed with one ml of bacterial serial dilutions prepared previously and then mixing homogeneously. The mixtures allowed standing for 5 minutes at 37 °C. A 0.1 ml of the mixture inoculated by spreading over nutrient agar plates and incubated at 37 °C for 24 hours and one colony inserted into each 1.5 ml Eppendorf tubes and DNA extraction was performed Reference).

RESULTS

Effect of benzalkonium chloride

All investigated bacteria (*Salmonella* Enitiridis, *Salmonella* Infantis & *Salmonella* Typhimorium) showed no observable growth after treatment with (1:100) diluted benzalkonium chloride for all treatment

periods at all treatment temperatures. When the bacteria treated with (1:200) the diluted benzalkonium chloride under the investigated treatment periods and temperatures, *Salmonella* Enteritidis did not show any observable growth but *Salmonella* Infantis exhibited uncountable growth after 5 minutes treatment time and 4°C incubation temperature. By increasing the treatment period to (5, 15, 60 minutes 6 and 24 hours) and the incubation temperature kept at 4°C growth of *Salmonella* Infantis showed steady decrease down to (15, 2, 2, 0) CFU (colony forming units) respectively. Increasing incubation temperature after treatment with (1:200) diluted benzalkonium chloride caused the reduction of CFU of *Salmonella* Infantis to 55 CFU after 5 minutes treatment period while no growth was observed when the remained treatment periods increased. Results showed no growth by both *Salmonella* Enteritidis & *Salmonella* Infantis after increasing incubation temperature up to 37°C. When *Salmonella* Typhimorium treated with (1:200) diluted benzalkonium chloride for 5 & 30 minutes and incubated at 4°C, its growth was uncountable while increasing treatment period to 60 minutes reduced the growth to 31 CFU & no growth was observed when the treatment period increased to 6 and 24 hours for the same treatment temperature (4°C). Increasing treatment temperature to 20°C did not cause any change in growth pattern of *Salmonella* Infantis when treatment period was 5 minutes but increasing treatment temperature to 37°C lowered the growth to 84 CFU. *Salmonella* Infantis showed no growth after increasing treatment temperature to 20 and 37°C with the application of the disinfectant for 30, 60 minutes and 6, 24 hours treatment periods. Using(1:400) of benzalkonium chloride dilution as disinfection agent did not show any growth inhibition with all investigated bacteria at all treatment periods and 4°C treatment temperature except *Salmonella* Enteritidis that did not give any growth after 24 hours treatment period. Increasing the treatment temperature caused growth inhibition for both *Salmonella* Enteritidis & *Salmonella* Infantis when the treatment time increased to 60 minutes and more but growth of *Salmonella* Typhimorium reduced to 60 CFU after 60 minutes of treatment period and completely inhibited at 6 and 24 hours treatment periods. The growth of *Salmonella* Typhimorium was not observable when the bacteria treated with (1:400) benzalkonium chloride for (60 minutes, 6 and 24 hours) at 37°C (Table 2).

Table 2 – Benzalkonium chloride.

Dilution	Incubation temperature	Bacteria														
		Salmonella Enteritidis (CFU)					*Salmonella* Infantis (CFU)					*Salmonella* Typhimurium (CFU)				
		5. min	30. min	60. min	6. hr	24. hr	5. min	30. min	60. min	6. hr	24. hr	5. min	30. min	60. min	6. hr	24. hr
1/100	4°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	20°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	37°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1/200	4°C	0	0	0	0	0	∞	15	2	2	0	∞	∞	31	0	0
	20°C	0	0	0	0	0	55	0	0	0	0	84	0	0	0	0
	37°C	0	0	0	0	0	0	0	0	0	0	∞	0	0	0	0
1/400	4°C	∞	∞	∞	∞	0	∞	∞	∞	∞	∞	∞	∞	∞	∞	∞
	20°C	∞	∞	0	0	0	∞	∞	0	0	0	∞	∞	60	0	0
	37°C	∞	4	0	0	0	∞	0	0	0	0	∞	∞	0	0	0

