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Antimicrobial effects of citrus oil on concurrent bacterial and fungal isolates from broiler chickens.

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ABSTRACT

Extensive antibiotic use in poultry has accelerated the emergence of multi-drug-resistant pathogens. This study investigated the prevalence of *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus spp.* in broiler chickens and evaluated the *in vitro* efficacy of citrus oil as a natural antimicrobial agent. Bacteriological and mycological examination of 63 samples from diseased broilers revealed a high prevalence of infection. The most common isolates were *P. aeruginosa* (23.8%, 15/63) and *E. coli* (19%, 12/63). Co-infections with both bacteria and fungi were also frequent, found in 19% (12/63) of samples. Antimicrobial testing revealed widespread resistance. *E. coli* isolates were 100% resistant to cephalosporins and showed high resistance (83.3%) to aminoglycosides, tetracyclines, and fluoroquinolones. *P. aeruginosa* isolates were 100% resistant to nearly all tested antibiotics, demonstrating a severe multi-drug-resistant phenotype.
Assessing the effect of 1% citrus oil on these resistant isolates, revealed inhibition of the growth of 50% of *E. coli* and 20% of *P. aeruginosa* isolates. Moreover, it had a powerful synergistic effect on grown isolates, re-sensitizing of two *E. coli* isolates that changed to susceptible to oxytetracycline, apramycin, and aztreonam. Whereas *P. aeruginosa*, four isolates changed to susceptible to tobramycin, ofloxacin, ceftazidime, and apramycin and two isolates changed to susceptible to streptomycin, cefotaxime sodium,doxycyclineHCl,chloramphenicol,sulfamethoxazole-trimethoprim, oxytetracycline, and cefuroxime sodium. Fungal isolates showed weak growth with 1% citrus oil and were completely inhibited at a 1.5% concentration.
In conclusion, citrus essential oil shows great promise as a therapeutic agent because it has antibacterial, antifungal effects and can re-sensitize highly resistant bacteria, such as *P. aeruginosa* and *E. coli*, conventional antibiotics These findings suggest a crucial new strategy for managing the global threat of antimicrobial resistance.

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INTRODUCTION

The poultry industry plays a crucial role in meeting consumer demand for high-quality food products and enhancing national food security. This sector not only provides essential protein sources but also contributes significantly to economic growth and rural development (**FAO, 2020**). Bacterial and fungal co-infections pose a significant threat to poultry health and productivity, particularly in intensive farming systems prevalent in Egypt and globally **(Abo-Aziz et al. 2020)**.

Escherichia coli and *Pseudomonas aeruginosa* are significant bacterial pathogens that pose serious health risks to poultry, leading to diseases that affect bird well-being and have economic implications for the poultry industry **(Kahn et al. 2019; Haque et al. 2020)**. *E. coli*, typically found in the intestines of healthy birds, can become pathogenic, avian pathogenic *E. coli* (APEC) and cause colibacillosis, resulting in respiratory distress, septicemia, diarrhea and/or enteritis, airsacculitis, perihepatitis, pericarditis and high mortality rates **(Solà-Ginés et al. 2015; Ghafari et al. 2021)**. Moreover, strains of *E. coli* also have zoonotic significance since they are known to cause infections both in humans and animals, including birds **(Kabiswa et al. 2018**).

P. aeruginosa, an opportunistic pathogen, primarily affects immunocompromised or stressed birds. Its antibiotic resistance complicates treatment, leading to decreased growth rates, poor feed conversion, increased mortality, and higher management costs **(Haque et al. 2020; Ghafari et al. 2021**).

The devastating impact of various fungal diseases, particularly those like aspergillosis and candidiasis, is significantly affecting the health and well-being of poultry populations. Candidiasis, caused by *Candida albicans*, can result in severe local infections in the mucosa of the upper digestive tract, further complicating the health challenges faced by poultry **(Shaapan and Girh, 2024)**.

