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Combating Multidrug-Resistant Avian pathogenic *E. coli*: Virulence Gene Profiling and the Potential of Chitosan Nanoparticles

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Abstract

AVIAN pathogenic *Escherichia coli* (APEC) is a leading cause of colibacillosis, contributing to significant economic losses in poultry production worldwide. This study aimed to investigate the prevalence of virulence and antimicrobial resistance genes in APEC isolates from different Egyptian governorates in addition evaluation the antimicrobial efficacy of chitosan nanoparticles (CNPs) as a potential alternative treatment. A total of 180 visceral organ samples were collected from broiler, layer, and breeder chickens suspected of colibacillosis; 158 (88%) yielded bacterial isolates, of which 85 (54%) were confirmed as *E. coli* biochemically, and 69 (81%) were serotyped. Antimicrobial susceptibility testing revealed high levels of antimicrobial resistance, with complete resistance to streptomycin and nalidixic acid, and notable resistance to ampicillin, tetracycline, and doxycycline. PCR detected three virulence genes (*papC*, *iss*, *iutA*) and three resistance genes (*blaCTX*-M, qnrA, and sul1), with sul1 present in all isolates. CNPs synthesized via ionic gelation showed a mean particle size of 56.6 nm and demonstrated effective antibacterial activity at a minimum inhibitory concentration of 50 mg/ml. These findings highlight the alarming prevalence of MDR APEC in Egyptian poultry and suggest that CNPs hold promise as a sustainable antimicrobial alternative.

Keywords: *E. coli*, antimicrobial resistance, virulence associated genes, PCR, Chitosan nanoparticles.

Introduction

Avian colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), is one of the most economically significant bacterial diseases affecting poultry production worldwide. It leads to high mortality rates, decreased productivity, increased condemnation of carcasses at slaughter, and substantial financial losses to the poultry industry [1, 2]. According to Guabiraba and Schouler [3], *E. coli* is a commensal bacterium of the gastrointestinal tract (GIT), as well as the pharynx and trachea of humans, animals, and birds. Avian pathogenic *Escherichia coli* (APEC) is an extra-intestinal pathogenic *Escherichia coli* (ExPEC) that can cause local or systemic infections in poultry, such as chickens, turkeys, ducks, and many other avian species [4]. APEC strains are typically associated with systemic infections, including pericarditis, perihepatitis, airsacculitis, and septicemia, particularly in broilers, layers, and breeders under conditions of stress or immunosuppression [5]. Colibacillosis necessitates predisposing conditions including *Mycoplasma gallisepticum* infections, weakened mucosal barriers, immunosuppression from stressors (viruses and vaccines), and inadequate ventilation and poor hygiene in hatcheries and chicken barns [6]**.** The pathogenicity of APEC is largely attributed to the

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presence of multiple virulence factors that facilitate adhesion, invasion, immune evasion, and nutrient acquisition. The virulence genes commonly associated with APEC include *papC* (P fimbriae assembly), *iss* (increased serum survival), and *iutA* (aerobactin receptor). The presence and expression of these genes are essential for the successful colonization and infection of host tissues, making them important targets for molecular identification and epidemiological studies [7-9]**.**

In parallel with their virulence, APEC strains are increasingly exhibiting multidrug resistance **(**MDR**)**, largely due to the misuse and overuse of antibiotics in the poultry industry. Resistance genes such as, *blaCTX-M, qnrA*, and sul1 are frequently detected in APEC isolates, conferring resistance to extended-spectrum β-lactams, quinolones, and sulfonamides, respectively [10,11]. The emergence and dissemination of such MDR strains pose a significant threat not only to animal health but also to public health, considering the zoonotic potential of APEC and the risk of gene transfer to human-pathogenic *E. coli* strains [12].