Effect of iodine

Using iodine as a disinfection agent was completely preventive for the growth of the three investigated bacteria when using (1:100 and 1:200) dilutions at all treatment periods and temperatures. Using more diluted iodine (1:400) did not exert any detectable inhibition effect over *Salmonella* Enteritidis at any treatment period and temperature. At 4°C temperature treatment, the growth of *Salmonella* Infantis decreased to 53, 37, 21, 12 CFU and no growth after treatment with (1:400) diluted iodine at treatment periods (5, 30, 60 minutes, 6 and 24 hours) respectively. Increasing treatment period caused inhibition for bacterial growth after the treatment for 6 hours at 20°C where bacterial count decreased to 260 CFU. Complete inhibition of growth occurred after the treatment for 24 hours at 37°C treatment temperature. *Salmonella* Typhimorium showed no growth inhibition after treatment with (1:400) diluted iodine at all treatment periods and treatment temperatures except when the treatment period increased to 24 hours where bacterial count was 20 CFU at 20°C treatment period and no observed growth at 37°C (Table 3).

Table 3 – Iodine.

Dilution	Incubation temperature	Bacteria														
		Salmonella Enteritidis (CFU)					*Salmonella* Infantis (CFU)					*Salmonella* Typhimurium (CFU)				
		5. min	30. min	60. min	6. hr	24. hr	5. min	30. min	60. min	6. hr	24. hr	5. min	30. min	60. min	6. hr	24. hr
1/100	4°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	20°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	37°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1/200	4°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	20°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	37°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1/400	4°C	∞	∞	∞	∞	∞	53	37	21	12	0	∞	∞	∞	∞	∞
	20°C	∞	∞	∞	∞	∞	∞	∞	∞	260	0	∞	∞	∞	∞	20
	37°C	∞	∞	∞	∞	∞	∞	∞	∞	∞	0	∞	∞	∞	∞	0

Effect of gluteraldehyde

The (1:100) diluted gluteraldehyde caused complete growth inhibition for all examined bacterial serotypes. The (1:200) diluted gluteraldehyde still bring complete growth inhibition against *Salmonella* Enteritidis & *Salmonella* Infantis but against *Salmonella* Typhmorium, it caused growth reduction down to 20 CFU at 4°C treatment temperature for 5 minutes. At the same 5 minutes treatment period, increasing treatment temperature to 20°C did not inhibit growth but increasing treatment period caused complete growth inhibition. Raising up treatment temperature to 37°C caused complete growth inhibition at all treatment periods. Using (1:400) diluted gluteraldehyde against *Salmonella* Enteritidis caused reduced growth (40 CFU) at treatment temperature 4°C and 5 minutes treatment period. Increasing treatment period or treatment temperature caused complete growth inhibition for the three bacterial serotypes in spite that 5 minutes treatment period was not effective against *Salmonella* Typhimorium at all treatment temperatures (Table 4).

Table 4 – Gluteradehyde

Dilution	Incubation temperature	Bacteria														
		Salmonella Enteritidis (CFU)					*Salmonella* Infantis (CFU)					*Salmonella* Typhimurium (CFU)				
		5. min	30. min	60. min	6. hr	24. hr	5. min	30. min	60. min	6. hr	24. hr	5. min	30. min	60. min	6. hr	24. hr
1/100	4°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	20°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	37°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1/200	4°C	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0
	20°C	0	0	0	0	0	0	0	0	0	0	∞	0	0	0	0
	37°C	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1/400	4°C	40	0	0	0	0	0	0	0	0	0	∞	0	0	0	0
	20°C	0	0	0	0	0	0	0	0	0	0	∞	0	0	0	0
	37°C	0	0	0	0	0	0	0	0	0	0	∞	0	0	0	0

Hydrogen peroxide effect

Results of the treatment with hydrogen peroxide showed no growth by the three investigated bacterial serotypes at all treatment conditions (Table 5).

Genotyping results of benzalkonium chloride resistance tests

The DNA of *Salmonella* Infantis, *Salmonella* Tuphimorium and *Salmonella* Enteritidis serotypes were extracted and then examined by PCR for the presence of the *qacEΔ1* gene. The genotyping tests were applied for the treated and non-treated bacteria with benzalkonium chloride. The results showed that *Salmonella* Thyphimorium contains the *qacEΔ1* gene whether the bacteria was treated or not treated with the disinfectant (see lane 5 & 8 in Figure 1). Results from the gel electrophoresis did not show any band related to the *qacEΔ1* gene in both *Salmonella* Enteritidisand *Salmonella* Infantis (see lane 6 & 7, Figure 1).

Table 5 – Hydrogen peroxide.