Aspergillosis, often called "brooder pneumonia" is a significant fungal disease in poultry that primarily affects the respiratory system. *Aspergillus niger* and *Aspergillus flavus* are important contributors to outbreaks in chicken and other avian species. They can cause respiratory aspergillosis, particularly when birds are exposed to high concentrations of its spores through contaminated litter, feed, or dust-laden air **(Shaapan and Girh, 2024).** Bacterial and fungal co-infections complex interaction often leads to more severe clinical signs, reduced performance, and increased mortality compared to single infections **(Adel and Mousa, 2019).**

In the poultry industry, antibiotics have been used in the treatment and control of poultry infections and in some countries, also as growth promoters **(Talebiyan et al. 2014).** Indiscriminate use of antibiotics resulted in the emergence of multi-drug-resistant strains. The increase in antimicrobial resistance patterns is also an emerging public health concern and there is an unresolved ongoing debate about the role of antimicrobials used in farm animal/ livestock production **(Nazzaro et al. 2017; Amor et al. 2023)**. Some plant products are sources of natural antimicrobials such as berry pomaces and citrus oil that show antimicrobial activity against various bacterial pathogens. **(Biswas et al. 2012; Yang et al. 2014).** Essential oils (EOs) are secondary metabolites of plants employed in folk medicine for a long time and recently in veterinary medicine too. The study of the antibacterial properties of EOs is of increasing interest because therapies with alternative drugs are welcome to combat infections caused by antibiotic-resistant strains.

Citrus essential oils, derived from the peels of fruits such as oranges, lemons, and grapefruits, contain potent antimicrobial compounds including limonene, citral, linalool, and gamma-terpinene. These oils are receiving increased interest as natural alternatives to synthetic antimicrobials, particularly considering growing concerns about drug-resistant microbes. **(Nazzaro et al. 2017)** Citrus oils show good activity against *E. coli.* They primarily work by damaging the bacterial cell membrane, which makes the cell leaky and kills it. *P. aeruginosa* can be more challenging to combat than *E. coli*. Nevertheless, research indicates these oils can still inhibit *P. aeruginosa* growth **(Nazzaro et al. 2017)**. Citrus oils are also effective against a variety of fungi and harmful yeasts like *Candida*. They typically work by disrupting the fungal cell membrane, like how they affect bacteria. These oils can also mess with how fungi process nutrients and stop their spores from sprouting, which prevents them from growing and spreading **(Li et al. 2015)**. Citrus oils show promise in combating antimicrobial resistance in bacteria and fungi by damaging microbial cell membranes and disrupting protective biofilms, which weakens pathogens. They may also disrupt resistance mechanisms, such as efflux pumps, thus reducing a microbe's inherent resistance **(Cai et al. 2024)**. Additionally, when used alongside traditional antimicrobials, citrus oils can provide synergistic effects, enable lower drug dosages and resensitize previously resistant microbes, thereby enhancing treatment efficacy **(Nazzaro et al. 2017; Amor et al. 2023).** This study focused on identifying single and mixed bacterial and fungal infections in diseased broiler chickens and assessing the antibacterial and antifungal effects of various citrus oil concentrations on the identified pathogens.

MATERIALS and METHODS

2.1 Sampling: Chicken samples (n=63) were gathered from randomly selected broiler flocks from Baniseuf city during 2024, chickens aged 1–21 days affected with diarrhea, unabsorbed yolk sac, enteritis, arthritis, and conjunctivitis.

2.2 Bacterial isolation: Gram-negative bacterial strains were recovered from naturally infected chicken, and these strains are *E. coli* and *P. aeruginosa*, the samples were collected in sterile 10 mL buffered peptone water (BPW) (Oxoid Ltd., Basingstoke, UK) tubes and transported in an icebox to the lab of the animal health research institute, Beni-Suef branch, for bacteriological analysis. Isolation, identification and serotyping of bacterial agents were done according to **(Quinn et al. 2011)** briefly, *E. coli* is isolated on MacConkey Agar, EMB and identified using a Gram stain and IMViC tests. *Pseudomonas*, on the other hand, were isolated on Cetrimide Agar and identified by a positive oxidase test and Gram stain.

