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Antimicrobial efficacy of quaternary ammonium compounds (QACs) against multidrug resistant bacterial species causing cellulitis in broiler chicken

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ABSTRACT

Avian cellulitis is one of the most important field problems facing the poultry sector. Sever financial losses resulted from the condemnation of the broiler carcasses infected with cellulitis lesions. In light of this, the current study was aimed to isolation and identification of the bacterial species causing cellulitis in broiler chickens in Dakahlia and Sharkia Governorates, Egypt. The bacterial isolates were tested for their antimicrobial susceptibility and molecular detection of some virulence and antimicrobial resistance genes. In addition to evaluate the antibacterial activity of quaternary ammonium compounds and glutaraldehyde (TH4 ®) against the bacterial isolates. Four bacterial species were isolated; *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus mirabilis* with percentages of (75%), (20%), (6%) and (5%) respectively. *E. coli* was recorded as the most predominant isolated bacteria in this study with12 different sero- groups (O1, O2, O26, O55, O78, O91, O121, O125, O126, O128, O153 and O158). *E. coli* O78 and O91 were the most prevalent identified sero- groups. The antimicrobial susceptibility testing revealed higher resistances against doxycycline and ampicillin (95.6%), amoxicillin (90.7%), norfloxacin (84%), oxytetracycline (79.1%) and amikacin (71.6%) in *E. coli*, doxycycline (73.3%), oxytetracycline (80%),ampicillin(75%), streptomycin (80%), erythromycin (73.3%), and oxacillin (86.7%) in *S. aureus*, doxycycline (83.3%), oxytetracycline (77.8%), ampicillin (83.3%), amoxicillin (88.9%), neomycin (72.2%) and erythromycin (77.8%) in *P. aeruginosa* and doxycycline, oxytetracycline, ampicillin, amoxicillin, streptomycin and erythromycin (100% for each of them) in *Proteus mirabilis*. All isolated bacterial species were multidrug resistance (MDR). The molecular identification showed the detection of virulence genes: *iut*A in *E. coli*, *nuc* in *S. aureus*, *tox*A in *P. aeruginosa* and *rsb*A in *Proteus mirabilis*., with percentage of (100%). bla_{TEM}, *tet*A (A), *qnr*A, *tet*K, *mec*A, *aac(6')aph (2")*, *ere*A and *aada1* resistance genes were reported in this study. Quaternary ammonium compounds in combination glutaraldehyde (TH4 ®) with 2% concentration showed the highest antibacterial activity against the examined multidrug resistant bacterial isolates. These results suggested for application of 2% TH4 ® to achieve effective disinfectant programs in poultry farms.

Introduction

Avian cellulitis is a serious problem for the broiler sector resulting in significant financial losses because of the higher condemnations of a part or the complete carcasses at processing (Radwan *et al*., 2018; Amer *et al*., 2020; Abd El-Ghany, 2023). Cellulitis is more prevalent in broilers (Abd El-Ghany, 2023) and it caused by bacteria which invading the subcutaneous tissues through the damaged skin (Amer *et al*., 2020). The disease condition is represented by an acute diffuse inflammation of subcutaneous tissues and muscles on different body areas, particularly the skin of thighs and abdomen. Yellowish patches with a pus plaque under the skin and hemorrhages in the underlying muscles were seen in the diseased birds. In the advanced cases, caseous, yellowish to green, dark red, or brown fetid gangrenous exudate might be observed (Abd El-Ghany, 2023). Cellulitis may be associated with other systemic lesions in the birds' internal organs (Radwan *et al*., 2018).

Several bacterial species were identified from the cellulitis lesions, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Clostridia, Aeromonas spp., Proteus mirabilis, Staphylococci, Streptococci* and *Enterobacter* species. The disease condition can be resulted from the infection with single or mixed bacteria (Amer *et al*., 2020) and *E. coli*, *P. mirabilis*, *S. epidermidis*, and *Manheimia haemolyica* (Santos *et al*., 2014). *E. coli* was mentioned by Singer *et al*. (2000) and Radwan *et al*. (2018) as the most common bacterial agent causing cellulitis. The ability of *E. coli* to invade the subcutaneous tissue of poultry resulting in cellulitis lesions may be attributed to the presence of *iut*A and iss virulence genes (Silva *et al*., 2021). *P. mirabilis* isolated from broiler chickens infected with cellulitis possessed *pmfA, mrpA, atfA, ucaA* (fimbriae), *hpm*A (hemolysin), *ptA* and *zapA* (proteases), and *ireA* (siderophore) genes (Sanches *et al*., 2020). The *rsb*A gene encodes a sensory protein that controls swarming behavior of *P. mirabilis* (AL-Dulaimy *et al*., 2023). Some predisposing factors such as immunosuppression (Amer *et al*., 2020), stock density, skin integrity, and litter conditions showed a higher risk of developing cellulitis lesions in chickens (Abd El-Ghany, 2023).

Antimicrobial resistance is one of the most serious public health issues all over the globe (Lemlem *et al*., 2023). The emergence of multidrug resistant (MDR) *E. coli* in the Egyptian poultry farms are of considerable concern which has been arise from the misuses of the antimicrobial agents (Abdel-Rahman *et al*., 2023). MDR *P. aeruginosa* is regarded as a serious bacterium that causes higher morbidity and mortality. It poses a risk to the poultry sector producing significant financial losses (Marouf *et al*., 2023). Among the identified bacterial species recovered from cellulitis, a higher incidence of MDR was found (Amer *et al*., 2019). Higher resistances of the bacterial species isolated from cellulitis to different antimicrobial agents were identified to lincomycin, streptomycin, spiramycin and trimethoprim/sulphamethoxazol in *E. coli* (Radwan *et al*., 2018), clindamycin, ampicillin and oxacillin in *Staphylococcus* species, enrofloxacin, tetracycline, chloramphenicol, vancomycin and oxacillin in *E. coli*, erythromycin and oxacillin in *P. mirabilis* and enrofloxacin, tetracycline, erythromycin and chloramphenicol in *P. aeruginosa* (Amer *et al*., 2019). The isolated bacteria from avian cellulitis showed the occurrence of some antimicrobial resistance genes such as *tet*B in *E. coli*, *P. mirabilis*,

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S. epidermidis, *Sul1* in *E. coli*, *P. mirabilis*, *S. epidermidis* and *Manheimia haemolytica*, *tet*A in *E. coli* and *S. epidermidis*, *SHV* in *E. coli*, *P. mirabilis S. epidermidis* and *cat1* in one *P. mirabilis* isolate (Santos *et al*., 2014).

Hygiene and sanitation play an important role in the disease control program in poultry industry (Kasˇkova´ *et al*., 2007). One of the commonly used disinfectants in poultry sector to maintain the farm hygiene is Quaternary ammonium compounds (QACs) (Maertens *et al*., 2020) which are antimicrobial chemicals controlling viruses and bacteria (Osimitz and Droege, 2021) and have antibacterial properties against wide range of microbes (Hegstad *et al*., 2010; Frolov *et al*., 2022).The development of alternative antibacterial therapies that do not readily lead to resistance, like quaternary ammonium salts (QAS), has attracted enormous research attention (Zhang *et al*., 2015). QACs adsorbed by large amounts to the bacterial cell membrane producing leakage and damage of cell membrane (Hamilton, 1968) which leads to leakage and coagulation of cytoplasm due to the interaction with phospholipids (Vijayakumar *et al*., 2012). Therefore, this work aimed to isolation and identification the bacterial species causing chicken cellulitis. *In-vitro* antimicrobial susceptibility and genotypic characterization of isolated bacteria. In addition to investigate the antibacterial activity of quaternary ammonium compounds and glutaraldehyde (TH4®) against the isolated bacteria.

