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ORIGINAL ARTICLE

Efficiency evaluation of some novel disinfectants and anti-bacterial nanocomposite on zoonotic bacterial pathogens in commercial Mallard duck pens for efficient control

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ABSTRACT

Objective: This work aimed to detect the frequency of pathogenic bacteria of zoonotic importance in ducks' dropping, their surrounding environment, and farmworkers in contact with them. Furthermore, the susceptibility pattern of isolated bacteria to antimicrobial drugs and the efficiency of disinfectants (CID 20, Durak® plus, and hydrogen peroxide (H_2O_2), nano zinc oxide (ZnO NPs), and hydrogen peroxide loaded nano zinc oxide (H_2O_2/ZnO NPs) composites against isolated bacteria were evaluated.

Materials and Methods: A total of 271 samples were collected from duck pens, including 35 fecal droppings, 200 environmental samples, and 36 from the hands of pen workers for isolation and identification of bacterial strains using standard microbiological procedures. After that, the antibiotic sensitivity testing of 40 bacterial isolates was carried out using disk diffusion assay. ZnO NPs and H_2O_2/ZnO NPs were characterized using Fourier-transform infrared spectrum and high-resolution transmission electron microscopy. The efficacy of disinfectants and nanocomposites was evaluated against enteropathogenic bacteria using the broth macro-dilution method.

Results: The results showed that the overall prevalence of pathogenic bacteria in duck pens was 62.73. The highest isolation rate was detected in duck fecal droppings (100%), while *Escherichia coli* was found to be the most isolated pathogen (56.47%), followed by *Pseudomonas aeruginosa* (21.8%), *Proteus mirabilis* (15.29), and *Salmonella* species (6.47%). Multidrug resistance (MDR) was detected in the majority of bacterial isolates. The efficiency of CID 20 and Durak® plus disinfectants against all bacterial isolates was highly susceptible (100%) after 120 min of exposure time compared to the effectiveness of H_2O_2 on enteropathogenic bacteria which did not exceeded 60% at 5% concentration. Meanwhile, the sensitivity of *Salmonella* spp. to Durak® plus did not exceeded 80%.

Conclusion: The duck fecal droppings are the primary source of bacterial isolates. MDR isolates were susceptible to both CID 20 and Durak® plus disinfectants after 120 min of exposure time at a concentration of 1:100 ml. Besides, H_2O_2/ZnO NPs composite proved its lethal effect against all testing strains at 0.02 mg/ml after 120 min of exposure. Strict biosecurity guidelines are required to mitigate and prevent the transmission of potentially zoonotic pathogens through the farm environment and/or duck droppings.

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Introduction

Duck rearing for meat or egg production is considered profitable livestock practice worldwide [1]. However, the duck industry is exposed to substantial economic losses due to virulent bacteria that cause severe mortality in ducks [2]. There are many bacterial pathogens, including *Pseudomonas*, *Escherichia coli* (*E. coli*), and *Salmonella* spp., responsible for health threats in ducks worldwide [2] that they also impart health risk to humans [3]. Food-borne pathogens are considered one of the leading causes of emerging disease outbreaks that affect millions worldwide [4]. The opportunistic pathogen *Pseudomonas aeruginosa* causes many signs in ducks as lameness, septicemia, and respiratory infection [5]. *E. coli* causes a

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serious illness in young ducks with a mortality rate of up to 43% [6]. Salmonellosis causes acute and chronic diseases depending on the age of ducks [7]. It also acts as a common food-borne pathogen in humans, causing illness and death [8].

Exposure of human beings to the risk of infection might be increased through contaminated food with food-borne pathogens besides contact with contaminated surfaces [9]. Recently, animal contact was documented as the key route in outbreak investigations for transmission of enteropathogens [10]. Duckling contact and/or consumption of contaminated duck meat might be responsible for salmonellosis, causing hospitalization and/or death of affected workers [11]. Moreover, it is estimated that 14% of enteropathogen illnesses are caused by contact with infected animals [12]. In animal farms, antimicrobial drugs' misuse could be strongly shared in increasing drug-resistant pathogens [13]. The application of proper hygienic measures is the crucial part for controlling bacterial pathogens' drug resistance in ducks [14].

Therefore, using different types of efficient disinfectants to prevent and/or inhibit microbial growth has become essential. The current products of interest require new approaches of disinfectant formula that has a low residual level as hydrogen peroxide [15]. Furthermore, it is found that hydrogen peroxide (H_2O_2) integrated with other anti-bacterial agents and facilitated its penetration power into the bacterial cells and/or enhanced the oxidizing action. Also, it has a lethal effect against *Staphylococcus aureus* and *P. aeruginosa* when combined with other products such as lactic acid (0.25%–4.1%) and sodium benzoate (0.25%–1.0%) [16].

One of the anti-bacterial agents causing growth inhibition of bacterial pathogens is nano-zinc oxide (ZnO NPs) particles permeating into the cell wall in addition to oxidative stress damages [17]. Moreover, in food packaging, nano-zinc oxide can be used as a food preservative [18]. Furthermore, it was found that ZnO NP has an antimicrobial effect against Gram-positive (*S. aureus* and *Salmonella typhimurium*) and negative bacteria (*Klebsiella pneumoniae* and *E. coli*.) [19,20]. Also, they revealed that the growth of micro-organisms was strongly inhibited by increasing NPs concentration (45 µg/ml). This work aimed to detect the spreading of zoonotic bacterial pathogens in Mallard duck feces, environment, and worker's hand swabs and assess some new disinfectants' efficacy against isolated pathogenic bacteria. The efficiency of H_2O_2 against the resistant isolated pathogens through capping it on ZnO NPs (hydrogen peroxide-loaded nano-zinc oxide H_2O_2/ZnO NPs composite) was also evaluated.