According to Ibrahim *et al* [13], the presence of resistance-encoding genes within plasmids or chromosomal genetic material causes antibiotic resistance with high complexity. These genes are found on mobile genetics elements which facilitate their quick spread among ExPEC strains. Additionally, human contamination may be caused by animal reservoirs, or the spread of commensal bacteria, including antibiotic-resistant APEC bacteria, through contaminated poultry food [14]**.** Although modern antibacterial drugs are available, bacterial infections remain a significant global health concern due to the rapid rise of multidrug-resistant strains. Nanoparticles have emerged as a promising and safe alternative to conventional antibiotics [15]**.** Therefore, there is an urgent need to explore alternative strategies to conventional antibiotics.

Among the most promising alternatives are nanotechnology-based antimicrobials, such as chitosan nanoparticles (CNPs)**.** Chitosan, a natural polysaccharide derived from chitin, has attracted increasing attention due to its biocompatibility, biodegradability, and broad-spectrum antimicrobial activity [16]**.** When converted into nanoparticulate form, chitosan exhibits enhanced interaction with bacterial membranes, leading to increased permeability, leakage of intracellular contents, and ultimately, bacterial cell death [17,18].

Therefore, the current study was designed to investigate the prevalence of virulence and antimicrobial resistance genes among APEC isolates obtained from poultry farms across different Egyptian governorates. Furthermore, it aims to evaluate the antibacterial efficacy of chitosan nanoparticles against multidrug-resistant APEC isolates, providing insights into their potential application as a novel bio-based antimicrobial agent for controlling colibacillosis in poultry.

Material and Methods

Sample collection and bacterial isolation and identification

A total of 180 recently deceased broiler, layer, and breeder chickens suspected of colibacillosis were randomly selected from various commercial poultry farms in three Egyptian governorates (Damietta, El Dakahlia, and Port Said). Visceral organs including the liver, lungs, air sacs, heart, and spleen were aseptically collected from each bird for microbiological analysis. Approximately 25 grams of tissue from each organ were pooled and pre-enriched in MacConkey broth. After incubation, 1 mL of the broth culture was streaked onto Eosin Methylene Blue (EMB) agar and MacConkey agar plates. The plates were incubated aerobically at 37°C for 24 hours. Isolated colonies were preserved in 20% (v/v) glycerol broth and stored at -70°C for subsequent analyses. A single representative colony from each sample was selected for further characterization. *Escherichia coli* isolates were initially identified based on morphological characteristics (e.g., Gram staining and motility testing) and further confirmed by standard biochemical tests, following the methodology described by [19]. For serological identification, isolates were examined using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) to determine their enteropathogenic serotypes, as outlined by [20]

Antimicrobial sensitivity testing:

The antimicrobial susceptibility of the *Escherichia coli* isolates was assessed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar**,** as recommended by the Clinical and Laboratory Standards Institute [21]. The methodology followed the standard procedures described by [22], with minor modifications. Briefly, bacterial suspensions were prepared from overnight cultures and adjusted to the 0.5 McFarland standard (equivalent to approximately 1×10^8 CFU/mL). Sterile cotton swabs were used to evenly inoculate the surface of Mueller-Hinton agar plates. After inoculation, commercially prepared antibiotic-impregnated discs (Oxoid Ltd., Basingstoke, Hampshire, UK) were placed on the agar surface, and the plates were incubated aerobically at 37°C for 18–24 hours**.** Zones of inhibition were measured in millimeters and interpreted as susceptible, intermediate, or resistant according to CLSI**.** Each isolate was tested against a panel of sixteen antibiotics representing various antimicrobial classes, including β-lactams, aminoglycosides, tetracyclines, fluoroquinolones, sulfonamides, and polymyxins. The antibiotics used and their respective disc potencies were as follows(Amikacin (AK) (30 μg/disc), Ampicillin (AM) (10 μg/disc), Amoxicillin/clavulanic acid (AMC) (30 μg/disc), Cefotaxime (CF) (30 μg/disc), Ceftazidime (Z) (10 μg/disc), Colistin (25 μg/disc), Doxycycline (DO) (30 μg/disc), Florfenicol (F) (30 μg/disc), Kanamycin (30 μg/disc), Gentamicin (10 μg/disc), Nalidixic acid (NA) (30 μg/disc), Streptomycin (S) (10 μg/disc), Sulfamethoxazole (SXT) (25 μg/disc), and Tetracycline(T)(30μg/disc).