Materials and methods

Study area and sampling

Three hundred diseased broiler chickens (age: 25 to 45 days) were collected randomly from 30 different broiler farms located in Sharkia and Dakahlia Governorates (15 farms for each governorate and ten chickens from each farm), Egypt. These chickens suffered from cellulitis lesions which represented by inflammation of subcutaneous tissues and muscles in the thighs and lower abdomen with yellowish fetid exudate. The lesion area showed thickened skin with red color and swelling. The samples were collected from July 2022 to July 2023. Samples from cellulitis lesions located in the muscles of thigh and lower abdomen were collected by inserting sterile swabs (containing 2 ml of Buffered Peptone Water) inside the lesions. All samples were labeled, identified and transported directly to the laboratory to complete further examinations.

Bacteriological examination

The collected swabs were subjected to the isolation and identification of 4 bacterial species (*E. coli*, *Proteus mirabilis*, *S. aureus*, *P. aeruginosa*). *E. coli* was isolated and identified according to Quinn *et al*. (2002) and serotyped according to Kok *et al*. (1996) by using rapid *E. coli* antisera (DENKA SEIKEN Co., Japan). From the collected samples, *Proteus* species was isolated and identified as described by Quinn *et al*. (2002). Furthermore, the isolation of *S. aureus* was carried out according to International Standard Office (2003). Also *P. aeruginosa* was isolated as described by MacFadden (2000).

Antimicrobial susceptibility testing

The susceptibility of the isolated bacterial species was tested against the commonly used antimicrobial agents in the broiler farms by using the disc diffusion method. For *E. coli*, *Proteus mirabilis* and *P. aeruginosa* isolates, the selected antimicrobial agents were tetracyclines (doxycycline; DO, 30 µg and oxytetracycline; OT, 30 µg), polypeptides (colistin sulphate; CT, 10 µg), penicillin (ampicillin; AMP, 10 µg and amoxicillin; AMX, 10 µg), aminoglycosides (streptomycin; S, 10 µg, amikacin; Ak, 30 µg), and neomycin; N, 30 µg), macrolide (erythromycin; E, 15 µg), and quinolone (norfloxacin; NOR, 10µg). Meanwhile, for *S. aureus*, tetracyclines (doxycycline; DO, 30 µg and oxytetracycline; OT, 30 µg), sulfonamides (trimethoprim-sulfamethoxazole; SXT, 1.25/23.75 µg), penicillin (ampicillin; AMP, 10 µg, amoxicillin; AMX, 10 µg and (oxacillin; OX, 1 µg, OX), polypeptides (colistin sulphate; CT, 10 µg), aminoglycosides (streptomycin; S, 10 µg) and macrolide (erythromycin; E, 15 µg).

The tested antimicrobial agents were categorized into susceptible or resistant according to CLSI (2020). Multidrug resistance (MDR) was detected in the basis of the resistance to more than 2 antimicrobial classes. In addition to the calculation of the multidrug resistance index (MARI).

Molecular identification

DNA extraction

Five isolates from each bacterial species were selected and examined for virulence and antimicrobial resistance genes. The samples were selected on the basis of exhibiting higher resistance to the used antimicrobial agents. The selected virulence genes were *iut*A for *E. coli*, *nuc* for *S. aureus*, 16S rDNA and *tox*A for *P. aeruginosa* and *rsb*A for *Proteus* Spp. The resistance genes bla_{TEM} encoded for ampicillin and amoxicillin, *tet*A (A) for oxytetracycline and doxycycline, *qnr*A for quinolones, *tet*K for oxytetracycline in *S. aureus*, *mec*A for oxacillin, *aac(6')aph (2")* for streptomycin in *S. aureus*, *ere*A for erythromycin and *aada1* for streptomycin resistance genes. DNA was extracted from the samples using the recommendations of QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications.

Oligonucleotide Primers: The primers used (Table 1) were supplied from Metabion (Germany).

PCR amplification

The primers were utilized in a reaction of 25- µl containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 5.5 µl of water, and 5 µl of DNA template. The reaction was conducted in a thermal cycler (Applied biosystem 2720).

Analysis of the PCR Products

The PCR products were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. The gel was analyzed as follow; 20 µl of the products was loaded in each gel slot. The fragment sizes were determined using a Generuler 100 bp ladder (Fermentas, Germany) and Gelpilot 100 bp ladder (Qiagen, Gmbh, Germany). The gel was photographed using a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed via computer software.

In vitro antibacterial activity of TH4® using agar diffusion assay

TH4 ® disinfectant product (INTERCOVA animal Health products, Batch no. 200350B) was used in this study. This product is a combination of Dialkyl dimethyl ammonium chloride (75 g), alkyl dimethyl benzyl ammonium chloride (50 g), glutaraldehyde (62.5 g) and excipients (surfactant, terpenic fragrances, water s.q. (1litre).

Agar well diffusion method was used for the evaluation of antimicrobial properties of quaternary ammonium compounds and glutaraldehyde (TH4) against the isolated *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus mirabilis* (5 isolates from each species) according to the recommendation of Clinical and Laboratory Standards Institute (CLSI, 2020). Ciprofloxacin disc (5µg) (oxoid) was used as a standard antibiotic which had a potential effect on the tested bacteria. The tested isolates were inoculated into 10 ml of sterile nutrient broth and incubated at 37ºC for 8 hours. After incubation 0.5 McFarland of the bacterial cultures were prepared and swabbed on the surface of sterile nutrient agar plates using a sterile cotton swab. Agar wells were performed with the help of sterilized cork borer with 10 mm diameter. By using a micropipette, 100 µl of different concentrations of the TH4® (2% -1% -0.5% -0.25%) and the ciprofloxacin disc were add-

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions.

Target	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
tetA(A)	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	570	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Randall *et al.* (2004)
qnrA	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Robicsek *et al.* (2006)
ereA	GCCGGTGCTCATGAACTTGAG CGACTCTATTCGATCAGAGGC	420	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Nguyen *et al.* (2009)
bla_{TEM}	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Colom *et al.* (2003)
aada1	TATCAGAGGTAGTTGGCGTCAT GTTCCATAGCGTTAAGGTTTCATT	484	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Randall *et al.* (2004)
E. coli iutA	GGCTGGACATGGGAACTGG CGTCGGGAACGGGTAGAATCG	300	94˚C 5 min.	94˚C 30 sec.	63˚C 30 sec.	72˚C 30 sec.	72˚C 10 min.	Yaguchi *et al.* (2007)
*Staph mec*A	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	72˚C 10 min.	McClure *et al.* (2006)
Staph nuc	ATATGTATGGCAATCGTTTCAAT GTAAATGCACTTGCTTCAGGAC	395	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	Gao *et al.* (2011)
Staph tetK	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	Duran *et al.* (2012)
Staph aac(6') aph (2")	GAAGTACGCAGAAGAGA ACATGGCAAGCTCTAGGA	491	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	
Proteus rsbA	TTGAAGGACGCGATCAGACC ACTCTGCTGTCCTGTGGGTA	467	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	Pathirana *et al.* (2018)
P. aeruginosa 16S rDNA	GGGGGATCTTCGGACCTCA TCCTTAGAGTGCCCACCCG	956	94˚C 5 min.	94˚C 30 sec.	52˚C 40 sec.	72˚C 1 min.	72˚C 10 min.	Spilker *et al.* (2004)
P. aeruginosa toxA	GACAACGCCCTCAGCATCACCAGC CGCTGGCCCATTCGCTCCAGCGCT	396	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	Matar *et al.* (2002)

ed to the plate. The agar plates were incubated for 24 hours at 37ºC and the diameters of the inhibition zones were measured by millimeter (mm) (Each test was conducted in triplicate). Less than 12 mm in diameter inhibition zones were considered as devoid from any antibacterial activity (Durairaj *et al*., 2009).