2.3 Antimicrobial susceptibility testing and determination of multi-drug resistance index (MDRI)

Antimicrobial susceptibility testing of 14 antibiotics had different disc content as shown: Streptomycin 10 (µg), Tobramycin 10(µg), Cefotaxime sodium 30(µg), Kanamycin 30 (µg), Doxycycline HCl 30(µg), Ofloxacin5 (µg), Chloramphenicol 30 (µg), Sulfamethoxazole-trimethoprim 25(µg), Ceftazidime 30 (µg), Oxytetracycline 30 (µg), Apramycin 15 (µg), Nalidixic acid 30 (µg), Aztreonam 30 (µg) and Cefuroxime sodium30(µg).

It was performed on all bacterial isolates using the Kirby-Bauer disc diffusion method on Mueller Hinton Agar, in accordance with **(CLSI, 2018)** standards. An isolate was classified as multi-drug resistant (MDR) if it exhibited resistance to at least three antibiotics from different categories. The multi-drug resistance index (MDRI) for each isolate was then calculated by dividing the count of resisted antimicrobials by the total number of antimicrobials tested. Following formula of **(Risso et al. 2008)**. Isolates with an MDRI exceeding 0.2 (or 20%) were designated as highly resistant. The MDRI was calculated using this formula.

MDRI= number of antibiotics to which the organism is resistant/total number of antibiotics tested against the organism.

Three replicates were used in antimicrobial testing

2.4 Fungal isolation samples were taken immediately and transferred directly into pre-enrichment broth malt extract broth (Oxoid) for yeast and at 25° C for 5-7 days for mold, then cultured on Sabouraud dextrose agar (SDA) medium (Oxoid) and incubated at 37° C for 24-48 h. The recovered fungi were identified morphologically according to **(Pitt and Hocking, 2009)**. Mycelial fungi were identified by examination of mycelial morphology and the reverse color as well as examination of colonial smears using lactophenol cotton blue stain. Yeast-like fungi were identified by colonial morphology. The appropriate API kit (API

20 C AUX, Oxoid) was used according to the manufacturer's instructions for biochemical identification of yeast isolates.

2.5 Determination of citrus oil effects.

2.5.1. citrus oil antibacterial activity:

Different concentrations of citrus oil from 0.5% to 1.5% were prepared by dilution with 1% DMSO. The antibacterial activity of the prepared concentrations was tested against MDR isolates according to **(Jeff-Agboola et al. 2012**). Briefly, the bacteria were cultivated on tryptone soya agar at 37º C for 24 h before being suspended in physiological saline (0.9% NaCl) and adjusted to $1{\times}10^8$ cfu. Muller Hinton agar was prepared and autoclaved at 121º C for 15 min. before being maintained at 55º C. The tested oils were then combined with Muller Hinton agar according to the tested concentrations. The oil-agar medium (10 ml) was then solidified in sterilized petri dishes. On the oil-agar plates, equal volumes of the bacterial suspensions were inoculated and spread. The plates were incubated at 37˚ C for 24-48 h. They were then examined for bacterial colony growth inhibition.

2.5.2. Synergistic effect with antimicrobials:

Plates showed obvious growth of MDR bacteria at concentration of 1% citrus oil were subjected to repeated antibiogram using same 14 antibiotics to evaluate the synergistic effects of citrus oil 1% with these antimicrobials.

2.5.3. Agar dilution method for detection of antifungal activity of citrus oil:

According to the method of **(Jeff-Agboola et al. 2012).** The antifungal activity of citrus oil against 18 selected fungal isolates was done. The tested isolates included *A. niger, A. flavus* and *Candida* species. Briefly, the tested fungi were grown on SDA at 37° C for 48 h, then cells were suspended in physiological saline (0.9% NaCl), and the suspension was adjusted to $1{\times}10^6$ CFU. SDA was prepared and autoclaved at 121˚ C for 15 minutes and kept at 55˚ C. Citrus oil was sterilized by filtration (pore size, 0.45 µm) and was mixed with SDA according to the tested concentrations (0.1, 0.25, 0.5, 1% and 1.5%). The oil-agar medium (10 ml) was then poured into sterile petri dishes and was solidified. Equal concentrations of the fungal suspensions were inoculated and spread onto the agar plates. The plates were then incubated at 37˚ C and 25˚ C for 24 h and 4-5 days respectively then examined daily for 8 days.