Molecular identification of virulence genes and antimicrobial genes by PCR

Rapid DNA extraction kits were used to extract bacterial DNA in accordance with the QIAamp DNA Mini Kit manufacturer's procedure (Catalogue No. 51304). The genotyping was determined by PCR. *E. coli* isolates were evaluated through PCR targeting three genes of virulence genes as Adhesins (*papC*) and iron acquisition/uptake systems (*Iss* and *iutA*) and three genes of antimicrobial resistance genes as *(qnrA*, *blaCTX-M and Sul1*). Six pairs of primers were supplied from metabion (Germany) or Biobasic (Canada). They have specific sequence and amplify specific products as shown in (Table 1).

The PCR reactions were carried out in a total volume of 25 µL, containing 12.5 µL of 2× PCR Master Mix, 0.5 µM of each primer, and 2 µL of template DNA. The PCR cycling conditions were as follows: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 45 seconds, with a final extension at 72°C for 7 minutes. PCR products were analyzed by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualized under UV illumination.

Preparation of the Chitosan Nanopartcles (CNPs):

CNPs were synthesized by the ionotropic-gelation method depending on electrostatic interaction between positive charged amino groups of chitosan and negatively charged groups of TPP (tripolyphosphate). Chitosan solution was made at 3 g dissolved in 600 ml of acidified distilled water (DW) with 6 ml Glacial acetic acid by vigorous stirring until a transparent solution was observed. The pH of the solution was adjusted up to 4.5-4.8 by using NaOH then the solution was filtered to remove all undissolved particles. TPP was made at 200 mg/200 ml DW and added dropwise at a consistent rate using titration pipette at the rate of 2 ml/min under continuous stirring for 2 h at room temperature then the mixture was sonicated for 10 mins [23]. The solution was centrifuged at 12000 rpm at 4 ◦C/15 min twice with washing. The supernatant was discarded, and the sediment dialyzed with DW then lyophilized and well ground to be used for further characterization. Average particle size and surface charge were determined using Zetasizer (Micotrac, wave, USA).

Determination of the Minimum Inhibitory Concentration (MIC) of Chitosan Nanoparticles (CNPs)

The minimum inhibitory concentration (MIC) of chitosan nanoparticles (CNPs) against *Escherichia coli* isolates was determined using a broth microdilution assay, following the method described by [24] with minor modifications. The assay was performed in sterile 96-well microtiter plates. Bacterial suspensions of *E. coli* were prepared from overnight cultures in tryptic soy broth and adjusted to logarithmic-phase growth (10^8 CFU/mL) using a spectrophotometer at 600 nm to match the 0.5 McFarland standard. A series of two-fold serial dilutions of CNPs were prepared in sterile Mueller-Hinton broth, covering a concentration range suitable for expected antimicrobial activity. To each well of the microtiter plate, 100 µL of CNP dilution was added, followed by 100 µL of bacterial suspension, yielding a final volume of 200 µL per well. Positive control wells (containing bacteria without CNPs) and negative control wells (media only, without bacteria or CNPs) were included. The plates were then incubated aerobically at 37°C for 24 hours**.** Following incubation, 10 µL of a 2.5 mg/mL tetrazolium salt solution (e.g., MTT or INT) was added to each well and incubated for an additional 4 hours at 37°C**.** This colorimetric assay allowed for visual assessment of bacterial viability: metabolically active (viable) cells reduce the tetrazolium salt to form a blue/purple-colored formazan**,** while inhibited bacterial growth results in colorless or pale wells. The MIC was defined as the lowest concentration of CNPs that completely inhibited visible bacterial growth**,** as indicated by the absence of color change. All tests were performed in duplicate, and results were recorded independently by two observers to ensure accuracy. For quality control, an *E. coli* ATCC 25922 strain was included as a reference organism.