Materials and Methods

Ethical approval

The present work was approved by Institutional Animal Care and Use Committee and Institutional Review Board, reference number: IORG 0009255,10 August 2019), Faculty of Medicine, Beni-Sue University, Egypt.

Study location and period

This work was conducted on private small commercial duck pens (n = 3) during the period from August 2019 to January 2020 in Beni-Suef (coordinates, 29° 04' N–31° 05' E) province, Egypt. Each duck farm contains two building units of 1-day-old Mallard ducks (*Anas platyrhynchus*). Sanitation measures inside the investigated building units were fair.

Samples' collection and preparation

Duck fecal droppings and environmental samples

All samples of duck fecal droppings (n = 35) and duck environment (n = 200) including air (n = 30), water supply (tap water, n = 30), feeds (n = 30), drinkers (n = 40), feeders (n = 40), litter (n = 30), and worker's hand swabs (n = 36) were collected. After that, all samples were kept in sterilized screw-capped bottles and plastic bags. Swabs were collected from drinkers and feeders. All swabs were moistened with 0.1% buffer peptone water (BPW: Oxoid, Ltd, Basingstoke, UK) immediately before sampling; then, the swabs were pre-enriched in BPW (10 ml). Ten gm of collected fecal droppings, feed, litter sample, and 10 ml of tap water supply were pre-enriched in 90 ml of BPW, according to Adzitey et al. [8]. For air sampling, sterilized Petri dishes containing different culture media (MacConkey agar and Brilliant green agar) were opened and distributed at the different corners, and middle areas were then left exposed for about 15–30 min and then the Petri dishes were closed and incubated at 35°C for 24 h according to the settling plate technique [21]. Samples of the worker's hand swabs were obtained from farmworkers who were in direct contact with ducks. After that, all swabs were moistened with 0.1% BPW (Oxoid, Ltd, Basingstoke, UK) immediately before sampling. Then, all swabs were pre-enriched in 10 ml BPW.

Isolation and identification of bacterial pathogens

All pre-enriched samples were incubated for 24 h at 37°C. After that, 0.1 ml of the incubated broth was added to 10 ml Rappaport Vassilidis and incubated at 42°C for 24 h. Then, one loopful was streaked onto Salmonella Shigella agar (SS agar: Oxoid®, CM 0099, Ltd, UK) and incubated at 37°C for 24 h for *Salmonella* isolation.

For isolation of other Gram-negative bacteria of family Enterobacteriaceae and *Pseudomonas* spp., one loopful from BPW-enriched broth inoculated into MacConkey agar (Oxoid®, CM 0007, Ltd, UK) plates and incubated at 37°C for 24 h. The pure separate colonies were picked up and inoculated into nutrient agar slope and incubate at 37°C for 24 h. The identification of bacterial colonies was based on colonial morphology, pigmentation, and Gram staining and biochemical reaction tests, according to Forbes et al. [22].

Serotyping of isolated *E. coli* species

The slide agglutination test was applied to isolated *E. coli* strains for its serological identification [23]. The serotyping was carried out at the Ministry of Health (the Central Health Laboratory), Egypt.

Sensitivity testing of isolated bacterial pathogens to antimicrobial drugs

The sensitivity of 40 bacterial strains (n = 10 each) to eight antimicrobial drugs was detected using a disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) [24]. The antimicrobial drugs include ciprofloxacin (CIP,15 ug), clarithromycin (CLR,15 ug), streptomycin (S, 10 ug), amikacin (AK, 10 µg), amoxicillin/clavulanic (AMC, 30 ug), chloramphenicol (C, 30 ug), ampicillin (Amp, 10 ug), and trimethoprim-sulfamethoxazole (STX, 30 ug). Suspension of each testing isolates was prepared according to McFarland standard (0.5). One loopful from bacterial suspension was inoculated onto agar media (Muller–Hinton), and placed the antibiotic discs onto the agar plates and incubated at 37°C for 24 h. According to the standard guideline, the zone of inhibition was measured then compared with the zone diameter's interpretation chart.

Assessing the biocidal effect of testing disinfectants

The anti-bacterial efficacy of some new disinfectants: CID 20 (alkyl-dimethyl benzyl ammonium chloride 61.5 gm/l, aldehyde 19.8 gm/l, formaldehyde 84.4 gm/l, glutaraldehyde 58.0 gm/l, isopropanol 37.6 gm/l, and pine oil 20 gm/l), Durak® plus disinfectant (didecyl dimethyl ammonium chloride 18.75 gm/l, alkyl-dimethyl benzyl ammonium chloride 50.0 gm/l, glutaraldehyde 62.50 gm/l, dioctyl dimethyl ammonium chloride 18.75 gm/l, octylnonyl dimethyl ammonium chloride 37.5 gm/l, pine essence 20.0 gm/l, and terpineol 20 gm/l), and hydrogen peroxide (6th October, 3rd Industrial Area, Egypt), ZnO NPs, and H_2O_2/ZnO NPs composite against 40 strains of enteropathogenic bacteria (*E. coli*, *Salmonella* spp., *Proteus mirabilis*, and *P. aeruginosa*) isolated from fecal droppings of Mallard ducks and their environment was evaluated using broth macro-dilution method according to Li et al. [25] at different concentrations and testing times (30, 60, and 120 min).

Preparation and characterization of testing nanomaterials

ZnO NPs (Loba, Chemi, Pvt. Ltd, India) were prepared using the high-energy ball milling technique, according to Salah et al. [26]. After that, to prepare H_2O_2 capping on ZnO NPs, hydrogen peroxide at 3% was added to different concentrations of ZnO NPs (0.01 and 0.02 mg/ml) and immediately pre-used. The mixture was shaken well continuously on the magnetic stirrer to reduce NPs agglomerations over the incubation periods (30, 60, and 120 min). Both ZnO NPs and H_2O_2/ZnO NPs were characterized using Fourier-transform infrared spectrum (FT-IR, VERTEX, 70) and high-resolution transmission electron microscopy (HR-TEM, a JEOL JEM 2000EX). HR-TEM micrographs were investigated in the Central lab of the Agriculture Faculty, Cairo University, Egypt, while FTIR spectra of the nanocomposite were examined at the Faculty of Postgraduate Studies of Advanced Science, Beni-Suef University, as shown in Figures 3 and 4.