Dilution	Incubation temperature	Bacteria														
		Salmonella Enteritidis (CFU)					*Salmonella* Infantis (CFU)					*Salmonella* Typhimurium (CFU)				
		5. min	30. min	60. min	6. hr	24. hr	5. min	30. min	60. min	6. hr	24. hr	5. min	30. min	60. min	6. hr	24. hr
1/100	4ºC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	20ºC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	37ºC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1/200	4ºC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	20ºC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	37ºC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1/400	4ºC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	20ºC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	37ºC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

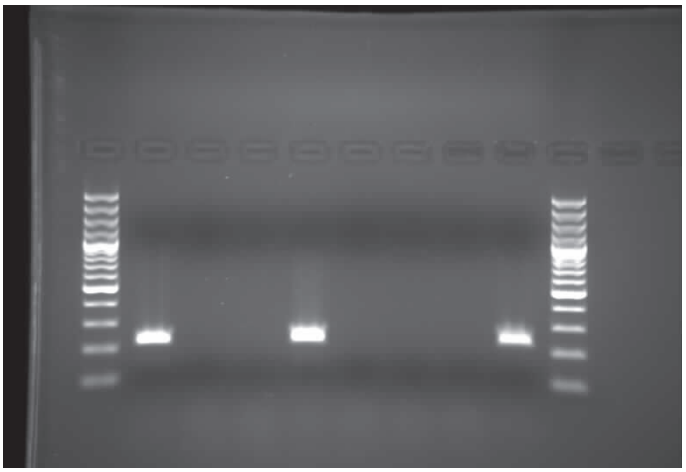

Figure 1 – Genotyping results of benzalkonium chloride resistance showing qacEΔ1 positive for *Salmonella* Thyphimorium before and after exposure to the benzalkonium chloride (lane 6 & 7) and the positive control (lane 1).

DISCUSSION

This study aimed to investigate the effects of some commonly used disinfectants on *Salmonella* Enteritidis, *Salmonella* Infantis and *Salmonella* Typhimurium, taking into account the duration of exposure to the disinfectants, environmental temperature and disinfectant concentration.

The implementation of effective sanitation programmes at poultry production sites is of great importance for consumers and for public health. The long-term use of disinfectants in poultry houses causes the development of resistance to the active ingredients in these products, so it is important to measure the effectiveness of commonly used disinfectants on the pathogenic microorganism and determine their effectiveness and conditions for their application to achieve the maximum benefit.

The results of treatment with benzalkonium chloride (BKC) showed that its killing ability depends firstly on the concentration and more diluted disinfectant needs longer time and higher temperature to work. Our results were in agreement with those obtained by Gehan *et al*. (2009), who studied the efficacy of five commercial disinfectant agents, two of them were quaternary ammonium chloride formulation in addition to hydrogen peroxide, against group of microorganisms including *Salmonella* Typhimurium. They concluded that increasing contact time or increasing concentration of the disinfectant is more effective against the microbes. Results of disinfection by hydrogen peroxide obtained by Sander *et al*. (2002) and Gehan *et al*. (2009) agreed with the results from this work but Gehan *et al*. (2009) indicated that the presence of organic matter brought the need for either higher disinfectant concentration or longer time exposure. Sander *et al*. (2002) showed short time killing (5 to 10 minutes) for bacteria including *Salmonella* isolates when using hydrogen peroxide which is agreeable with results of this work. The results of gluteraldehyde showed excellent killing capacity for all used concentrations against all the serotypes under all treatment conditions but decreasing its concentration invited the need for longer exposure time. Weber *et al*. (2007) reported that glutaraldehyde preparations containing high levels of oil showed effect within 20-45 minutes. McLaren *et al*. (2011) showed that killing capacity of disinfectants containing glutaraldehyde, iodine and hydrogen peroxide to *Salmonella* Typhimurium and *Salmonella* Enteritidis isolates was a time dependant as in the results observed in this work. The outcomes of this work were similar to reported results of Gehan *et al*. (2009) for the effects of hydrogen peroxide at 30 and 60 min, and Sander *et al*. (2002) for 5-10 min treatment with hydrogen peroxide. This study also found to concur with the results of (McLaren *et al*., 2011) for 30 min