Results

Isolation and phenotypic characterization of isolates:

Out of 180 collected samples, 158 (88%) were positive for *E. coli* using standard culture techniques. Initial identification of isolates was based on microscopic morphology and motility testing. Gram staining revealed medium-sized, Gram-negative, uniformly pigmented coccobacilli, consistent with the morphological characteristics of *Escherichia coli* [25]. Motility was confirmed by the presence of an oval-shaped diffused growth pattern extending from the stab line, indicating active flagellar movement. For preliminary culture-based differentiation, isolates were grown on MacConkey agar, where they produced characteristic pink to red colonies, indicative of lactose fermentation (Fig. 1.A). When sub-cultured on Eosin Methylene Blue (EMB) agar, colonies exhibited the typical metallic green sheen, a hallmark trait of strong lactose-fermenting *E. coli* strains (Fig. 1.B).

Further biochemical identification was performed using the [19] system. Among the 158 presumptive isolates, 85 samples (54%) exhibited biochemical profiles consistent with *E. coli*. The isolates tested positive for indole production, methyl red reaction, nitrate reduction, and ONPG (ortho-nitrophenyl-β-galactoside) activity, confirming their β-galactosidase enzyme activity. Conversely, the isolates were negative for the Voges-Proskauer test, citrate utilization, urease activity, H_2S production, and gelatin liquefaction, which supports differentiation from other Enterobacteriaceae. Taken together, the cultural appearance, Gram stain morphology, motility, and biochemical characteristics confirmed the identity of the isolates as *Escherichia coli*.

Serological identification:

Out of the 85 biochemically confirmed *E. coli* isolates, 69 samples (81%) were successfully identified through serological typing. Among these, 12 isolates were classified as O78 (enterotoxigenic *E. coli* - ETEC**)**, while 10 isolates belonged to O91:H21 (enterohemorrhagic *E. coli* **-** EHEC)**.** Seven isolates were identified as O128:H2 (ETEC)**,** and six isolates as O1:H7 (enteropathogenic *E. coli* **-** EPEC). Additionally, five isolates were typed as O26:H11 (EHEC)**,** O2:H6 (EPEC)**,** and O146:H21 (EPEC)**,** respectively. Other identified serotypes included O55:H7 (EHEC) with four isolates, O121:H7 (EPEC) with three isolates, and O159 (Enteroinvasive *E. coli* **-** EIEC) with two isolates. A further two isolates each were classified as O119:H6 (EPEC)**,** O17:H18 (EPEC)**,** O153:H2 (EPEC), and O127:H6 (EPEC)**.** Finally, single isolates were identified as O124 (EIEC) and O103:H2 (EHEC) as shown in (Fig. 2)**.**

These findings reflect the diversity of *E. coli* pathotypes present among the isolated strains, with notable representation from ETEC, EHEC, EPEC, and EIEC serogroups.