Assessing the method of testing disinfectants and nanomaterials

One hundred microliters of different bacterial strains (1×10^{-6} CFU/ml) were inoculated with CID 20 disinfectant at concentrations of 1:100 and 1:200 ml, Durak® plus the same concentrations, hydrogen peroxide (3% and 5%), ZnO NPs (0.01 and 0.02 mg/ml), and H_2O_2/ZnO NPs composite (0.01 and 0.02 mg/ml) in Mueller–Hinton broth (MHB) onto a 96-well plate (Sarstedt, Nu¨mbrecht, Germany) according to Li et al. [25]. Furthermore, the negative control was prepared by added one µl of broth culture to MHB without testing materials; meanwhile, testing disinfectants and nanomaterials in MHB used as a positive control. All tested materials were incubated at 37°C for 24 h. The *in-vitro* trial was conducted in triplicate. From each well, one loopful was inoculated on Mueller–Hinton agar to observe the presence and/or absence of microbial growth at different concentrations of testing compounds according to guidelines of CLSI [27].

Data analyses

All data were collected for statistical analyses using the Statistical Package for the Social Sciences software. A non-parametric test (chi-square test) was used for analyzing the prevalence of enteropathogenic bacteria in ducks' droppings, their distribution in the surrounding environment, and workers' hand swabs besides the anti-bacterial activity of disinfectants and nanocomposites against all bacterial isolates. The one-way analysis of variance analysis was used to analyze the inhibition zone diameter of testing compounds against enteropathogenic bacterial isolates.

Results

Prevalence and frequent distribution of enteropathogenic bacteria in the examined duck pens

The prevalence of enteropathogenic bacterial isolates in examined duck pens was (62.73%; 170/271), whereas the highest isolation rate was detected in duck fecal droppings (100%; 35/35), followed by the surrounding environment (58.5%; 117/235). Concerning workers' hand swabs, 50% (18/36) of bacterial isolates was found. Interestingly, *E. coli* represented the most isolated entero-pathogen (56.47%; 96/170), followed by *P. aeruginosa* (21.8%; 37/170), *P. mirabilis* (15.29%; 26/170), and *Salmonella* spp. (6.47%; 11/170), at chi-square association, $\chi^2 = 23.4$ and $p \leq 0.01$ as shown in Table 1.

The frequency distribution of isolated pathogens revealed that *E. coli* poly III O:25 K: II was found in the percentage of 9.4% (16/170) in all examined samples (duck fecal droppings, workers' hand swabs, drinker, litter, feed, and feeders, respectively). Oppositely, *E. coli* poly III O:142 K:86 was detected in all examined samples include fecal droppings, ducks' litter, feed, feeders, drinkers, and worker's hand swabs at a percentage of 8.8% (15/170). The other isolated strains of *E. coli* spp. were untypeable. On the other hand, *Salmonella* spp. were isolated at the highest rate in duck feeders (9.09%; 3/33), followed by the fecal dropping, ducks' litter, drinkers, and workers' hand swabs (8.57%, 3/35; 8%; 2/25; 5.55%, 2/36; and 5.5%, 1/18, respectively). Besides, *Salmonella* spp. was not isolated from both ducks' feed and tap water supply. Concerning *P. aeruginosa* was isolated at the highest rate in the tap water supply (30%, 3/10), followed by duck fecal droppings, hand swabs, feeders, feed, drinkers, and ducks' litter (28.57%,10/35; 27.7%, 5/18; 24.24%, 8/33; 23.1%, 3/3; 19.44%, 7/36; and 4.0%;1/25, respectively) at $\chi^2 = 16.84$ and $p \leq 0.05$. Besides, *P. mirabilis* showed the highest isolated rate in ducks' feed, followed by ducks' litter, tap water supply, and drinkers (30.8%, 4/13; 24%, 6/25; 20%, 2/10; 16.7%, 6/36, respectively). While feeders, workers' hand swabs, and duck fecal droppings revealed the least isolated rate (12.12%, 4/33; 11.15%, 2/18; and 5.71%, 2/35, respectively) as revealed in Table 2.

Table 1. Prevalence rate of enteropathogenic bacteria in commercial duck pens.

Bacterial findings of examined samples	Total		Prevalence rate of isolated bacterial pathogens No. (%)			
	Examined (No.)	Positive No. (%)	*E. coli*	*Salmonella* spp.	*P. mirabilis*	*P. aeruginosa*
I- Duck fecal droppings	35	35 (100.0)	20 (57.14)	3 (8.6)	2 (5.7)	10 (28.57)
II- Farm environment	200	117 (58.5)	66 (56.4)	7 (5.98)	22 (18.8)	22 (18.8)
III- Worker's hand swabs	36	18 (50.0)	10 (55.6)	1 (5.5)	2 (11.1)	5 (27.7)
Total	**271**	**170 (62.73)**	**96 (56.47)**	**11 (6.47)**	**26 (15.29)**	**37 (21.8)**

Table 2. Frequent distribution of enteropathogenic bacteria in commercial duck pens.