of exposure to glutaraldehyde, iodine and hydrogen peroxide. (McLaren *et al*., 2011) investigated the effects of exposure to variable concentrations of glutaraldehyde, hydrogen peroxide and iodine at 37°C for 30 min, 2 h and 4 h on *Salmonella* Typhimurium and *Salmonella* Enteritidis isolates from chicken and turkey houses. It was ascertained that exposure to glutaraldehyde for all three of the indicated time periods at 37°C had prevented the growth of *Salmonellae*. On the other hand, while exposure to iodine and hydrogen peroxide for 30 min at 37°C related to bacterial growth, exposure periods of 2 h and 4 h ascertained to have prevented the growth of *Salmonellae*. In another research, Stringfellow *et al*. (2009) concluded that the temperature was largely effective factor in the disinfection activity against the bacteria which is agreeable with the results of the present work. The temperatures at which no growth was determined to have occurred in the present study are similar to the results of glutaraldehyde, but different from those reported for iodine and hydrogen peroxide by McLaren *et al*. (2011). Concentration of the disinfectant agent observed as a strong factor in determining the effectiveness of the disinfectant agent. This conclusion was also supported by the results of Møretrø *et al*. (2012). McLaren *et al*., (2011) found that gluteraldehyde was the most effective bactericidal agent against *Salmonella* Typhimurium and *Salmonella* Enteritidis isolated from chicken and turkey houses. In the present study, glutaraldehyde showed to be a strong disinfectant that is valid for the use at all temperatures for nearly short times not exceeding 30 minutes. Results from (Gehan *et al*., 2009) pointed that the most effective disinfectant was H_2O_2. In the present study, the most effective disinfectant was identified as hydrogen peroxide. This result is in covenant with the report of (Gehan *et al*., 2009), but contrasts from the results reported by (McLaren *et al*., 2011) and (Sander *et al*., 2002) who applied protocols aimed to determine the effectiveness of wet and dry models for eliminating the microbes that are different from the present working protocol. In spite of that, McLaren *et al*. (2011) showed that both concentration and time of exposure were a strong determinant factor on the effectiveness of the disinfectant. Huberman & Terzolo (2008) found that 25-50 ppm dose of N-alkyl dimethyl benzyl ammonium chloride added to the drinking water of chickens to protect against Typhoid reduced the mortality down to 30% of the chickens compared with chickens provided with water free from disinfectant. Due to the dilutions of the disinfectant solutions prepared in the present study being higher than the disinfectant concentrations used in studies of (McLaren *et al*., 2011) and (Huberman & Terzolo, 2008), a comparison was not able to be made. However, the results of the present study found to be similar to those reported by McLaren *et al*. (2011) in that these researchers determined a moderate disinfection may be achieved with the use of iodine and peroxide and an effective (good) disinfection may be achieved with the use of glutaraldehyde. A comparison could not made with the results obtained by Huberman & Terzolo (2008) due to different active substances in the used disinfectant.

Resistance mechanisms, and in particular multiple antibiotic resistance, observed in enteric microorganisms, including *Salmonellae*, are known to be associated with integrons. Integrons described as genes, which harbour the integrase gene (intI1) in addition to antibiotic resistance gene cassettes (attI). They integrate with open reading frames (ORF) where eventually functionalize them. Integrons also harbour genes, which encode resistance to disinfectants (qacE Delta1) and sulfonamides (sul1) (Maguire *et al*., 2001).

In their research on 156 zoonotic *Salmonella* Enteritidis and *Salmonella* Typhimurium isolates obtained from Norwegian hospitals, (Lindstedt *et al*., 2003) the existence of integrons was detected in 64 out of 66 *Salmonella* Typhimurium isolates (97%), and in 20 out of 90 *Salmonella* Enteritidis isolates (22.2%). The sensitivity toward clinically important antibiotics and benzalkonium chloride, a quaternary ammonium compound, was investigated in 122 *Salmonella enterica* isolates from pigs and poultry. All isolates were examined for the existence of qacE, qacEΔ1 and intI1 genes (class 1 integrase). While the gene of qacEΔ1 was found in 27% of the isolated bacteria, the occurrence of the gene intI1 confirmed in 18.9%. On the other hand, the gene of qacE was not detected in any isolate (Chuanchuen *et al*., 2007). In another study conducted to detect the presence of integron-associated gene cassettes, harbouring genes coding resistance to disinfectants (qacEΔ1) and sulfonamides (sul1) in randomly isolates of *Salmonella enterica* serotypes and *Salmonella* Typhimurium. Out of the 226 *Salmonella* Typhimurium serotypes around 183 (81%) confirmed to contain class 1 integrons. These integrons examined for the existence of the qacEΔ1 gene and the positivity rate was determined to be the same (Daly & Fanning, 2000). In this work, the occurrence of the qacEΔ1 gene was determined using the polymerase chain reaction (PCR) technique, in the chromosomal DNA of *Salmonella* Enteritidis, *Salmonella*