3. RESULTS

3.1. Bacterial and fungal isolates from affected chickens

Out of 63 samples, 27 (42.8%) bacterial isolates and 6 (9.5%) fungal isolates while 12 (19%) bacterial and fungal co infection isolates were recovered. Bacterial isolates include 12 *E. coli* and 15 *P. aeruginosa* with percentages of 19% and 23.8%. Regarding fungal isolates were 3 *C.* albicans and 3 *A. niger* with a percentage of 4.7% for each while co-infection including *E. coli* with *A. niger*, *P. aeruginosa* with *C. albicans*, *E. coli* and *P. aeruginosa* with *A. niger*, and *E. coli* with *A. niger* and *A. flavus* were recovered with a percentage of (4.7% for each) shown in **Table 1**.

Table 1. Recovery rates of bacterial and fungal isolates from broiler chickens:

Type of isolate	Total sample No.	Positive isolation No.	%
Bacterial isolates	63		
E. coli		12	19
P. aeruginosa		15	23.8
Total		27	42.8
Fungal isolates			
C. albicans		3	4.7
A. niger		3	4.7
Total		6	9.5
bacterial and fungal co infection isolates			
E. coli with *A. niger*		3	4.7
P. aeruginosa with *C. albicans*		3	4.7
E. coli and *P. aeruginosa* with *A. niger*		3	4.7
E. coli with *A. niger* and *A. flavus*		3	4.7
Total		12	19

% was calculated according to the total number (No.) of samples (n=63).

3.2. Antimicrobial susceptibility profile of bacterial isolates:

Results of in-vitro sensitivity tests demonstrated that *E. coli* isolates were highly resistant (100%) to cefotaxime sodium, ceftazidime, cefuroxime sodium, followed by streptomycin, tobramycin, kanamycin, doxycycline HCl, ofloxacin, chloramphenicol, sulfamethoxazole-trimethoprim, oxytetracycline, nalidixic acid with a prevalence of (83.3% for each) and to **(**aztreonam and apramycin) (66.6% and 50%) , respectively. On the other side, *P. aeruginosa* isolates were completely resistant (100%) to streptomycin, tobramycin, cefotaxime sodium, kanamycin, doxycycline HCl, chloramphenicol, sulfamethoxazole-trimethoprim, ceftazidime, oxytetracycline, nalidixic acid, and cefuroxime sodium, followed by ofloxacin, apramycin (86.6% for each) while they were highly sensitive to aztreonam (73.3%) **(Table 2)**.

Table 2. Antimicrobial susceptibility testing results of *E. coli* and *P. aeruginosa* isolates from affected chickens:

Antibacterial agents	Disc content (µg)	*coli* isolates (*n*=12) R No.	%	S No.	%	*P. aeruginosa* isolates (*n*=15) R No.	%	S No.	%
Streptomycin	10	10	83.3	2	16.6	15	100	0	0
Tobramycin	10	10	83.3	2	16.6	15	100	0	0
Cefotaxime sodium	30	12	100	0	0	15	100	0	0
Kanamycin	30	10	83.3	2	16.6	15	100	0	0
Doxycycline HCl	30	10	83.3	2	16.6	15	100	0	0
Ofloxacin	5	10	83.3	2	16.6	13	86.6	2	13.3
Chloramphenicol	30	10	83.3	2	16.6	15	100	0	0
Sulfamethoxazole-trimethoprim	25	10	83.3	2	16.6	15	100	0	0
Ceftazidime	30	12	100	0	0	15	100	0	0
Oxytetracycline	30	10	83.3	2	16.6	15	100	0	0
Apramycin	15	6	50	6	50	13	86.6	2	13.3
Nalidixic acid	30	10	83.3	2	16.6	15	100	0	0
Aztreonam	30	8	66.6	4	33.3	4	26.6	11	73.3
Cefuroxime sodium	30	12	100	0	0	15	100	0	0

3.3. Effect of citrus oil 1% on MDR *E. coli*, MDR *P. aeruginosa* and fungal isolates:

Antibacterial activity and synergistic effect of citrus oil 1% against both MDR *E. coli* and *P. aeruginosa* isolates.