Bacterial findings	Frequent distribution of enteropathogenic bacteria No. (%)							
	Total		*E. coli spp.*			*Salmonella spp.*	*P. mirabilis*	*P. aeruginosa*
Examined sample	Examined No.	PositiveNo. (%)	*E. coli* poly III O:25 K: II	*E. coli* poly III O: 142 K: 86	Un-typeable			
I-Duck fecal droppings	35	35 (100)	7 (35)	5 (25)	8 (40)	3 (8.57)	2 (5.71)	10 (28.57)
II-Duck's environment								
Air	30	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Tap water supply	30	10 (37.3)	0 (0.0)	0(0.0)	5 (50)	0 (0.0)	2 (20.0)	3 (30.0)
Drinkers	40	36 (90.0)	3 (8.33)	2 (5.55)	16 (44.44)	2 (5.55)	6(16.66)	7 (19.44)
Feeders	40	33 (82.5)	1 (3.03)	2 (6.06)	15 (45.45)	3 (9.09)	4 (12.12)	8 (24.24)
Ducks' litter	30	25 (83.3)	2 (8)	4 (16)	10 (40)	2 (8.0)	6 (24.0)	1 (4.0)
Ducks' feed	30	13 (43.3)	1(7.69)	1 (7.69)	4 (30.76)	0 (0.0)	4 (30.76)	3 (23.1)
III-Workers hand swabs	36	18 (50)	2 (11.1)	1 (5.5)	7 (38.88)	1 (5.5)	2 (11.1)	5 (27.7)
Total	271	170 (62.73)	16 (9.41)	15 (8.82)	65 (38.23)	11 (6.47)	26 (15.29)	37 (21.76)

The chi-square association between frequency distribution of enteropathogenic bacteria varies significantly at $\chi^2 = 16.84$ ($p \leq 0.05$).

Antimicrobial susceptibility pattern of isolated enteropathogenic bacteria

The susceptibility of isolated enteropathogenic bacteria to antimicrobial drugs revealed multidrug-resistant (MDR) patterns in the majority of isolates, whereas *E. coli* species showed complete resistance to STX, AMC, and Amp (100%). In comparison, the sensitivity to AK and CIP was slightly moderate (33.3%). Oppositely, *Salmonella* species were sensitive to CIP and AK drugs (66.6%); meanwhile, their resistance to CLR, STX, AMC, Amp, and chloramphenicol was 100%. On the other hand, *P. mirabilis*, besides *P. aeruginosa* exhibited their resistance (100%) to CLR, STX, AMC, Amp, and streptomycin. Additionally, *P. mirabilis* revealed a complete resistance to chloramphenicol. Both isolates of both species appeared moderate to complete sensitivities (66.6%, and 100%, respectively) to AK (Table 3 and Fig. 1).

Table 3. Antibiotics susceptibility pattern of enteropathogenic bacteria.

Bacterial isolates antimicrobial agents	Tested conc. (µg)	Susceptibility of enteropathogenic bacteria (%)								
		E. coli		*Salmonella spp.*		*P. mirabilis*		*P.aeruginosa*		*p value*
		S	R	S	R	S	R	S	R	
CIP	15	66.6	33.3	66.6	33.3	33.3	66.6	33.3	66.6	0.03
CLR	15	33.3	66.6	0.0	100	0.0	100	0.0	100	0.05
STX	30	0.0	100.0	0.0	100	0.0	100	0.0	100	N
AMC	30	0.0	100.0	0.0	100	0.0	100	0.0	100	N
Amp	10	0.0	100.0	0.0	100	0.0	100	0.0	100	N
Streptomycin (S)	10	33.3	66.6	33.3	66.6	0.0	100	0.0	100	0.07
AK	10	66.6	33,3	66.6	33.3	66.6	33.3	100	0.0	0.1
Chloramphenicol (C)	30	33.3	66.6	0.0	100	0.0	100	0.0	66.6	0.06

S = Susceptible; R = resistant; N = means no statistics are computed.