Infantis and *Salmonella* Typhimurium isolates. The bacteria was grown in the presence and absence of the benzylkonium ammonium chloride. Of the strains grown in the presence of benzylkonium ammonium chloride, *Salmonella* Enteritidis and *Salmonella* Infantis were negative for the qacEΔ1 gene, whilst *Salmonella* Typhimurium was positive for the qacEΔ1 gene. When grown without treatment with benzylkonium chloride, *Salmonella* Enteritidis & *Salmonella* Infantis were negative for the qacEΔ1 gene, whilst *Salmonella* Typhimurium was positive. Comparing results without the disinfectants obtained by (Daly & Fanning, 2000; Chuanchuen *et al*., 2007) the output results from this study were similar in that *Salmonella* Typhimurium isolates harboured the qacEΔ1 gene. Making comparison against the qacE gene was not possible, because no data was accessible for incidence of such gene in the isolates grown without the disinfectants. In spite of that *Salmonella* Enteritidis, *Salmonella* Infantis and *Salmonella* Typhimurium showed reduced susceptibility to the benzylkonium chloride after decreasing the concentration of the disinfectant, the presence of the qacEΔ1 gene proved only in *Salmonella* Typhimurium. This may indicate that the reduction of susceptibility to the benzalkonium chloride may be attributed to some physiological reasons like changes in the structure of the cell wall (McDonnell & Russell, 1999).

CONCLUSION

Examining the resistance of three *Salmonella* serotypes (*S.* Typhimurium, *S.* Infantis and *S.* Enteridis) against four commonly used disinfectants (benzalkonium chloride, hydrogen peroxide, iodine and gluteraldehyde) showed that the killing activity of the disinfectant was dependent on three factors including concentration, treatment temperature and treatment period. Increasing any of the three factors resulted in increasing killing activity of the disinfectant. The type of the disinfectant was a strong factor in its biocidal activity. Hydrogen peroxide proved to be the strongest disinfectant followed by gluteraldehyde, iodine and benzalkonium chloride. A connection between the resistance of the bacteria against benzalkonium chloride and genotype proved in *S.* Typhimurium serotype by presence of the *qacEΔ1* gene within the genome of the bacteria. The results of the study recommend an evaluation of the conditions (treatment temperature, treatment period and concentration of the disinfectant) to ensure effective application of the disinfectant. In addition, it is required to evaluate the presence of genetically based resistance of some *Salmonella* serotypes against quaternary ammonium chloride and its derivatives including benzalkonium chloride before using to ensure effectiveness of the disinfection protocol.

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REFERENCE

Ángel M, Fuentes F, Morente EO, Abriouel H, Pulido RP, Gálvez A. Antimicrobial resistance determinants in antibiotic and biocide-resistant gram-negative bacteria from organic foods. Food Control 2014; 37:9–14.

Arda M. Mikrobiyal üremenin kontrolü. Temel Mikrobiyoloji. Medisan Yayın Serisi. Ankara: Universitesi Veterinir Fakültesi; 2006.

Back KH, Ha JW, Kang DH. Effect of hydrogen peroxide vapor treatment for inactivating Salmonella Typhimurium, Escherichia coli O157: H7 and Listeria monocytogenes on organic fresh lettuce. Food Control 2014;44:78–85.

Barrow PA, Neto OCF. Pullorum disease and fowl typhoid—new thoughts on old diseases: a review. Avian Pathology 2011;40(1):1–13.

Bäumler AJ, Tsolis RM, Ficht TA, Adams LG. Evolution of host adaptation in Salmonella enterica. Infection and Immunity 1988;66(10):4579-4587.

Bengtsson-Palme J, Boulund F, Fick J, Kristiansson E, Larsson DGJ, Knapp CW, et al. Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India. Frontiers in Microbiology 2014;2(5):648.