The antibacterial activity of citrus oil (1%) against multi-drug-resistant MDR isolates (12 *E. coli* and 15 *P. aeruginosa* isolates) is summarized in **Table 3**. These results revealed that citrus oil 1% inhibited the growth of *E. coli* and *P. aeruginosa* isolates with a prevalence of 50% and 20% respectively.

Table 3. Antibacterial activity of citrus oil against *E. coli* and *P. aeruginosa* isolates:

Conc.	Tested isolates.	*E. Coli* isolates (*n*=12)		*P. Aeruginosa* isolates (n=15)	
		Growth	No growth	Growth	No growth
citrus oil	0.5%	12(100%)	-	15(100%)	-
citrus oil	1%	6 (50%)	6 (50%)	12 (80%)	3 (20%)
citrus oil	1.5%	-	12(100%)	-	15(100%)

3.3.1.2. The synergistic effect of both citrus oil (1%) and same antimicrobials on previously tested MDR *E. coli* and *P. aeruginosa* isolates:

Considering the results of antibiogram in table (2), MDR *E. coli* isolates (n=6) and MDR *P. aeruginosa* isolates (n=12) table (3) were tested to evaluate the synergistic effect of citrus oil with previously tested antibacterials. Two *E. coli* isolates changed to susceptible to oxytetracycline, apramycin, and aztreonam. Whereas *P. aeruginosa*, four isolates changed to susceptible to tobramycin, ofloxacin, ceftazidime, and apramycin and two isolates changed to susceptible to streptomycin, cefotaxime sodium, doxycycline HCl, chloramphenicol, sulfamethoxazole-trimethoprim, oxytetracycline, and cefuroxime sodium as presented in **Table 4**.

Table 4. Antimicrobial susceptibility of MDR *E. coli* and *P. aeruginosa* isolates with citrus oil 1%.

Antimicrobial Agents	Disc cont.(µg)	*E. Coli* isolates (n=6) Pre	Post	*P. aeruginosa* isolates (n=12) Pre	Post
		Exposure to citrus oil		Exposure to citrus oil	
Streptomycin	10	R	R	R	S (16.6%)
Tobramycin	10	R	R	R	S (33.3%)
Cefotaxime sodium	30	R	R	R	S (16.6%)
Kanamycin	30	R	R	R	R
Doxycycline HCl	30	R	R	R	S (16.6%)
Ofloxacin	5	R	R	R	S (33.3%)
Chloramphenicol	30	R	R	R	S (16.6%)
Sulfamethoxazole/trimethoprim	25	R	R	R	S (16.6%)
Ceftazidime	30	R	R	R	S (33.3%)
Oxytetracycline	30	R	S (33.3%)	R	S (16.6%)
Apramycin	15	R	S (33.3%)	R	S (33.3%)
Nalidixic acid	30	R	R	R	R
Aztreonam	30	R	S (33.3%)	S	S
Cefuroxime sodium	30	R	R	R	S (16.6%)

Abbreviations: R; resistant- S; susceptible

The effect of different citrus oil dilutions against different fungal isolates:

The more the concentration of citrus oil on fungal isolates was subjected to, the more inhibition that occurred to them as shown in **table (5).**

Table 5. The antifungal effect of diluted citrus oil 1.5%, 1%, 0.5%, 0.25% and 0.1%:

Sample	*A. niger* (n=12)	*A. flavus (n=3)*	*C. albicans (n=6)*
Citrus 1.5%	-	-	-
Citrus 1%	+	+	+
Citrus 0.5%	++	++	++
Citrus 0.25%	+++	+++	+++
Citrus 0.1%	++++	++++	++++

++++: heavy growth ++: medium growth + : light growth -:no growth

DISCUSSION

The poultry industry is a vital contributor to global food security and economic development (**FAO, 2020; Birhanu et al. 2023**). However, the intensification of poultry farming has led to an increased prevalence of bacterial and fungal co-infections, which pose a significant threat to poultry health and productivity worldwide (**Abo-Aziz et al. 2020; kim et al. 2020; Thøfner and Christensen, 2021**).