Antimicrobial sensitivity Profile of E. coli Isolates

The antimicrobial susceptibility of the 69 serotyped *E. coli* isolates was assessed using the disc diffusion method. The isolates demonstrated a wide range of resistance patterns across the tested antimicrobial agents. Complete resistance (100%) was observed against streptomycin, indicating its ineffectiveness against all tested isolates. High levels of resistance were also recorded for nalidixic acid (92.7%), ampicillin (76.8%), and tetracycline (65.2%). A similarly elevated resistance rate was observed for doxycycline (60.9%). Moderate resistance was detected against kanamycin (47.8%), ceftazidime (36.2%), amoxicillin-clavulanic acid (30.4%), and neomycin (29.0%). Resistance to sulphamethoxazole-trimethoprim (SXT) was 26.1%, while cefotaxime showed a relatively lower resistance rate of 20.3%. In contrast, low resistance levels were observed for florfenicol (13.0%), colistin (8.7%), gentamicin (5.8%), and amikacin (4.4%). The highest susceptibility was recorded for meropenem, with 97.1% of isolates sensitive to the antibiotic and only 1.5% showing resistance. Overall, the isolates exhibited multidrug resistance, particularly against several first-line and commonly used antibiotics, while maintaining high susceptibility to meropenem, amikacin, and gentamicin—suggesting these as potentially effective treatment options against the resistant strains as shown in (Table 2 and Fig. 3). The average number of Multidrug resistance (MDR) in our study was (0.386).

Molecular identification of virulence genes and antimicrobial genes by PCR:

All 69 *E. coli* isolates tested positive by PCR for the presence of three virulence-associated genes: ***papC*** (adhesin), **iss**, and ***iutA*** (iron acquisition and uptake systems) (Fig. 4). *All 69 E. coli isolates were screened by PCR for the presence of three antibiotic resistance genes: (BlaCTX-M) (β-lactamase encoding gene), qnrA (plasmid-mediated quinolone resistance gene), and sul1 (sulfonamide resistance gene) (Fig. 5). Out of the tested isolates, 24 samples (35%) were positive for all three resistance genes.*

Use of Chitosan Nanoparticles (CNPs) as a Potential Antimicrobial Agent against E. coli

Chitosan nanoparticles (CNPs) were evaluated for their antimicrobial activity against *Escherichia coli*. The synthesized nanoparticles had an average particle size of 56.6 nm**,** a polydispersity index (PDI) of 0.4**,** indicating moderate size uniformity, and a positive surface charge (zeta potential) of +32.3 mV, which enhances interaction with the negatively charged bacterial cell membranes. The minimum concentration of CNPs that exhibited a measurable antibacterial effect against *E. coli* was 50 mg/mL**,** demonstrating their potential as an effective nano-based antimicrobial agent.

Discussion

The increasing prevalence of multidrug-resistant (MDR) *Escherichia coli* in poultry poses a critical concern for both animal health and public safety, particularly due to the zoonotic potential of avian pathogenic *E. coli* (APEC) strains [12]**.** In the present study, *E. coli* was isolated from 88% of colibacillosis-suspected birds, which is consistent with previous reports from Egypt [26-28]. Such high prevalence suggests poor biosecurity measures and excessive antibiotic use in poultry farms. Variations in isolation rates across studies [29,30] may be attributed to differences in sampling strategies, regional management practices, and laboratory methodologies. Serotyping revealed dominance of O78, O91, O128, O1, and O2 strains, reflecting the global distribution of highly virulent APEC serotypes [31-33]. Notably, the detection of multiple pathotypes including ETEC, EHEC, EPEC, and EIEC underscores the genetic diversity and adaptability of APEC strains. This observation is supported by studies demonstrating the emergence of novel serotypes across different Egyptian regions [34,35].

Because of the wide use of antibiotics in the food animal sector, little attention has been paid to how antibiotic use in farm animals contributes to the problem of antibiotic resistance, especially in developing countries [36,37]**.** The antimicrobial susceptibility profiles of isolates were alarming. All isolates were resistant to streptomycin, with high resistance rates also reported for nalidixic acid (92.7%), ampicillin (76.8%), tetracycline (65.2%), and doxycycline (60.9%). These findings reflect the widespread misuse of antibiotics in poultry and confirm similar trends observed in local and international studies [38-41]. Although low resistance was noted for meropenem and amikacin, the potential transmission of resistance genes from animal to human strains through food remains a major concern [42,43].