Results

Incidence of different bacterial species isolated from broiler chicken farms

Out of the examined 300 diseased broiler chickens in Dakahlia and Sharkia Governorates (150 chickens for each), 4 bacterial species included *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus mirabilis* were isolated with percentages of 75%, 20%, 6% and 5% respectively. The isolated bacterial species from Dakahlia revealed the detection of *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus* with percentages of (78.7%), (18.7%), (5.3%) and (6%) meanwhile these bacterial species were isolated from Sharkia governorate with (71.3%), (21.3%), (6.7%) and (4%) respectively. *E. coli* is the most predominant isolated bacteria followed by *S. aureus* and finally *P. aeruginosa* and *Proteus* (Fig. 1).

From Figure 2, our findings showed that *E. coli* isolated from Dakahlia and Sharkia Governorates were serologically differentiated into 12 serogroups (O1, O2, O26, O55, O78, O91, O121, O125, O126, O128, O153 and O158). O78 and O91 were the most predominant sero- groups in this study. All of the sero- groups were recorded in Dakahlia meanwhile 8 sero- groups (O1, O2, O78, O91, O125, O126, O153 and O158) were recorded in Sharkia.

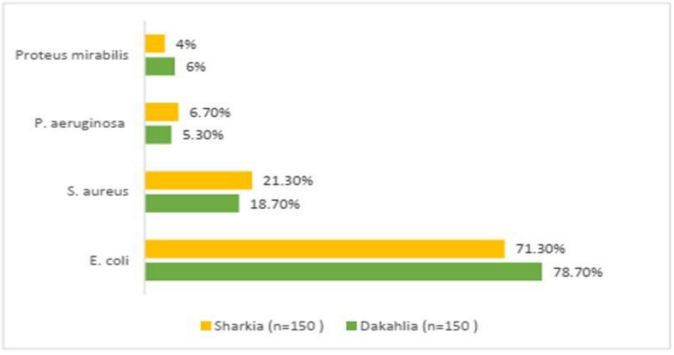

Fig. 1. Incidence of different bacterial species isolated from broiler chickens in Dakahlia and Sharkia Governorates.

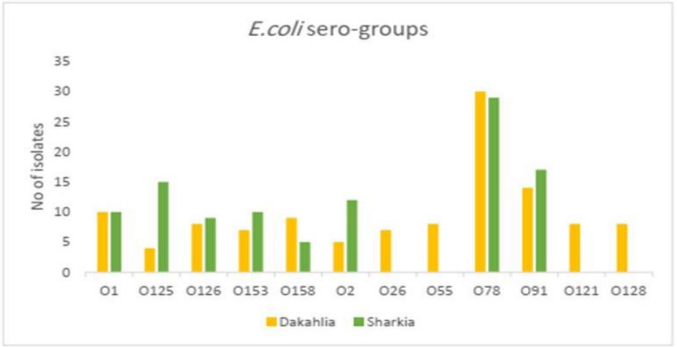

Fig. 2. Distribution of *E. coli* sero-groups isolated from broiler chickens in Dakahlia and Sharkia Governorates.

Antimicrobial susceptibility pattern of the isolated E. coli, S. aureus P. aeruginosa and Proteus mirabilis

The disc diffusion tests (Table 2) showed higher resistances of the *E. coli* isolates against the used antimicrobial agents; doxycycline and ampicillin (95.6%), amoxicillin (90.7%), norfloxacin (84%), oxytetracycline (79.1%) and amikacin (71.6%). The complete resistances in the identified sero- groups were recorded in O1 and O2 to doxycycline, ampicillin, amoxicillin and amikacin, O26 and O55 to doxycycline, oxytetracycline, ampicillin, amoxicillin and norfloxacin, O91 to ampicillin, O121 to doxycycline, oxytetracycline, ampicillin and amoxicillin, O125 and O126 to doxycycline, ampicillin and norfloxacin, O128 to doxycycline, ampicillin, amoxicillin, neomycin and norfloxacin, O153 to doxycycline and ampicillin and finally O158 to doxycycline, oxytetracycline, amoxicillin, amikacin and norfloxacin. Complete sensitivity was observed in O26 to colistin sulphate and neomycin and in O55 to streptomycin and neomycin.

The antimicrobial susceptibility of *S. aureus* revealed that the recorded resistances for doxycycline, oxytetracycline, colistin sulphate, ampicillin, amoxicillin, streptomycin, erythromycin, trimethoprim-sulfamethoxazole and oxacillin were (73.3%), (80%), (66.7%), (75%), (65%), (80%), (73.3%), (63.3%) and (86.7%) respectively (Fig. 3).

The reported resistances of *P. aeruginosa* isolates to doxycycline, oxytetracycline, colistin sulphate, ampicillin, amoxicillin, streptomycin, amikacin, neomycin, erythromycin and norfloxacin were (83.3%), (77.8%), (61.1%), (83.3%), (88.9%), (61.1%), (55.6%), (72.2%), (77.8%) and (66.7%) respectively. For *Proteus marbilis* complete resistances (100%) were recorded for doxycycline, oxytetracycline, ampicillin, amoxicillin, streptomycin and erythromycin (Fig. 3).

Multidrug resistance (MDR) to 3 or more antimicrobial agent classes was recorded in all of the isolated *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus marbilis*. Twenty nine antimicrobial agent pattern profiles were recorded in *E. coli* isolates in addition to 14 patterns in *S. aureus* isolates,

Table 2. Antimicrobial resistance among different serogroups of *E. coli* isolates.

Sero-group (no.)	DO	OT	CT	AMP	AMX	S	AK	N	E	NOR
O1 (20)	20	15	5	20	20	20	20	5	20	10
O2 (17)	17	8	4	17	17	8	17	9	8	13
O26 (7)	7	7	0	7	7	3	4	0	7	7
O55 (8)	8	8	4	8	8	0	4	0	4	8
O78 (59)	54	49	39	54	54	40	34	39	35	49
O91 (31)	26	26	23	31	27	26	22	21	26	26
O121 (8)	8	8	5	8	8	5	6	6	7	6
O125 (19)	19	14	9	19	14	14	14	10	5	19
O126 (17)	17	13	9	17	13	13	9	13	0	17
O128 (8)	8	4	4	8	8	4	4	8	4	8
O153 (17)	17	12	5	17	14	12	13	8	12	12
O158 (14)	14	14	5	9	14	5	14	9	9	14
Total (225)	215	178	112	215	204	150	161	128	137	189
Resistant (%)	95.6	79.10	49.80	95.60	90.70	66.70	71.60	56.90	60.90	84

Doxycycline: DO, Oxytetracycline: OT, Clostin sulphate: CT, Ampicillin: AMP, Amoxicillin: AMX, Streptomycin: S, Amikacin: Ak, Neomycin: N, Erythromycin: E, Norfloxacin: NOR.

Table 3. Genotypic and phenotypic characteristics of virulence and antimicrobial resistance for *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus mirabilis* isolates.