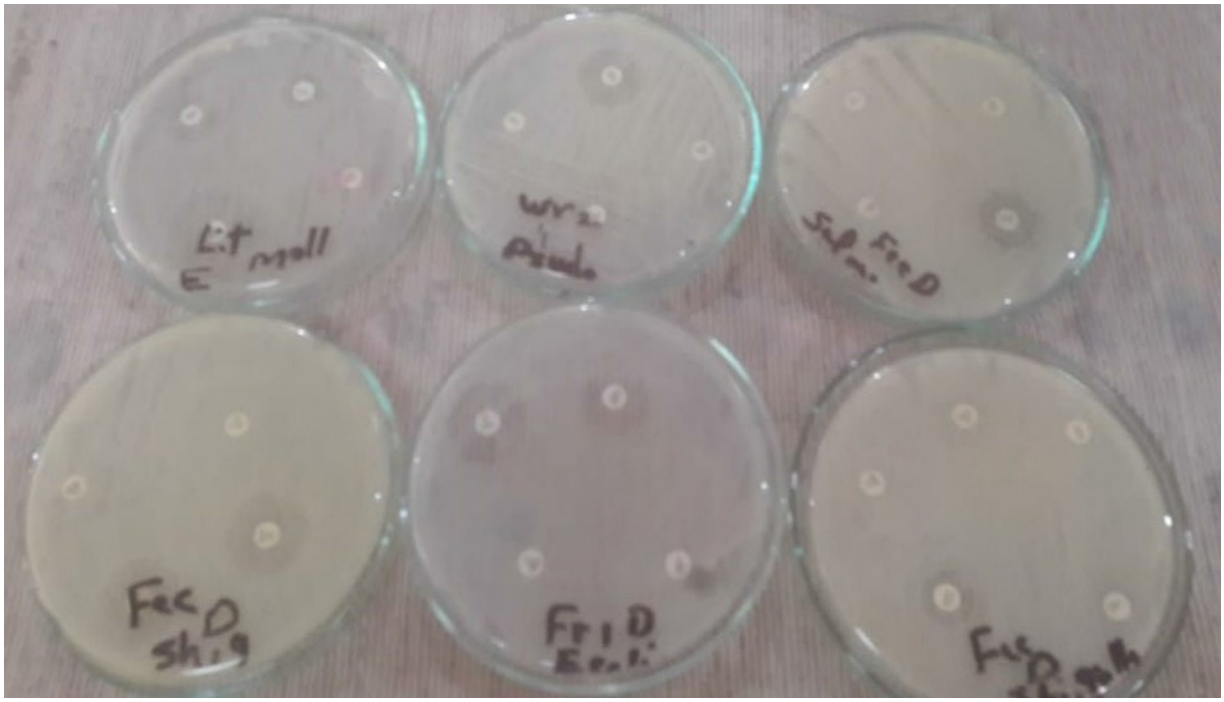

Figure 1. The susceptibility of enteropathogenic bacteria to antimicrobial drugs revealed that *E. coli* spp. was highly resistant to STX, AMC, and Amp. While *Pseudomonas aeruginosa* showed its resistance to CLR, STX, AMC, Amp, and streptomycin**.** Contrarily, both *Salmonella* spp. and *Proteus mirabilis* were highly resistant to most testing antibiotics.

Antimicrobial efficiency of disinfectants and nanocomposite

Antimicrobial activity of testing disinfectants (CID 20, Durak® plus, and H_2O_2), ZnO NPs, and H_2O_2/ZnO NPs composite against enteropathogenic bacteria in Table 4 and Figure 2 clarified that all were isolated bacteria, *E. coli*, *Salmonella* spp., *P. aeruginosa*, and *P. mirabilis*, was susceptible to CID 20 at a concentration of 1:100 ml after 120 min of exposure time at $p \leq 0.05$. Besides, the sensitivity of both *E. coli* and *P. aeruginosa* did not exceeded 70% at the least concentration (1:200 ml) after 120 min of contact time. Meanwhile, *Salmonella* spp. and *P. mirabilis*'s sensitivity pattern was 90% each compared to 30- and 60-min contact time. On the other hand, Durak® plus disinfectant was highly effective (100%) against *E. coli*, *P. mirabilis*, and *P. aeruginosa* at a concentration of 1:100 ml after 120 min contact time at $p \leq 0.05$. In comparison, the effectiveness against *Salmonella* spp. was not exceeded 80% compared to 1:200 ml concentration at different contact times. On the contrary, the sensitivity testing of enteropathogenic bacterial isolates to H_2O_2 was significantly low at different contact times and did not exceed 60 % at 5% concentration after 120 min of exposure at $p \leq 0.01$ compared to the lowest concentration of 3%. Conversely, ZnO NPs proved its bactericidal effect (100%) on *E. coli*, *P. mirabilis*, and *P. aeruginosa*, followed by *Salmonella* spp. (90%) at 0.02 mg/ml after 120 min of exposure. Interestingly, increasing the penetrating power of hydrogen peroxide to bacterial cells using ZnO NPs. It has been found that hydrogen peroxide loaded on ZnO NPs was highly effective (100%) against all bacterial isolates at 0.02 mg/ml after 120 min of exposure compared to other concentrations. Interestingly, the characterization of testing nanomaterials using TEM microscopy, as shown in Figure 3 clarified the morphological feature of ZnO NPs (Fig. 3a) was hexagonal, and the NPs diameter ranged from 75.08 to 100.58 nm (Fig. 3b). Furthermore, TEM micrographs of H_2O_2/ZnO NPs showed a change in nanoparticle shape to pentagonal (Fig. 3c), and the diameter size of NPs ranged from 5.48 to 34.6 nm (Fig. 3d). Oppositely, the FTIR spectrum of ZnO NPs, hydrogen peroxide, and H_2O_2 loaded on ZnO NPs as shown in Figure 4. ZnO NPs showed intense absorption peaks at 3,435, 2,375, 1,637, 1,044, 723, and 535 cm^{-1} (Fig. 4a). While H_2O_2 clarified broad absorption peaks that attributed to the hydroxyl groups (O–H) absorption. Characteristic peaks appeared at 3,261, 2,353, 2,122, 1,636, 1,387, 1,210, and 600 cm^{-1}, respectively (Fig. 4b). Furthermore, H_2O_2/ZnO NPs composite (Fig. 4c) showed the strongest peak was moved to 3,271 and 2,350 cm^{-1}, besides characteristic peaks of stretching mode vibration at 1,346 and 615 cm^{-1} confirmed the interaction between ZnO NPs and tested disinfectant (H_2O_2).

Discussion

Lacking quantitative data about the levels of enteropathogenic bacteria shed by ducks and focusing only on fecal flora of those birds without much attention to their surrounding environment might expose the human health and environment to hazards. Contamination of the ducks'

Table 4. Efficiency of disinfectants and nanoparticles composite against enteropathogenic bacteria.

Bacterial isolates (n = 40)	Sensitivity pattern of enteropathogenic bacteria at various exposure times												*p value*
Testing compounds (Conc.)	*P. aeruginosa*			*P. mirabilis*			*Salmonella* spp.			*E. coli*			
CID 20	120 min	60 min	30 min	120 min	60 min	30 min	120 min	60 min	30 min	120 min	60 min	30 min	
1:100 ml	100	90	80	100	90	70	100	80	60	100	60	60	
1:200 ml	70	50	50	90	80	60	90	80	50	70	50	40	**0.05**
Durak® plus													
1:100 ml	100	90	50	100	80	60	80	60	50	100	70	50	**0.03**
1:200 ml	90	70	50	70	70	40	70	60	50	70	50	30	
H_2O_2													
5%	60	50	30	60	60	40	30	20	0.0	50	30	20	**0.01**
3%	60	50	20	50	50	30	20	20	0.0	30	10	0.0	
ZnO NPs													
0.02 mg/ml	100	90	60	100	100	70	90	70	60	100	80	60	**0.05**
0.01 mg/ml	100	70	60	80	80	60	80	50	50	80	70	60	
H_2O_2/ZnO NPs													
0.02mg/ml	100	90	70	100	100	70	100	100	80	100	90	90	**0.02**
0.01mg/ml	100	70	50	90	60	50	70	70	50	90	80	70	

Min = minute.