All 69 isolates harbored the virulence genes *papC*, *iss*, and *iutA*, essential for colonization, iron acquisition, and immune evasion [7,8]. Their universal presence confirms the pathogenic potential of the isolates and aligns with previous Egyptian reports [43,44]. The co-occurrence of virulence and resistance genes within the same strains emphasizes the selective advantage and increased fitness of MDR APEC, which are often propagated through horizontal gene transfer [13,45]**.**

The high prevalence of resistance genes *sul1* (100%), *qnrA* (74%), and *blaCTX-M* (48%) confirms the presence of mobile resistance determinants conferring sulfonamide, quinolone, and β-lactam resistance. These findings are consistent with reports from [46-48], and they illustrate the increasing dissemination of extended-spectrum β-lactamase (ESBL) genes, especially *blaCTX-M*, among *E. coli* strains [49,50]**.**

Although bacterial virulence and antibiotic resistance have been thoroughly investigated separately, little is known about their interaction. Our findings demonstrated the strong correlation between antibiotic resistance and virulence gene presence in resistant strains; MDR E. coli serovars have at least two virulence and antibiotic resistance genes. Therefore, in order to overcome the MDR problem, the world has recently resorted to using strict hygienic measures in conjunction with the use of safe antibiotic alternatives. These alternatives include the use of natural, safe products like hyperimmune serum [51], herbal extracts, organic acids, essential oils, prebiotics, probiotics, symbiotics, postbiotics, and nano-preparations to improve the avian gut microbiome in order to compete with pathogens and ultimately improve birds' [52].

Given the limitations of conventional antibiotic therapies, this study evaluated chitosan nanoparticles (CNPs) as a natural antimicrobial alternative. The synthesized CNPs exhibited favorable physicochemical properties small particle size (~56.6 nm), moderate polydispersity, and high positive zeta potential conducive to strong interactions with bacterial membranes [17,53]. The observed MIC of 50 mg/mL aligns with prior studies demonstrating the antimicrobial efficacy of CNPs against Gram-negative pathogens [23,54].

Chitosan's antimicrobial activity involves multiple mechanisms, including disruption of bacterial membranes, chelation of essential metals, and interference with nutrient uptake and DNA replication [55]. Its biodegradability, safety profile, and ability to combat resistant strains make it a viable candidate for incorporation into poultry disease management strategies, particularly amid growing restrictions on antibiotic usage.

Conclusion

The widespread distribution of MDR APEC harbouring both virulence and resistance genes in Egyptian poultry highlights an urgent need for stricter antibiotic usage, enhanced biosecurity, and adoption of safe antimicrobial alternatives. CNPs hold significant potential as a novel bio-based therapeutic, which need further in vivo investigation and integration into national poultry health programs.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

The study was following the Institutional Animal Care and Use Committee at the Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. The ethics approval number (Vet CU 290720251233).

TABLE 1. Oligonucleotide primers sequences of virulence associated genes and antimicrobial resistance genes.

Gene	Primer sequence (5'-3')	Length of amplified product	Reference
papC	TGATATCACGCAGTCAGTAGC CCGGCCATATTCACATAA	501 bp	[56]
Iss	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATACCC	266 bp	[57]
iutA	GGCTGGACATGGGAACTGG CGTCGGGAACGGGTAGAATCG	300 bp	
qnrA	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516 bp	[58]
blaCTX-M	ATGTGCAGYACCAGTAARGTKATGGC TGGGTRAARTARGTSACCAGAAYCAGCGG	593 bp	[59]
sul1	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	433 bp	[60]

TABLE 2. Antimicrobial resistance profile of avian pathogenic *E. coli* isolates.