Bacterial species	isolates	Phenotypic profile of resistance	Virulence gene	Resistant genes	MDRI
E. coli	1	CT-AMP-AMX-S-AK-N-E-NOR	*iut*A	bla_{TEM}, *tet*A (A), *qnr*A	0.8
	2	DO-OT-AMP-AMX-N-NOR	*iut*A	bla_{TEM}, *tet*A (A), *qnr*A	0.6
	3	DO-OT-AMP-AMX-S-AK-N-E-NOR	*iut*A	bla_{TEM}, *tet*A (A), *qnr*A	0.9
	4	DO-OT-AMP-AMX-N-E-NOR	*iut*A	bla_{TEM}, *qnr*A	0.7
	5	DO-OT-CT- AMP-AMX-S-AK-N-E-NOR	*iut*A	bla_{TEM}, *tet*A(A), *qnr*A	1
S. aureus	1	DO-OT-AMP-AMX-S-E-SXT-OX	*nuc*	*tet*K, *mec*A	0.9
	2	CT-AMP-E-SXT	*nuc*	*tet*K, *mec*A, aac(6')aph (2''	0.4
	3	DO-AMP-AMX-S-E-OX	*nuc*	*tet*K, *mec*A, aac(6')aph (2''	0.7
	4	DO-OT-AMP-AMX-S-E-OX	*nuc*	*tet*K, *mec*A, aac(6')aph (2''	0.8
	5	DO-OT-CT-AMP-AMX-S-E-SXT-OX	*nuc*	*tet*K, *mec*A, aac(6')aph (2''	1
P. aeruginosa	1	DO-AMP-AMX-S-N-E-NOR	16S *rDNA*, *tox*A	bla_{TEM}, *tetA*(A), *ere*A	0.7
	2	DO-AMP-AMX-S-N-NOR	16S *rDNA*, *tox*A	bla_{TEM}, *tetA*(A)	0.6
	3	DO-AMP-AMX-S-NOR	16S *rDNA*, *tox*A	bla_{TEM}, *tetA*(A), *ere*A	0.5
	4	DO-OT-AMX-S-AK-N-E	16S *rDNA*, *tox*A	bla_{TEM}, *tetA*(A), *ere*A	0.7
	5	DO-OT-CT-AMP-AMX-AK-E-NOR	16S *rDNA*, *tox*A	bla_{TEM}, *ere*A	0.8
Proteus mirabilis	1	DO-OT-AMP-AMX-S-AK-E	*rsb*A	bla_{TEM}, *tetA*(A), *Aada1*	0.7
	2	DO-OT-AMP-AMX-S-AK-N-E	*rsb*A	bla_{TEM}, *tetA*(A), *Aada1*	0.8
	3	DO-OT-AMP-AMX-S-E	*rsb*A	bla_{TEM}, *tetA*(A), *Aada1*	0.6
	4	DO-OT-CT-AMP-AMX-S-AK-N-E	*rsb*A	bla_{TEM}, *tetA*(A), *Aada1*	0.9
	5	DO-OT-CT-AMP-AMX-S-E	*rsb*A	bla_{TEM}, *tetA*(A), *Aada1*	0.7

10 patterns in *P. aeruginosa* isolates and finally 7 patterns in *Proteus mirabilis* isolates. The most recorded patterns were (DO-OT-AMP-AMX-S-AK-N-E-NOR), (DO-OT-CT-AMX-S-SXT-OX), (DO-OT-CT-AMP-AMX-AK-E-NOR) and (DO-OT-AMP-AMX-S-AK-E) for the isolated *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus* species respectively. High multidrug resistance index (MDRI) was calculated in the current study and the recorded findings showed that which the index was ranged from (0.6 to 1), (0.4 to 1), (0.5 to 0.8) and (0.6 to 0.9) for *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus mirabilis* respectively (Table 3).

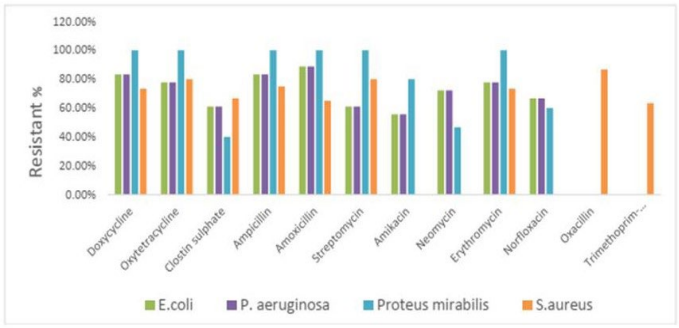

Fig. 3. Antimicrobial resistant profiles of *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus mirabilis* isolates.

Molecular detection of virulence and resistance genes in the isolated bacterial species

The detection of virulence genes; *iut*A in *E. coli* (Fig. 4), *nuc* in *S. aureus* (Fig 5a), 16S rDNA and *tox*A in *P. aeruginosa* (Fig. 6a) and *rsb*A in *Proteus mirabilis* (Fig. 6b), were recorded in all of the examined isolates with percentage of (100%).

The antimicrobial resistance genes identified that bla_{TEM} (Fig. 7a), *tet*A (A) (Fig. 7b), and *qnr*A (Fig. 4) genes were detected in *E. coli* isolates with percentages of (5/5, 100%), (4/5, 80%) and (5/5, 100%) respectively. In *S. aureus* isolates, 3 resistance genes were recorded; *tet*K, *mec*A and *aac(6') aph (2")* with percentages of (5/5, 100%), (5/5, 100%) and (4/5, 80%) respectively (Fig. 5b). In *P. aeruginosa* bla_{TEM} (Fig. 7a), *tet*A(A) (Fig. 7b), and *ere*A (Fig. 6a), genes were detected with percentages of (5/5, 100%), (4/5, 80%) and (4/5, 80%) respectively. bla_{TEM}, *tet*A(A) and *aada1* genes were reported in all of the examined *Proteus mirabilis* with percentage of (5/5, 100%) for each of them (Fig. 7a, 7b and 6b respectively).

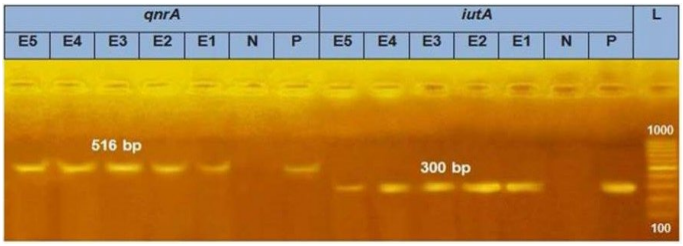

Fig. 4. Agarose gel electrophoresis of the PCR products for *E. coli* isolates to detect *iut*A (300 bp) and *qnr*A genes (516 bp) in genomic DNA. Lane L: DNA ladder, P: Positive control, N: Negative control, Lanes: E1 to E5: examined samples.

In Vitro Antibacterial activity of TH4® against the isolated bacterial species

The antibacterial activity of quaternary ammonium compounds and glutaraldehyde (TH4®) showed that all of the examined isolates were susceptible to TH4® (Table 4 and Fig. 8). All examined concentration of TH4® showed antibacterial effects however the inhibition zones diameters in case of 2% concentration in all of the tested bacterial species was higher than other concentrations (1%, 0.5% and 0.25%). Interestingly, TH4® showed good antibacterial activities results when compared to ciprofloxacin.

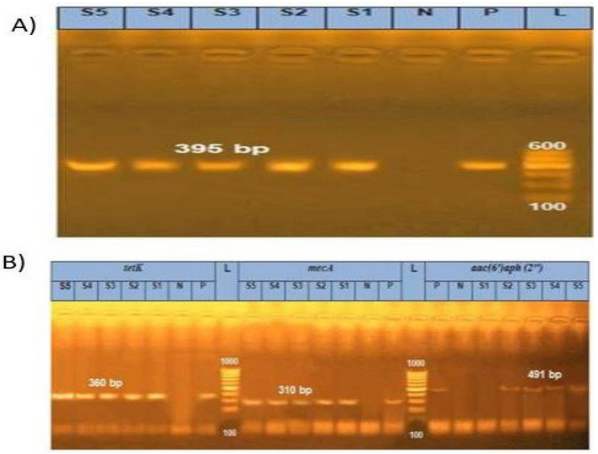

Fig. 5. A) Agarose gel electrophoresis of the PCR products for *S. aureus* isolates to detect *nuc* (395 bp) in genomic DNA. Lane L: DNA ladder, P: Positive control, N: Negative control, Lanes: S1 to S5: examined samples.B) Agarose gel electrophoresis of the PCR products for S. aureus isolates to detect tetK (360 bp), mecA (310 bp) and aac(6')aph (2'') (491 bp) genes in genomic DNA. Lane L: DNA ladder, P: Positive control, N: Negative control, Lanes: S1 to S5: examined samples.