Our investigation into microbial infections in chickens revealed a high prevalence of pathogens, with 71.4% of samples yielding microbial growth. This high rate of infection highlights a significant disease burden in the studied population. Bacterial isolates were predominant, accounting for 42.8% of the total isolates, with *P. aeruginosa* (23.8%) and *E. coli* (19%) being the most frequently identified species. The isolation of *E. coli* from systemic organs is consistent with its role as an opportunistic pathogen causing colibacillosis (**Nazzaro et al. 2017**). The presence of *P. aeruginosa* is particularly concerning given its intrinsic and acquired multi-drug resistance (MDR) and biofilm-forming capabilities, which complicate treatment and contribute to increased mortality (**Amor et al. 2023**).

Fungal isolates, although less frequent in single infections (9.5%), included *C. albicans* (4.7%) and *A. niger* (4.7%). The recovery of these fungi from internal organs indicates systemic infections such as candidiasis and aspergillosis, which can be devastating to poultry health, particularly when birds are immunosuppressed or housed in poor hygienic conditions (**Tokarzewski, 2015; Shaapan and Girh, 2024**).

A crucial finding was the high incidence of mixed bacterial and fungal isolates (19%), surpassing single fungal infections. This co-occurrence suggests a synergistic relationship between pathogens, where bacterial infections can facilitate fungal proliferation and vice versa **(Dhama et al. 2014; Nazzaro et al. 2017**).

For instance, *Aspergillus* species common in contaminated environments, can cause respiratory damage that predisposes birds to secondary bacterial infections like Avian Pathogenic *E. coli* (APEC), leading to more severe outcomes and higher mortality than single infections (**Hamila et al. 2024**). Similarly, *C. albicans* overgrowth in the gut, often triggered by antibiotic use or poor hygiene, can damage the intestinal lining, making it vulnerable to harmful enteric bacteria such as *Salmonella* (**Ceolin et al. 2012**). The high prevalence of co-infections, particularly involving *P. aeruginosa*, highlights the need for comprehensive diagnostic and integrated disease management strategies that address these complex interactions and the challenge of antimicrobial resistance (AMR) (**Li et al. 2015; Amuzie and Abas, 2017; Amor et al. 2023; Teke and Oloche, 2024**).

The sensitivity test results revealed a severe AMR problem in poultry isolates. *P. aeruginosa* exhibited a broader and higher degree of resistance compared to *E. coli* against commonly used antibiotics, complicating treatment selection. This underscores the urgent need for robust antimicrobial stewardship, enhanced biosecurity, and the exploration of alternative therapeutic agents like essential oils (**Li et al. 2015; Nazzaro et al. 2017**). The potential for these resistant strains to transfer to humans highlights a critical 'One Health' implication (**Al-Talib et al. 2024**). Specifically, *E. coli* showed 100% resistance to cephalosporins, indicative of ESBL production, a global concern **(Shaapan and Girh, 2024).** High resistance was also observed against aminoglycosides, tetracyclines, fluoroquinolones, and chloramphenicol, consistent with widespread antibiotic use and selective pressure (**Nazzaro et al. 2017; Al-Ajeeli et al. 2023; Amor et al. 2023**). While lower, resistance to aztreonam and apramycin is also developing **(El-Latif et al. 2021).** *P. aeruginosa* isolates demonstrated near-complete (100%) resistance to almost all tested antibiotics, representing a severe MDR phenotype. This alarming level of resistance, though inherent to *P. aeruginosa*, is higher than some reported averages, suggesting a particularly severe local epidemiological context **(Amor et al. 2023; Luan et al. 2023; Shaapan and Girh, 2024; Wang et al. 2022).** These findings emphasize the critical need for rigorous antimicrobial stewardship and alternative control measures in poultry production **(Amor et al. 2023).**

The study also investigated the antibacterial activity of 1% citrus oil, finding a promising 50% inhibition rate against *E. coli*. This aligns with previous research demonstrating citrus essential oils' ability to inhibit *E. coli* growth by disrupting cell membranes and interfering with metabolic processes **(Wang et al. 2022; Cai et al. 2024; Hamila et al. 2024).** While the 20% inhibition rate against *P. aeruginosa* was relatively low, it is still significant given this bacterium's inherent resistance to many antimicrobial agents, including essential oils **(Edogbanya et al. 2019)**. Some studies indicate citrus oils can inhibit *P. aeruginosa* growth or interfere with quorum sensing and biofilm formation **(Civilica, 2024).** These findings suggest citrus oils as valuable alternative or complementary tools for poultry health management, especially against antimicrobial resistance, warranting further investigation into optimal concentrations and synergistic interactions.