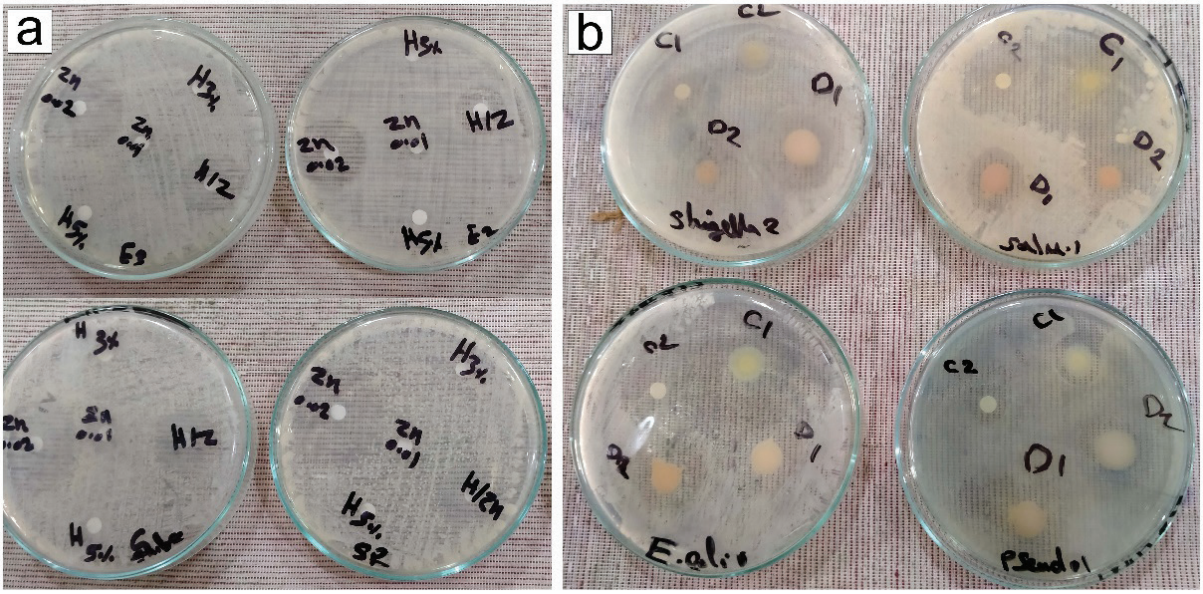

Figure 2. The sensitivity testing of enteropathogenic bacterial isolates to H_2O_2 was significantly low at 3% and 5% concentrations (**a**) compared to the antimicrobial activity of both ZnO NPs and H_2O_2/ZnO NPs composite that approved its lethal effect against all bacterial isolates at 0.02 mg/ml. Contrarily, all isolated bacteria *E. coli*, *Salmonella* spp., *P. aeruginosa*, and *Proteus mirabilis* were susceptible to CID 20 and Durak® plus disinfectant (**b**) at a concentration of 1:100 ml after 120 min of exposure time.

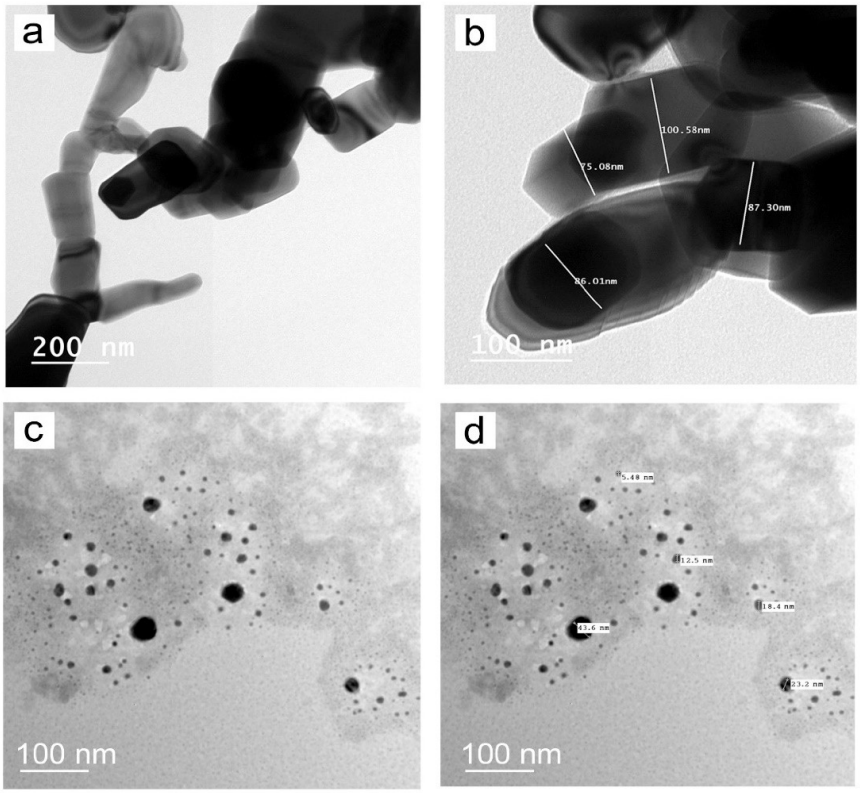

Figure 3. Image of transmission electron microscopy of nano-zinc oxide (a,b) clarified the hexagonal shape of NPs (a) and the diameter of NPs ranged between 75.08 and 100.58 nm (b). Furthermore, micrographs of H_2O_2/ZnO NPs showed the change in nanoparticles shape to pentagonal (c) and the diameter size of NPs was ranged from 5.48 to 34.6 nm (d).