Antimicrobial agent	S		I		R	
	NO	%	NO	%	NO	%
Streptomycin (S)	-	-	-	-	69	100
Nalidixic acid (NA)	2	2.9	3	4.4	64	92.7
Ampicillin (AM)	11	15.9	5	7.3	53	76.8
Tetracycline (T)	19	27.5	5	7.3	45	65.2
Doxycycline (DO)	25	36.2	2	2.9	42	60.9
Kanamycin (K)	32	46.4	4	5.8	33	47.8
Ceftazidime (Z)	43	62.3	1	1.5	25	36.2
Amoxycillin-Clavulanic acid (AMC)	45	65.2	3	4.4	21	30.4
Neomycin (N)	47	68.1	2	2.9	20	29.0
Sulphamethoxazol (SXT)	47	68.1	4	5.8	18	26.1
Cefotaxime (CF)	52	75.4	3	4.4	14	20.3
Florfenicol (F)	56	81.2	4	5.8	9	13.0
Colistin (CO)	61	88.4	2	2.9	6	8.7
Gentamicin (G)	65	94.2	-	-	4	5.8
Amikacin (AK)	65	94.2	1	1.5	3	4.4
Meropenem (M)	67	97.1	1	1.5	1	1.5

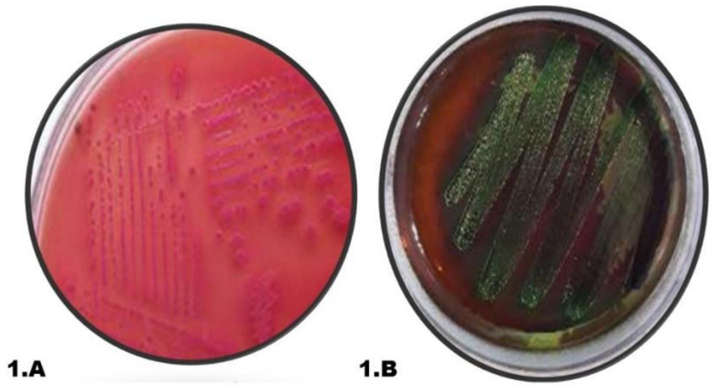


Fig.1. A: show colony of *E.coli* on MacConky agar that typically appear as bright pink or red, smooth, and circular B: show colony of *E.coli* on (EMB) that typically appear dark, often blue-black, colonies with a metallic green sheen.

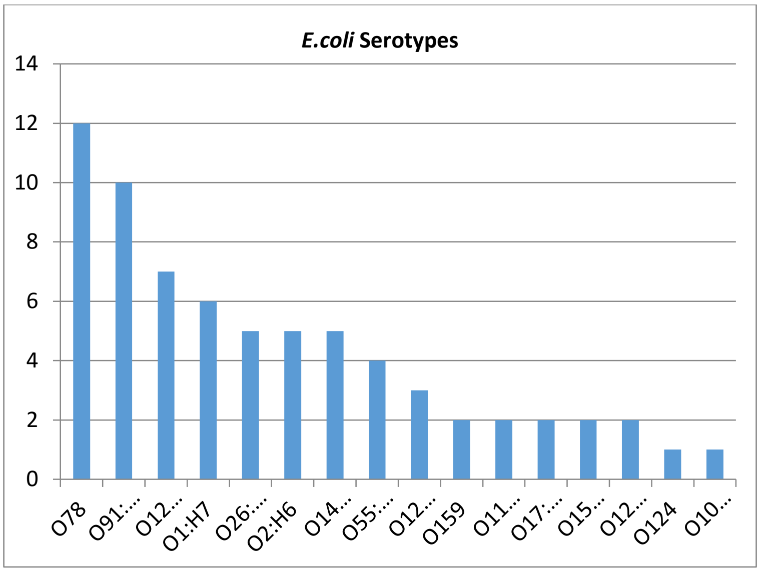


Fig. 2. *E.coli* isolates serotypes identification according to (MacFaddin, 2000)