Bessems E. The effect of practical conditions on the efficacy of disinfectants. *International* Biodeterioration & Biodegradation 1998;41(3–4):177–183.

Bouzoubaa K, Lemainguer K, Bell JG. Village chickens as a reservoir of Salmonella pullorum and Salmonella gallinarum in Morocco. Preventive Veterinary Medicine 1992; 12(1–2):95–100.

Cardoso M, Ribeiro A, Santos L, Borsoi A, Pilotto F, Rocha S, et al. In vitro efficiency of disinfectants against salmonella enteritidis samples isolated from broiler carcasses. *Revista* Brasileira de Ciência Avícola 2008;10(2):139–141.

Chapman JS. Biocide resistance mechanisms. International Biodeterioration & Biodegradation 2003;51(2):133-138.

Chuanchuen R, Khemtong S, Padungtod P. Occurrence of qacE/qacEDelta1 genes and their correlation with class 1 integrons in salmonella enterica isolates from poultry and swine. The Southeast Asian Journal of Tropical Medicine and Public Health 2007;38(5):855–562.

Coble DJ, Sandford EE, Ji T, Abernathy J, Fleming D, Zhou H, et al. Impacts of *Salmonella enteritidis* infection on liver transcriptome in broilers. Genesis 2013;51(5):357–364.

Colinon C, Jocktane D, Brothier E, Rossolini GM, Cournoyer B, Nazaret S. Genetic analyses of *Pseudomonas aeruginosa* isolated from healthy captive snakes: evidence of high inter- and intrasite dissemination and

occurrence of antibiotic resistance genes. Environmental Microbiology 2010;12(3):716–729.

Daly M, Fanning S. Characterization and chromosomal mapping of antimicrobial resistance genes in Salmonella enterica serotype typhimurium. Applied and Environmental Microbiology 2000;66(11):4842–4848.

Davison S, Benson CE, Munro DS, Rankin SC, Ziegler AF, Eckroade RJ. The role of disinfectant resistance of salmonella enterica serotype enteritidis in recurring infections in pennsylvania egg quality assurance program monitored flocks. Avian Diseases 2003;47(1):143–148.

DeMarco CE, Cushing LA, Frempong-Manso E, Seo SM, Jaravaza TAA, Kaatz, GW. Efflux-related resistance to norfloxacin, dyes, and biocides in bloodstream isolates of Staphylococcus aureus. Antimicrobial Agents and Chemotherapy 2007;51(9):3235–3239.

Denyer SP, Stewart GSAB. Mechanisms of action of disinfectants. *International* Biodeterioration & Biodegradation 1998;41(3–4):261–268.

El-Sharkawy H, Tahoun A, El-Gohary AE-GA, El-Abasy M, El-Khayat F, Gillespie T, et al. Epidemiological, molecular characterization and antibiotic resistance of Salmonella enterica serovars isolated from chicken farms in Egypt. Gut Pathogens 2017:9(1):8.

Espedido BA, Partridge SR, Iredell, JR. Bla(IMP-4) in different genetic contexts in Enterobacteriaceae isolates from Australia. Antimicrobial Agents and Chemotherapy 2008;52(8):2984–2987.

Fluit AC, Schmitz, F-J. Resistance integrons and super-integrons. Clinical Microbiology and Infection 2004;10(4):272–288.

Gaze WH, Abdouslam N, Hawkey PM, Wellington EMH. Incidence of class 1 integrons in a quaternary ammonium compound-polluted environment. Antimicrobial Agents and Chemotherapy 2005;49(5):1802–807.

Gehan ZM, Anwer W, Amer HM, El-Sabagh IM, Rezk A, Badawy EM. In vitro efficacy comparisons of disinfectants used in the commercial poultry farms. International Journal of Poultry Science 2009;8(3):237-241.

Gorman SP, Scott EM, Russell AD. Antimicrobial Activity, Uses and Mechanism of Action of Glutaraldehyde. Journal of Applied Bacteriology 1980;48(2):161–190.

Hegstad K, Langsrud S, Lunestad BT, Scheie AA, Sunde M, Yazdankhah SP. Does the wide use of quaternary ammonium compounds enhance the selection and spread of antimicrobial resistance and thus threaten our health? Microbial Drug Resistance 2010;16(2):91–104.