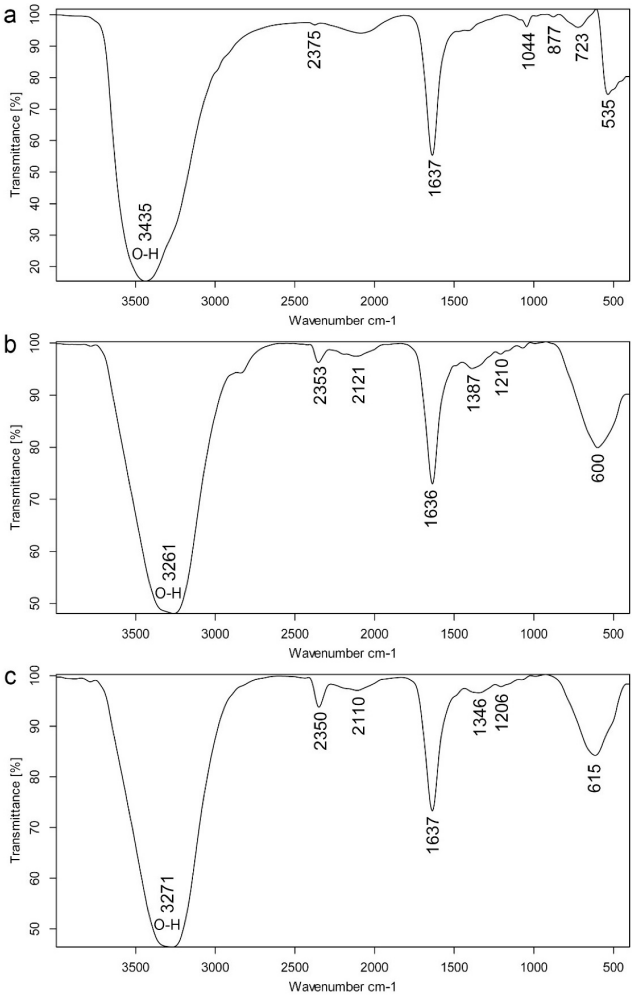

Figure 4. (a) FTIR spectrum of nano-zinc oxide, (b) hydrogen peroxide, and (c) hydrogen peroxide/ZnO NPs composite.

environment by pathogenic bacteria is the leading cause of higher mortality rates besides significant economic losses in duck farms. In the examined duck pens, the spreading of enteric bacteria was significantly higher. Besides, the highest rate was isolated from duck fecal droppings, followed by the ducks' environment that could be attributed to environmental contamination with bird fecal droppings and reflecting lower sanitation measures applied in these farms. The *E. coli* spp. were the most isolated pathogens at 56.47%, whereas Enteropathogenic *E. coli* was the predominant pathogenic *E. coli* O: 25 and O: 142 that significantly isolated from duck fecal droppings, followed by their litter. Banerjee et al. [1] and Eid et al. [5] exhibited that the enteropathogenic bacteria isolated from fecal droppings of apparently healthy duck at the percent of 53.96% and 26.8%, respectively. Besides, Eid et al. [5] showed that *E. coli* was isolated at the least rate of 3.6% from duck droppings.

On the other hand, Majumder et al. [28] recorded that *E. coli* isolated at the highest rate of 43.33% from duck droppings. In Egypt, Byomi et al. [29] found it in duck fecal droppings at the rate of 57.7%, while Adzitey et al. [8] revealed that it was 78% when isolated from two duck pens in Malaysia.

In the current context, *E. coli* spp. were isolated in the highest percentage from the ducks' environment include drinkers, tap water supply, feed, feeders, and litters. It can be concluded that there is cross-contamination from duck fecal droppings to litters and subsequently reach to drinkers and feeders in front of ducks. Silvaraj et al. [30] pointed to direct or indirect contact with ducks and their contaminated environment with *E. coli* spp. act as a source of infection and can be transmitted to farmworkers. Thus, strict biosecurity guidelines are required to mitigate and prevent the transmission of potentially zoonotic pathogens throughout the ducks' environment. In the current context, it has been found that the farmworkers' hand swabs contaminated with *E. coli*, *Salmonella* spp., *P. aeruginosa*, and *P. mirabilis*. Noble et al. [11] revealed that ducks' contact and/or consumption of contaminated duck meat was responsible for Salmonellosis and associated with affected workers' death. Besides, contact with young hatching ducks might pose human beings to hazardous risks [31]. Oppositely, *Salmonella* spp. was isolated from the fecal dropping of healthy duck at 5.26% [32].

Meanwhile, Rahman et al. [33] exhibited that *Salmonella* spp. was isolated from ducks at a rate of 39.58%. Interestingly, *P. aeruginosa* is one of the predominant bacterial isolates found in duck droppings besides *P. mirabilis* during our study. Eid et al. [5] isolated *P. aeruginosa* from duck droppings at the rate of 2%. Moreover, Harmsen et al. [34] found that the predominant isolated bacteria were *P. aeruginosa,* and the resistance pattern to disinfectants was high and could be adhered to surfaces from clinical samples. Furthermore, Silvaraj et al. [30] recorded that *P. aeruginosa* toxins are responsible for respiratory manifestations in young chick birds. On the other hand, Armbruster [35] found that *P. mirabilis* is an opportunistic zoonotic bacterial pathogen responsible for urinary tract infection isolated from the examined farm at 15.29% and caused wound infection in human beings [36]. Meanwhile, Olaitan et al. [3] revealed that *P. mirabilis* was isolated in a higher percentage (38.3%) than duck droppings, while Nahar et al. [37] found it at a similar percentage of 39.0% in chickens' droppings.