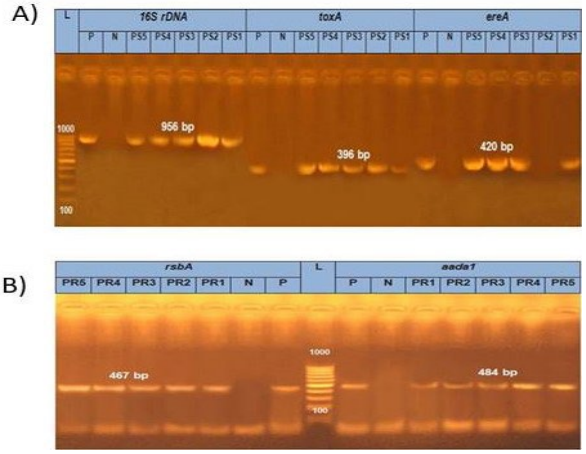

Fig. 6. a) Agarose gel electrophoresis of the PCR products for *P. aeruginosa* isolates to detect 16S rDNA (956 bp), *tox*A (396 bp) and *ere*A genes (420 bp) in genomic DNA. Lane L: DNA ladder, P: Positive control, N: Negative control, Lanes: PS1 to PS5: examined samples.b) Agarose gel electrophoresis of the PCR products for *Proteus* Spp. isolates to detect *rsb*A (467 bp) and *aada1* genes (484 bp) in genomic DNA. Lane L: DNA ladder, P: Positive control, N: Negative control, Lanes: PR1 to PR5: examined samples.

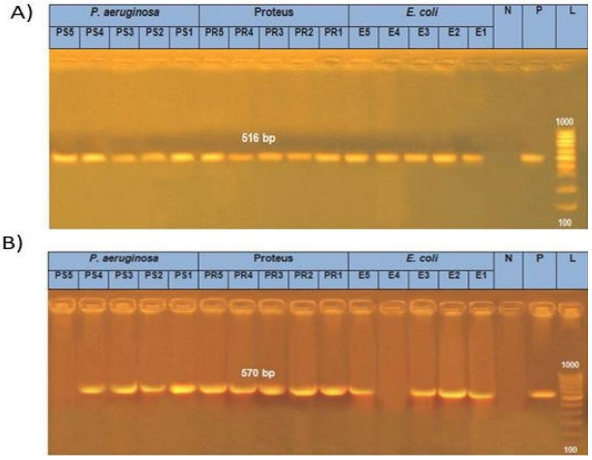

Fig. 7. a) Agarose gel electrophoresis of the PCR products for *P. aeruginosa*, *Proteus* spp. and *E. coli* isolates to detect bla_{TEM} (516 bp) in genomic DNA. Lane L: DNA ladder, P: Positive control, N: Negative control, Lanes: PS1 to PS5, PR1 to PR5 and E1 to E5: examined samples. b) Agarose gel electrophoresis of the PCR products for *P. aeruginosa*, *Proteus* Spp. and *E. coli* isolates to detect *tet*A(A) (570 bp) in genomic DNA. Lane L: DNA ladder, P: Positive control, N: Negative control, Lanes: PS1 to PS5, PR1 to PR5 and E1 to E5: examined samples

Discussion

Nowadays, cellulitis is an important cause of broilers condemnation in slaughterhouses through the globe, resulting in significant financial losses (Fard *et al.*, 2007). Although several bacterial agents, both Gram

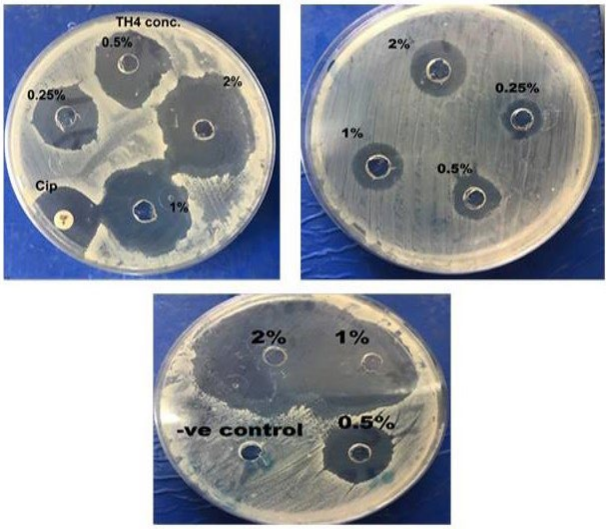

Fig. 8. Antimicrobial activity of different concentrations of TH4® against bacterial species isolated from cellulitis lesions.

Table 4. Antibacterial activity of TH4® and ciprofloxacin against bacteria causing cellulitis in chicken (n=5).

	Inhibition zone diameter(mm)*				
	TH4 ® concentration				Ciprofloxacin
Isolates	2%	1%	0.50%	0.25%	(5µg/disc)
E. coli	27.6±2.07	22.6±1.8	15.0±1.58	8.0± 1.58	27.2± 1.9
S. aureus	30.0±3.16	26.0±2.9	17.8±2.58	8.0±1.87	26.0±2.9
P. aeruginosa	28.0± 2.0	23.8±2.86	14.4±1.5	8.4±1.14	28.4±2.07
Proteus mirabilis	27.4±3.02	22.2±2.48	16.4±1.67	10.4±1.14	27.2±1.48

positive and Gram negative, were identified from cellulitis lesions and thought to be the cause of the disease, *E. coli* is still the main infectiousagent and is considered to be the cause of coliform cellulitis in chickens (Saif *et al*., 2003). In this study *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus mirabilis* were isolated from 300 broiler chickens suffered from cellulitis in two Egyptian Governorates (Dakahlia and Sharkia) with percentages of 75%, 20%, 6% and 5% respectively. *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus mirabilis* were isolated with (78.7%, 18.7%, 5.3% and 6%) and (71.3%, 21.3%, 6.7% and 4%) from Dakahlia and Sharkia respectively. *E. coli* is the most predominant isolated bacteria followed by *S. aureus* and finally *P. aeruginosa* and *Proteus mirabilis*. This result was in line with earlier research that found that the most common microorganism isolated from cellulitis lesions was *E. coli* (Fard *et al*., 2007). Also, our results nearly agreed with Radwan *et al*. (2018) who recovered *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus* species from cellulitis in chickens collected from Beni-Suef and El-Fayoum Governorates, Egypt with percentages of (80.3%), (5.1%), (4.5%) and (5.7%) respectively and Silva *et al*. (2021) who isolated *E. coli* in (82.0%) of cellulitis samples. Four species [*E. coli* (44%), *S. epidermidis* (36%), *P. mirabilis* (28%) and strains of *Manheimia haemolytica* (12%)] were identified by Santos *et al*. (2014) as bacterial causes of avian cellulitis. Furthermore, Alfifi *et al*. (2022) detected *E. coli* (60.9%), *P. mirabilis* (12.5%) and *S. aureus* (1.6%) from broiler carcasses infected with cellulitis. Higher incidences of *E. coli* isolated from cellulitis lesions in chickens were recorded by Gomis *et al*. (2000) and Derakhshanfar and Ghanbarpou (2002) with (92%) and (91.8%) respectively. Different incidences from our results were reported by Amer *et al*. (2019) in broiler diseased with cellulitis; *E. coli* (45.2%), staphylococci (33.2%), *P. aeruginosa* (2.2%) and *P. mirabilis* (4.4%).

Serotyping results of examined *E. coli* isolates identified 12 different sero- groups (O1, O2, O26, O55, O78, O91, O121, O125, O126, O128, O153 and O158). O78 and O91 were the most predominant sero- groups in this study. Similar findings were obtained by Jeffrey *et al*. (2002), Derakhshanfar and Ghanbarpou (2002) and Amer *et al*. (2019) whereas *E. coli* (O78) was recorded as a most predominant sero- group isolated from cellulitis in chickens. Also, Fard *et al*. (2007) mentioned that serogroups O78, O1, and O2 were the most predominated isolates from cases of cellulitis. Additionally, Ngeleka *et al*. (1996) found that O25 and O78 were the most frequently observed. On the other hand, Wang *et al*. (2010); Tana *et al*. (2013) and Radwan *et al*. (2018) found that O65, O86 and O125 were the most prevalent serogroups, respectively.