Citrus essential oils, rich in compounds like limonene, citral, and linalool, influence microbial resistance through several mechanisms. They exhibit direct antimicrobial action against resistant bacterial and fungal strains to antibiotics or antifungals by damaging cell membranes and disrupting vital cellular **(Nazzaro et al. 2017)**. Furthermore, citrus oils, particularly limonene, can prevent and break down biofilms, thereby increasing microbial susceptibility to antibiotics and immune responses **(Li et al. 2015)**. They may also interfere with specific resistance mechanisms, such as inhibiting efflux pumps that actively remove antibiotics from bacterial cells, and increasing cell membrane permeability, allowing conventional antimicrobials to enter more easily (**Burt, 2004; Nazzaro et al. 2013; Chear et al. 2022**).

Citrus oils show significant promise in combination with conventional antimicrobials, offering synergistic effects that can reduce required antibiotic doses, minimize side effects, and slow resistance development. Crucially, they can re-sensitize previously resistant microbial strains by targeting different pathways or disrupting resistance mechanisms, making traditional antimicrobials effective again **(Amuzie and Abas, 2017; Teke and Oloche, 2024)**.

Our study demonstrated a notable increase in antimicrobial susceptibility in both *E. coli* and *P. aeruginosa* isolates after treatment with 1% citrus oil. Specifically, two previously resistant *E. coli* isolates became susceptible to oxytetracycline, apramycin, and aztreonam. Even more pronounced effects were observed in *P. aeruginosa*, with four isolates becoming susceptible to tobramycin, ofloxacin, ceftazidime, and apramycin, and an additional two isolates showing broad-spectrum susceptibility to antibiotics including streptomycin, cefotaxime sodium, doxycycline HCl, chloramphenicol, sulfamethoxazole-trimethoprim, oxytetracycline, and cefuroxime sodium. These results strongly suggest that citrus oil can modulate bacterial resistance, thereby enhancing the efficacy of conventional antimicrobial agents **(Gislene et al. 2012; Nazzaro et al. 2013; Blair et al. 2014; Pang et al. 2019; Svetaz et al. 2020; Yang et al. 2020; Mahato et al. 2021; Chear et al. 2022; WHO, 2024).** The differential re-sensitization patterns observed, particularly the broader impact on *P. aeruginosa*, indicate that citrus oil may disrupt its resistance mechanisms more effectively than those of *E. coli.*

Raising the concentration of citrus oil typically results in more pronounced inhibition of fungal development. This direct relationship, where higher doses lead to increased antifungal effectiveness, is consistent with **(Yang et al. 2020)** findings. While promising, further research is needed to elucidate the exact molecular interactions, establish dose-response relationships, identify active compounds, and assess efficacy and safety in living organisms. Citrus oil holds great potential as antibacterial and antifungal as well as an adjuvant therapy against antimicrobial resistance, especially for difficult-to-treat pathogens like *P. aeruginosa*, warranting extensive future investigation.

CONCLUSION

This study highlights the significant challenge posed by bacterial and fungal co-infections and widespread antimicrobial resistance (AMR) in poultry, particularly involving *P. aeruginosa* and *E. coli.* The high prevalence of these infections and the alarming rates of multi-drug resistance underscore a critical threat to poultry health, productivity, and potentially public health through the 'One Health' pathway. Crucially, the research demonstrates the promising potential of citrus essential oils as an alternative or complementary therapeutic strategy. Our findings indicate that citrus oil not only possesses direct antibacterial and antifungal activity but also exhibits a remarkable ability to re-sensitize highly resistant bacterial strains, such as *P. aeruginosa and E. coli*, to conventional antibiotics. This re-sensitization is attributed to various mechanisms, including membrane disruption, efflux pump inhibition, and anti-biofilm properties, suggesting a novel approach to combating the global AMR crisis.

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