The pervasion of MDR bacteria is representing a global threat to a public health concern. Regrettably, in the current study, the majority of tested microorganisms exhibited an MDR pattern. The MDR is defined by European Center for Disease Control and Prevention as the resistance of one microbe (any agent) to three or more antibiotic classes

[38]. *E. coli* spp. revealed resistance to STX, AMC, and Amp. Furthermore, Xu et al. [39] found that *E. coli* isolates from animal and human sources were highly resistant to tetracycline (54.7%), Amp (49.4%), and streptomycin (46.1%). Previous literature showed a variable degree of resistance profile in *E. coli* isolated from ducks [5,33,40]. On the contrary, *P. aeruginosa* revealed its resistance to CLR, STX, AMC, Amp, and streptomycin. These results were in line with Basak [41] and Eid et al. [5], who found that the isolated *P. aeruginosa* strains were highly resistant to penicillin, streptomycin, erythromycin, and sulfamethoxazole-trimethoprim. Oppositely, *Salmonella* spp. were highly resistant to most testing antibiotics (chloramphenicol, CIP, STX. and Amp). *P. mirabilis* also revealed a complete resistant pattern to CLR, STX, AMC, Amp, streptomycin, and chloramphenicol that following Wong et al. [42] and Nahar et al. [37].

Sensitivity pattern of enteropathogenic bacteria for testing quaternary ammonium compounds (CID 20 and Durak® plus disinfectants) discovered that all testing bacterial strains (*E. coli*, *Salmonella* spp., *P. mirabilis*, and *P. aeruginosa*) were significant highly sensitive to testing disinfectant CID 20 after 120 min of exposure time at a concentration of 1:100 ml, while the Durak® plus effectiveness on *Salmonella* spp. did not exceeded 80% after the same concentration and exposure time compared to other testing concentrations at different exposure times. The current context was in line with Widmer and Frei [43], who found that after 5 min of exposure time, the most susceptible bacteria to quaternary ammonium compound (QAC) and aldehyde disinfectants was *S. typhi*. Furthermore, *P. aeruginosa*, *E. coli*, and *S. aureus* were highly sensitive to glutaraldehyde-based disinfectants [44], while the QACs have the lowest efficiency against *P. aeruginosa* and Gram-negative microorganisms. Besides, they discovered that the QACs have a lethal effect on *S. typhimurium* and *S. aureus* in the absence of organic matter [45]. Oppositely, our study was found that the effectiveness of hydrogen peroxide on enteropathogenic bacterial isolates was significantly lower at different contact times and did not exceed 60% at 5% concentration after 120 min of exposure. While Rutala and Weber [46] pointed to the superior disinfectant among the oxidizing agents was H_2O_2 at 7.5% concentration. Wirtanen et al. [47] observed that hydrogen peroxide-based product was effective against *P. aeruginosa* at 0.5% after 30 min of exposure. On the contrary, Rios-Castillo et al. [16] found that H_2O_2 integrated with cationic polymer at the same concentration was highly effective after 5 min of exposure. Lineback et al. [48] H_2O_2 disinfectant was highly effective against *P. aeruginosa* and *S. aureus* biofilms compared to QACs.

The anti-bacterial effect of ZnO NPs was assessed against *E. coli*, *P. mirabilis*, and *P. aeruginosa* and proved its lethal effect (100%), followed by *Salmonella* spp. (90%) at 0.02 mg/ml after 120 min of exposure. This study gave us the chance to increase the penetrating power of hydrogen peroxide to bacterial cells using ZnO NPs. It has been found that hydrogen peroxide/ZnO NPs composite was highly efficient against all bacterial isolates at 0.02 mg/ml after 120 min of exposure compared to a low concentration of 0.01 mg/ml; besides, the average size of NPs ranged from 5.48 to 34.6 nm. The biocidal effect of ZnO NPs occurred through an accumulation of nanoparticles in the cytoplasm and/or outer bacterial cell wall and make Zn^{2+}release, which led to membrane protein damage and consequently the death of the microbial cell [49,50]. ZnO NPs have potential antimicrobial efficiency at 30 nm average size that caused bacterial cell death by destroying the cell wall's integrity [51]. Siddiqi et al. [52] stated that ZnO NPs particles were efficiently high against both *S. aureus* and *E. coli* at 125 µg/ml, while for *P. aeruginosa* it was at 500 µg/ml.

Conclusion

The prevalence rate of pathogenic bacteria was significantly high in duck fecal droppings and their surrounding environment. Most of the isolated bacteria were highly resistant to different testing antimicrobial drugs. Testing strains of *E. coli*, *Salmonella* spp., *P. mirabilis*, and *P. aeruginosa* were highly susceptible to testing disinfectant CID 20 after 120 min of exposure time at a concentration of 1:100 ml while the efficiency of Durak® plus on *Salmonella* spp. was not exceeded 80% at the same concentration and exposure time. At all testing contact times, the effectiveness of H_2O_2 on enteropathogenic bacteria was not exceeded 60% at 5% concentration. Interestingly, the H_2O_2/ZnO NPs composite has the potential anti-bacterial activity against enteropathogenic bacteria at 0.02 mg/ml after 120 min of exposure time.

List of abbreviations

H_2O_2: Hydrogen peroxide, ZnO NPs: Nano-zinc oxide, H_2O_2/ZnO NPs: Hydrogen peroxide-loaded nano zinc oxide, FT-IR: Fourier-transform infrared spectrum, HR-TEM: High-resolution transmission electron microscopy, *E. coli*: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. mirabilis*: *Proteus mirabilis*, BPW: Buffer peptone water, MHB: Mueller–Hinton broth.

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Conflict of interest

Related to this work, the authors declared that they have no conflict of interest.

Authors' contributions

All the authors contributed to this work equally in study design planning, sample collection, preparation, microbial investigation, sensitivity testing, nanomaterial preparation and characterization, statistical analysis, and manuscript text writing. All the authors approved the article publication.

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