Heir E, Sundheim G, Holck AL. The *Staphylococcus qacH* gene product: a new member of the SMR family encoding multidrug resistance. FEMS Microbiology Letters 1998;163(1):49–56.

Huberman YD, Terzolo HR. Fowl typhoid: assessment of a disinfectant oral dose to reduce horizontal spread and mortality. Avian Diseases 2008;52(2):320–323.

Jaglic Z, Cervinkova D. Genetic basis of resistance to quaternary ammonium compounds – the qac genes and their role: a review. Veterinární Medicína 2012;57(6):275–281.

Jeong JH, Shin KS, Lee JW, Park EJ, Son S-Y. Analysis of a novel class 1 integron containing metallo-β-lactamase gene VIM-2 in Pseudomonas aeruginosa. The Journal of Microbiology 2009;47(6):753–759.

Kaura YK, Singh J, Kaushok RK, Kulshrestha RCM. Salmonella gallinarum var. duisburg: An emerging biotype causing heavy mortality in poultry birds in northern India. Indian Journal of Animal Science 1990;60(2):127-130

Kazama H, Hamashima H, Sasatsu M, Arai T. Characterization of the antiseptic-resistance gene qacE Î" 1 isolated from clinical and environmental isolates of Vibrio parahaemolyticus and Vibrio cholerae non-O1. FEMS Microbiology Letters 1999;174(2):379–384.

Kim H, Lee J, Jang Y, Chang B, Kim A, Choe, N. Prevalence and antimicrobial resistance of Salmonella spp. and Escherichia coli isolated from ducks in Korea. Journal of Veterinary Research 2016;56(2):91–95.

Labas MD, Zalazar CS, Brandi RJ, Cassano AE. Reaction kinetics of bacteria disinfection employing hydrogen peroxide. Biochemical Engineering Journal 2008;38(1):78–87.

Lambert PA. Mechanisms of action of biocides. In: Russell H. Ayliffe's principles and practice of disinfection, preservation & sterilization. Oxford: Blackwell Publishing; 2008. p.139-153.

Layton BM. Disinfectants and salmonella: a study showing the effectiveness of disinfectants against the bacteria salmonella. Saint Martin's University Biology Journal 2006;1:96-106.

Lindstedt B-A, Heir E, Nygård I, Kapperud G. Characterization of class I integrons in clinical strains of Salmonella enterica subsp. enterica serovars Typhimurium and Enteritidis from Norwegian hospitals. Journal of Medical Microbiology 2003;52(2):141–149.

Maguire AJ, Brown DF, Gray JJ, Desselberger U. Rapid screening technique for class 1 integrons in Enterobacteriaceae and nonfermenting gram-negative bacteria and its use in molecular epidemiology. Antimicrobial Agents and Chemotherapy 2001;45(4):1022–1029.

McDonnell G, Russell AD. Antiseptics and Disinfectants: Activity, Action, and Resistance. Clinical Microbiology Reviews 1999;12(1):147–179.

McLaren I, Wales A, Breslin M, Davies R. Evaluation of commonly-used farm disinfectants in wet and dry models of *Salmonella* farm contamination. Avian Pathology 2011;40(1):33–42.

Mereghetti L, Quentin R, Marquet-Van Der Mee N, Audurier A. Low sensitivity of Listeria monocytogenes to quaternary ammonium compounds. Applied and Environmental Microbiology 2000;66(11):5083–5086.

Miles AA, Misra SS, Irwin JO. The estimation of the bactericidal power of the blood. Epidemiology & Infection 1938;38(6):732-749.

Minami A, Chaicumpa W, Chongsa-Nguan M, Samosornsuk S, Monden S, Takeshi K, et al. Prevalence of foodborne pathogens in open markets and supermarkets in Thailand. Food Control 2010;21(3):221–226.

Møretrø T, Heir E, Nesse LL, Vestby LK, Langsrud S. Control of Salmonella in food related environments by chemical disinfection. Food Research International 2012;45(2):532–544.

Mueller-Doblies D, Carrique-Mas JJ, Sayers AR, Davies RH. A comparison of the efficacy of different disinfection methods in eliminating *Salmonella* contamination from turkey houses. Journal of Applied Microbiology 2010;109(2):471–479.