In poultry farms, antibiotics are usually utilized as growth promoters and therapeutics. However, overuse of antibiotics poses a serious risk because it can lead to the emergence of antibiotic-resistant bacterial strains, which can infect humans through the food chain (Nemati *et al*., 2008; Suleiman *et al*., 2013).With regard to the antimicrobial susceptibility findings in the present study, higher resistances of *E. coli* isolates were recorded against doxycycline and ampicillin (95.6%), amoxicillin (90.7%), norfloxacin (84%), oxytetracycline (79.1%) and amikacin (71.6%). These results were coincided with findings of Gomis *et al*. (2000) who demonstrated that resistance of *E. coli* to erythromycin, neomycin, doxycycline, streptomycin, and ampicillin were (97.1%), (89.5%), (84.7%), (83.7%), and (71.1%) respectively, Abdel-Rahman *et al*. (2023) who recorded higher resistance to tetracycline and ampicillin in *E. coli* isolated from broiler chickens in Egypt, and Ibrahim *et al*. (2019) who recorded complete resistance to ampicillin. The antimicrobial susceptibility results for *S. aureus* isolates exhibited resistances for doxycycline (73.3%), oxytetracycline (80%), colistin sulphate (66.7%), ampicillin (75%), amoxicillin (65%), streptomycin (80%), erythromycin (73.3%), trimethoprim-sulfamethoxazole (63.3%) and oxacillin (86.7%). These outcomes lined up with Amer *et al*. (2019) who found that the antibiogram profile of *Staphylococcus* spp. isolates from cellulitis showed high resistance rate to many antibiotics. The resistances results of *P. aeruginosa* were reported to doxycycline, oxytetracycline, colistin sulphate, ampicillin, amoxicillin, streptomycin, amikacin, neomycin, erythromycin, norfloxacin were (83.3%), (77.8%), (61.1%), (83.3%), (88.9%), (61.1%), (55.6%), (72.2%), (77.8%) and (66.7%) respectively. And *Proteus mirabilis* showed complete resistances (100%) to doxycycline, oxytetracycline, ampicillin, amoxicillin, streptomycin and erythromycin. The antibiotic sensitivity of some bacterial species isolated from cellulitis in broiler chickens was previously determined by Amer *et al*. (2019) who found that *E. coli* exhibited resistance to tetracycline (89.1%), ampicillin (67.4%), and erythromycin (42.9%), *Staphylococcus* species showed resistance to ampicillin (97.0%), oxacillin (76.3%) and tetracycline (40.7%), *P. aeruginosa* showed resistance to tetracycline (100%), erythromycin (88.8%) and ampicillin (77.7%) and *P. mirabilis* demonstrated resistance to erythromycin (77.7%) and ampicillin (27.7%).

Multidrug resistance (MDR) to 3 or more antimicrobial agent classes were recorded in all of the isolated *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus mirabilis* in the present study. Our results agreed to some extent with Radwan *et al*. (2018) who detected MDR *E. coli* isolates with MDR (92.1%) and Radwan *et al*. (2014) who showed that the percentage of MDRI for *E. coli* isolates was 90.4% along with increasing occurrences of antibiotic-resistant *E. coli* strains isolated from chickens with cellulitis. Also, Sharada *et al*. (2001) found that no single antimicrobial medication was 100% effective against isolates of *E. coli*, which may be the result of antibiotic resistance developing as a result of overuse. Marouf *et al*. (2023) recorded MDR in *P. aeruginosa* isolated from avian species. In case of *P. mirabilis* previous research work of Magiorakos *et al*. (2012) recorded a high frequency of MDR (78.13%) among *P. mirabilis* isolates. According to our research, the high percentages of resistance found in antibiotics are likely the result of miss use of antibiotics. These findings corroborated those made by Blanco *et al*. (1997), who linked the development of antibiotics resistance to frequent use of drugs at suboptimal concentrations in veterinary practices. It is strongly advised that these antimicrobial agents be used cautiously in veterinary medicine as they have the potential to cause cross-resistance with human enteric pathogens.

In this study, the detection of virulence genes; *iut*A in *E. coli*, *nuc* in *S. aureus*, 16S rDNA and *tox*A in *P. aeruginosa* and *rsb*A in *Proteus mirabilis* were recorded in all of the examined isolates with percentage of (100%). These results align with earlier research by Silva *et al*. (2021) showing that 97.6% of *E. coli* recovered from cellulitis lesions had the *iut*A gene; AL-Dulaimy *et al*. (2023) finding that 100% of *P. mirabilis* isolates had the *rsb*A gene; Abou-Khadra *et al*. (2021) demonstrating that the *nuc* gene was present in all examined *S. aureus* isolates; and Abou-Khadra *et al*. (2019) indicating that 100% of *P. aeruginosa* isolates had the *tox*A gene.

The detection of antimicrobial resistance genes in the current study showed that bla_{TEM}, *tet*A (A), and *qnr*A genes were detected in *E. coli* isolates with percentages of (5/5, 100%), (4/5, 80%) and (5/5, 100%) respectively. In *S. aureus* isolates, *tet*K, *mec*A and *aac(6')aph (2")* were reported with percentages of (5/5, 100%), (5/5, 100%) and (4/5, 80%) respectively. In *P. aeruginosa* bla_{TEM}, *tet*A(A) and *ere*A genes were detected with percentages of (5/5, 100%), (4/5, 80%) and (4/5, 80%) respectively. bla_{TEM}, *tet*A(A) and *aada1* genes were reported in all of the examined *P. mirabilis* with percentage of (5/5, 100%) for each of them. In a previous study conducted by Alfifi *et al*. (2022) *tet*(A) and bla_{TEM-1B} genes were also detected in *E. coli* isolated from cellulitis. Abdel-Rahman *et al*. (2023) reported that *E. coli* isolated from chickens harbored bla_{TEM} in (93%) of isolates and *qnr*A in 2 isolates only. *tet*A gene was detected in *E. coli* isolates with (91.18%) (Ibrahim *et al*., 2019), with (100%) (EL-Azzouny *et al*., 2020) and with (45.4%) (Lemlem *et al*., 2023). Earlier studies of Abou-Khadra *et al*. (2019) and Marouf *et al*. (2023) found that bla_{TEM} gene was the most detected gene in *P. aeruginosa* with (100%). Interestingly, we detected *mec*A

gene in all examined *S. aureus* isolates which is in accordance with the previous study of Abou-Khadra *et al*. (2021) who found that all examined *S. aureus* isolates harbored *mec*A and considered as Methicillin-resistant *S. aureus* (MRSA).

Quaternary ammonium-based disinfectants are a great antimicrobial agent because of their strong biocide activity, extended shelf life, and environmental friendliness (Massicote, 2009). The in vitro antibacterial activity of TH4® (quaternary ammonium compounds and glutaraldehyde) showed susceptibility of the examined isolates to different concentration particularly in case of 2% concentration. These findings disagreed with mentioned previously by Alexander *et al*. (1991) who found that highly antibiotic-resistant organisms are generally more disinfectant-resistant. The obtained results illustrated the better antibacterial effect of the TH4® containing quaternary ammonium compounds and glutaraldehyde on bacterial causing cellulitis in chicken (Table, 5). Our findings were supported to that previously mentioned by Ohta *et al*. (2008); Hegstad *et al*. (2010); Ahmed *et al*. (2017) and Frolov *et al*. (2022). Also, Chen *et al*. (2021) stated that to create a perfect disinfectant for chicken farms quaternate ammonium salts were mixed with two other disinfectants (glutaraldehyde and chlorhexidine acetate). The susceptibility of MDR pathogens tested against TH4® that is shown here is beneficial for the effective application of the disinfection procedure in the infection control program.