Paulsen IT, Littlejohn TG, Rådström P, Sundström L, Sköld O, Swedberg G, Skurray RA. The 3′ conserved segment of integrons contains a gene associated with multidrug resistance to antiseptics and disinfectants. Antimicrobial Agents and Chemotherapy 1993;37(4):761–768.

Randall LP, Cooles SW, Piddock LJV, Woodward, MJ. Effect of triclosan or a phenolic farm disinfectant on the selection of antibiotic-resistant Salmonella enterica. Journal of Antimicrobial Chemotherapy 2004;54(3):621–627.

Rumi N, Rahman M, Akter M, Fakhruzzaman M, Hossain M. Evaluation of the effectiveness of commercially available disinfectants against salmonellae isolated from internal organs of dead chickens. Bangladesh Journal of Veterinary Medicine 2012;9(1):43–52.

Russell AD. Factors influencing the efficacy of antimicrobial agents. In: Russell H. Ayliffe's principles and practice of disinfection, preservation & sterilization. Oxford: Blackwell Publishing; 2004. p.98-127.

Sander JE, Hofacre CL, Cheng I-H, Wyatt RD. Investigation of resistance of bacteria from commercial poultry sources to commercial disinfectants. Avian Diseases 2002;46(4):997–1001.

Sheela RR, Babu U, Mu J, Elankumaran S, Bautista DA, Raybourne RB, et al. Immune responses against salmonella enterica serovar enteritidis infection in virally immunosuppressed chickens. Clinical and Vaccine Immunology 2003;10(4):670–679.

Sing A. Zoonoses with public health relevance in poultry. In: Hafez MH, Haug R. Zoonoses: infections affecting humans and animals. Dordrecht: Springer Netherlands; 2015. p.103-123.

Smith K, Gemmell CG, Hunter IS. The association between biocide tolerance and the presence or absence of qac genes among hospital-acquired and community-acquired MRSA isolates. Journal of Antimicrobial Chemotherapy 2007;61(1): 78–84.

Stringfellow K, Anderson P, Caldwell D, Lee J, Byrd J, McReynolds J, et al. Evaluation of disinfectants commonly used by the commercial poultry industry under simulated field conditions. Poultry Science 2009;88(6):1151–1155.

Sundheim G, Langsrud S, Heir E, Holck AL. Bacterial resistance to disinfectants containing quaternary ammonium compounds. International Biodeterioration & Biodegradation 1998;41(3–4):235–239.

Swayne DE. A laboratory manual for the isolation and identification of avian pathogens. Philadelphia: American Association of Avian Pathologists; 1998.

Ta YT, Nguyen TT, To PB, Pham DX, Le HTH, Alali WQ, et al. Prevalence of Salmonella on Chicken Carcasses from Retail Markets in Vietnam. Journal of Food Protection 2012;75(10):1851–1854.

Taheri N, Ardebili A, Amouzandeh-Nobaveh A, Ghaznavi-Rad E. Frequency of antiseptic resistance among staphylococcus aureus and coagulase-negative staphylococci isolated from a University Hospital in Central Iran. Oman Medical Journal 2016;31(6):426–432.

The Community Summary Report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in the European Union in 2008. EFSA Journal 2010;8(1):1496.

Theis T, Skurray RA, Brown MH. Identification of suitable internal controls to study expression of a Staphylococcus aureus multidrug resistance system by quantitative real-time PCR. Journal of Microbiological Methods 2007;70(2):355–362.

Vali L, Davies SE, Lai LLG, Dave J, Amyes SGB. Frequency of biocide resistance genes, antibiotic resistance and the effect of chlorhexidine exposure on clinical methicillin-resistant Staphylococcus aureus isolates. Journal of Antimicrobial Chemotherapy 2008;61(3):524–532.

Wales A, Breslin M, Davies R. Assessment of cleaning and disinfection in Salmonella-contaminated poultry layer houses using qualitative and semi-quantitative culture techniques. Veterinary Microbiology 2006;116(4):283–293.

Weber DJ, Rutala WA, Sickbert-Bennett EE. Outbreaks associated with contaminated antiseptics and disinfectants. Antimicrobial Agents and Chemotherapy 2007;51(12):4217–4224.

Zhao W-H, Chen G, Ito R, Kimura S, Hu, Z-Q. Identification of a plasmid-borne blaIMP-11 gene in clinical isolates of Escherichia coli and Klebsiella pneumoniae. Journal of Medical Microbiology 2012;61(2):246–251.