Conclusion

Cellulitis is a serious problem facing the poultry sector. Some bacterial species such as *E. coli*, *S. aureus*, *P. aeruginosa* and *Proteus* miarbilis were isolated from the cellulitis lesions. *E. coli* is the most prevalent bacterial agent causing cellulitis particularly sero- groups O78 and O91. All isolated bacterial species were multidrug resistant (MDR). TH4® containing quaternary ammonium compounds and glutaraldehyde showed a better antibacterial activity on the isolated bacteria particularly in a concentration of 2%. This study recommends avoiding the misuses of the antimicrobial agents and conducting sensitivity tests before the treatment of the diseased broiler chickens. In addition to the application of biosecurity measures and effective disinfectant product such as TH4® containing quaternary ammonium compounds and glutaraldehyde.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Abd El-Ghany, W.A., 2023. Avian cellulitis: a skin affection associated with economic losses in broiler chickens. J. Anim. Feed Res. 13, 34-38.

Abdel-Rahman, M.A.A., Hamed, E.A., Abdelaty, M.F., Sorour, H.K., Badr, H., Hassan, W.M., Shalaby, A.G., Halem, A.A.E., Soliman, M.A., Roshdy, H., 2023. Distribution pattern of antibiotic resistance genes in *Escherichia coli* isolated from colibacillosis cases in broiler farms of Egypt. Vet. World 16, 1–11.

Abou-Khadra, S.H., El-Amin, A.M., Al-Otaibi, S., Fahmy, H.A., 2021. The antibacterial and antibiofilm activities of silver nanoparticles on staphylococci isolates from cow milk. Slov. Vet. Res. 58]

Abou-Khadra, S.H., El-Azzouny, M.M., Salah-Eldein, W.M., Ahmed, W.F., 2019. Phenotypic and genotypic detection of antibiotic resistant *Pseudomonas aeruginosa* isolated from some meat products. Anim. Health Res. J. 7, 830-841.

Ahmed, N., Ismail, H., Mohammed, A., 2017. Laboratory Evaluation of Some Disinfectants Used in Poultry Farms Against Some Bacterial Isolates. J. Appl. Sci. Res. 5, 32-41.

AL-Dulaimy, I.M., Saleem, A.J., Al-Taai, H.R.R., 2023. Detection of flaA, fliC, mrpA and *rsb*A Gene in *Proteus mirabilis* Multidrug Resistance Isolated from Different Clinical Sources in Baquba City. Egypt. J. Hospital Med. 90, 2831-2838.

Alexander, D.M., Jeawon, R.H., Persad, S., 1991. Disinfection resistance in antibiotic resistant organisms. SA. J. Sci. 87, 614–617.

Alfifi, A., Christensen, J.P., Hounmanou, Y.M.G., Sandberg, M., Dalsgaard, A., 2022. Characterization of *Escherichia coli* and other bacteria isolated from condemned broilers at a Danish abattoir. Front. Microbiol. 13, 1020586.

Amer, M.M., Mekky, H.M., Fedawy, H.S., Elbayoumi, K.M., Sedeek, D.M., 2019. Antibiotic Profile of Bacterial Species Isolated from Broiler Chickens with Cellulitis. World Vet J. 9, 268-279.

Amer, M.M., Mekky, H.M., Fedawy, H.S. Amer, A.M. and Elbayoumi, K.M., 2020. Cellulitis in Broiler Chickens. Kor. J. Food Health Converg. 6, 1-10.

Blanco, J.E., Blanco, M., Mora, A., Blanco, J., 1997. Prevalence of Bacterial Resistance to Quinolones and Other Antimicrobials among Avian *Escherichia coli* Strains Isolated from Septicemic and Healthy Chickens in Spain. J. Clin. Microbiol. 35, 2184-2185.

Chen, N., Qin, P., Liu, Y., Yang, Y., Wen, H., Jia, L., Li, J., Zhu, Z., 2021. Influence of New Compound Disinfectant From N-Dodecyl-2-(Piridin-1-Ium) Acetamide Chloride on Pathogenic Microorganisms in Poultry Houses. Front. Microbiol. 12, 735859.

Clinical and Laboratory Standards Institute (CLSI), 2020. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

Colom, K., Pèrez, J., Alonso, R., Fernández-Aranguiz, A., Lariño, E., Cisterna, R., 2003. Simple and reliable multiplex PCR assay for detection of bla_{TEM}, blaSHV and bla_{OXA-1} genes in Enterobacteriaceae. FEMS Microbiol. Lett. 223, 147-151.

Derakhshanfar, A., Ghanbarpou, R. 2002. A study on avian cellulitis in broiler chickens. Veterinarski Arhiv. 72, 277-284.

Durairaj, S., Srinivasan, S., Lakshmanaperumalsamy, P., 2009. In vitro Antibacterial Activity and Stability of Garlic Extract at Different pH and Temperature. Electr. J. Biol. 5, 5-10.

Duran, N., Ozer, B., Duran, G.G., Onlen, Y., Demir, C., 2012. Antibiotic resistance genes & susceptibility patterns in staphylococci. Ind. J. Med. Res. 135, 389-396.

El-Azzouny, M., Elhady, M., Abou-Khadra, H.S., 2020. Factors Affecting Calf Enteritis Infection Caused By *Salmonellae* And *Escherichia coli*. Assiut Vet. Med. J. 66, 21-43]

Fard, B., Karimi, M.H., Fathi, V., Behmanesh, R.E., 2007. Bacteriologic survey on infectious cellulitis in broiler chickens in Masjid Soleiman slaughterhouse, Iran. Arch. Razi Instit. 62, 91-95.

Frolov, N., Detusheva, E., Fursova, N., Ostashevskaya, I., Vereshchagin, A., 2022. Microbiological Evaluation of Novel Bis-Quaternary Ammonium Compounds: Clinical Strains, Biofilms, and Resistance Study. Pharmaceuticals 15, 514.

Gao, J., Ferreri, M., Liu, X.Q., Chen, L.B., Su, J.L., Han, B., 2011. Development of multiplex polymerase chain reaction assay for rapid detection of *Staphylococcus aureus* and selected antibiotic resistance genes in bovine mastitic milk samples. J. Vet. Diag. Investg. 23, 894–901.

Gomis, S.M., Gomis, A.I.U., Horadagoda, N.U., Wijewardene, T.G., Allan, B.J. and Potter, A.A., 2000. Studies on cellulitis and other disease syndromes caused by *Escherichia coli* in broilers in Sri Lanka. Trop. Anim. Health and Prod. 32, 341-351.

Hamilton, W.A., 1968. The mechanism of the bacteriostatic action of tetrachlorosalicylanilide: a membrane-active antibacterial compound. J. Gen. Microbiol. 50, 441-458.

Hegstad, K., Langsrud, S., Lunestad, B.T., Scheie, A.A., Sunde, M., Yazdankhah, S.P., 2010. Does the Wide Use of Quaternary Ammonium Compounds Enhance the Selection and Spread of Antimicrobial Resistance and Thus Threaten Our Health?. Microb Drug Resist. 16, 91-104.

Ibrahim, W.A., Marouf, S.A., Erfan, A.M., Nasef, S.A., El Jakee, J.K., 2019. The occurrence of disinfectant and antibiotic-resistant genes in *Escherichia coli* isolated from chickens in Egypt. Vet. World 12, 141-145.

International Standard Office, 2003. Microbiology of food and animal feeding stuff-horizontal method for enumeration of coagulase positive Staphylococci, EN ISO 6888-1 (*Staphylococci* and other species).

Jeffrey, J.S., Nolan, L.K., Tonooka, K.H., Wolfe, S., Doetkott, C., 2002. Virulence Factors of *Escherichia coli* from Cellulitis or Colisepticemia Lesions in Chickens. Avian Dis. 46, 48-52.

Kasˇkova´, A., Ondrasˇovicˇova´, O., Vargova´, M., Ondrasˇovicˇ M., Venglovsky, J., 2007. Zoonoses Public Health 54, 125–130.

Kok, T., Worswich, D., Gowans, E.M., 1996. Some serological techniques for microbial and viral infections. In Practical Medical Microbiology (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A., eds.), 14th ed., Edinburgh, Churchill Livingstone, UK.

Lemlem, M., Aklilu, E., Mohammed, M., Kamaruzzaman, F., Zakaria, Z., Harun, A., Devan., S.S., 2023. Molecular detection and antimicrobial resistance profiles of Extended-Spectrum Beta-Lactamase (ESBL) producing *Escherichia coli* in broiler chicken farms in Malaysia. PLoS ONE. 18, e0285743.

MacFadden, J.F., 2000. Biochemical tests for identification of medical bacteria 3rd ED. The Williams & Wilkins Co. USA, pp. 689- 691.

Maertens, H., Demeyere, K., De Reu, K., Dewulf, J., Vanhauteghem, D., Van Coillie E., Meyer, E.. 2020. Effect of sub inhibitory exposure to quaternary ammonium compounds on the ciprofloxacin susceptibility of *Escherichia coli* strains in animal husbandry. BMC Microbiol. 20, 155.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Monnet, D.L., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18, 268–281.

Marouf, S., Li, X., Salem, H.M., Ahmed, Z.S., Nader, S.M., Shaalan, M., Awad, F. H., Zhou, H., Cheang, T., 2023. Molecular detection of multidrug-resistant *Pseudomonas aeruginosa* of different avian sources with pathogenicity testing and in vitro evaluation of antibacterial efficacy of silver nanoparticles against multidrug-resistant *P. aeruginosa*. Poult. Sci. 102, 102995.

Massicote, R., 2009. Désinfectants et désinfection en hygiène et salubrité: principes fondamentaux, Ministère de Santé et des Services.

Matar, G.M., Ramlawi, F., Hijazi, N., Khneisser, I., Abdelnoor, A.M., 2002. Transcription Levels of *Pseudomonas aeruginosa* Exotoxin A Gene and Severity of Symptoms in Patients with Otitis Externa. Curr. Microbiol. 45, 350–354.

Nemati, M., Hermans, K., Lipinska, U., Denis, O., Deplano, A., Struelens, M., Devriese, L.A., Pasmans, F., Haesebrouck, F., 2008. Antimicrobial Resistance of Old and Recent *Staphylococcus aureus* Isolates from Poultry: First Detection of Livestock Associated Methicillin-Resistant Strain ST398. Antimicrob. Agents Chemother. 52, 3817-3819.

Nguyen, M.C.P., Woerther, P., Bouvet, M., Andremont, A., Leclercq, R., Canu, A., 2009. *Escherichia coli* as reservoir for macrolide resistance genes. Emerg. Infect. Dis. 15, 10.

Ohta , Y., Kondo, Y., Kawada, K., Teranaka, T. Yoshino, N., 2008. Synthesis and antibacterial activity of quaternary ammonium salt-type antibacterial agents with a phosphate group. J. Oleo Sci. 57, 445-452.

Osimitz, T.G., Droege, W., 2021. Quaternary ammonium compounds: perspectives on benefits, hazards, and risk. Toxicol. Res. Appl. 5, 1–16.

Pathirana, H.N.K.S., De Silva, B.C.J., Wimalasena, S.H.M.P., Hossain, S., Heo, G.J., 2018. Comparison of virulence genes in *Proteus* species isolated from human and pet turtle. Iranian Journal of Veterinary Research, Shiraz University, 19-1, 48-52.

uinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J., Leonard, F.C., 2002. Veterinary Microbiology and Microbial Disease.1st ed.. Cornwall, Great Britain. Blackwell Science Ltd., pp. 43-122

Radwan, I.A., Hassan, H.S., Abd-Alwanis, S.A., Yahia, M.A., 2014. Frequency of some virulence associated genes among multidrug-resistant *Escherichia coli* isolated from septicemic broiler chicken. Int. J. Adv. Res. 2, 867-874.

Radwan, I.A., Abed, A.H., Abd Allah, M.M., Abd El-Latif, M.A.A., 2018. Bacterial pathogens associated with cellulitis in chickens. J. Vet. Med. Res. 25, 68 – 79.

Randall, L.P., Cooles, S.W., Osborn, M.K., Piddock, L.J.V., Woodward, M.J., 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of Salmonella enterica isolated from humans and animals in the UK. J. Antimicrob. Chemother. 53, 208–216.

Robicsek, A., Strahilevitz, J., Jacoby, G.A., Macielag, M., Abbanat, D., Park, C.H., Bush, K., Hooper, D.C., 2006. Fluoroquinolone modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. Nat. Med. 12, 83-88.

Saif, Y.M., Barnes, H.J., Glisson, J.R., Fadly, A.M., Dougland, L.R., Swayne, D.E., 2003. Diseases of Poultry (11th ed.). Press Iowa State, USA. pp. 562-566.

Sanches, M.S., Baptista, A.A.S., de Souza, M., Menck-Costa, M.F., Justino, L., Nishio, E.K., Oba, A., Bracarense, A.P., Rocha, S.P.D., 2020. *Proteus mirabilis* causing cellulitis in broiler chickens. Braz. J. Microbiol. 51, 1353–1362.

Santos, M.M., Alcântara, A.C.M., Perecmanis, S., Campos, A., Santana A.P., 2014. Antimicrobial Resistance of Bacterial Strains Isolated from Avian Cellulitis. Braz. J. Poult. Sci. 16, 13-18.

Sharada, R., Krishnappa, G., Upendra, H.A., 2001. Serological (O) grouping and drug susceptibility of *Escherichia coli* strains from chicken. Ind. J. Vet. 78, 78-79.

Silva, R.M., Silva, I.M.M., Jesus, M.C., Fernandes, M.D.B., Oliveira, F.S., Evêncio-Neto, J., 2021. Co-relationship between *Escherichia coli* in broiler cellulitis and liver lesions. Braz. J. Biol. 81, 714-718.

Singer , R.S., Jeffrey, J.S., Carpenter, T.E., Cooke, C.L., Atwill, E.R., Johnson, W.O., Hirsh, D.C., 2000. Persistence of cellulitis-associated *Escherichia coli* DNA fingerprints in successive broiler chicken flocks. Vet. Microbiol. 75, 59-71.

Spilker, T., Coenye, T., Vandamme, P., LiPuma, J.J., 2004. PCR-Based Assay for Differentiation of *Pseudomonas aeruginosa* from Other Pseudomonas Species Recovered from Cystic Fibrosis Patients. J. Clin. Microbiol. 2004, 2074–2079.

Suleiman, A., Zaria, L.T., Grema, H.A., Ahmadu, P., 2013. Antimicrobial resistant coagulase positive *Staphylococcus aureus* from chickens in Maiduguri, Nigeria. Sokoto J. Vet. Sci. 11, 51-55.

Vijayakumar, G., Tamilarasan, R., Dharmendirakumar, M., 2012. Adsorption, Kinetic, Equilibrium and Thermodynamic studies on the removal of basic dye Rhodamine-B from aqueous solution by the use of natural adsorbent perlite. J. Mater. Environ. Sci. 3, 157-170.

Yaguchi, K., Ogitani, T., Osawa, R., Kawano, M., Kokumai, N., Kaneshige, T., Noro,T., Masubuchi, K., Shimizu, Y., 2007. Virulence Factors of Avian Pathogenic *Escherichia coli* Strains Isolated from Chickens with Colisepticemia in Japan. Avian Dis. 51, 656-62.

Zhang, Y., Li, Y.Y., Li, J.H., 2015. Synthesis and antibacterial characterization of waterborne polyurethanes with gemini quaternary ammonium salt. Sci. Bull. 60, 1114–21.