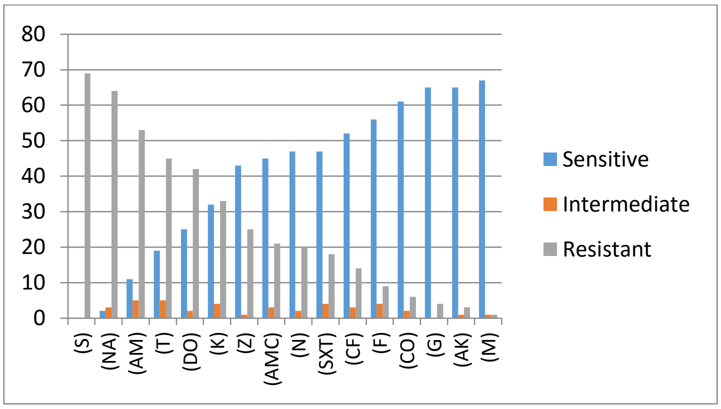


Fig. 3. Sensitivity and resistance to different antimicrobial agents.

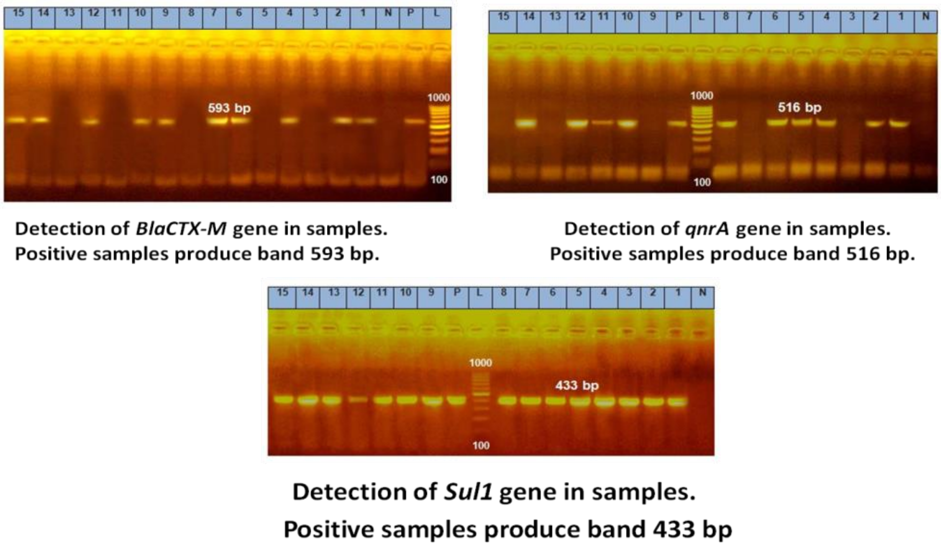
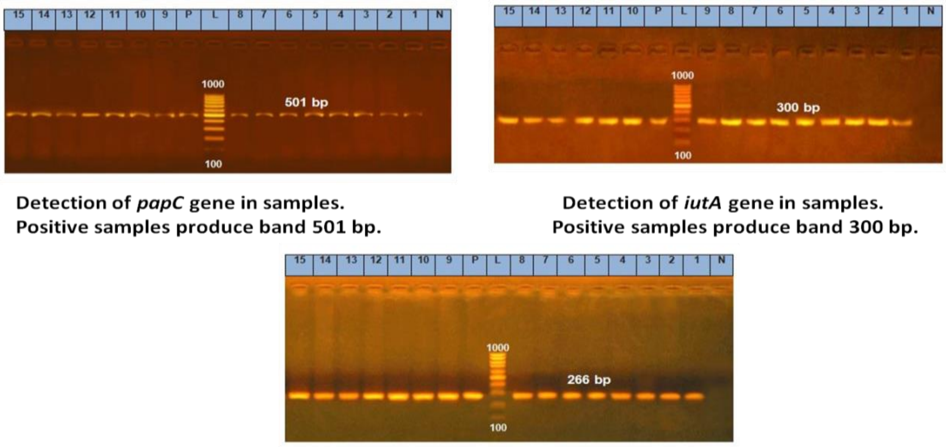


Fig. 4. PCR bands for (*BlaCTX-M*, *qnrA* and *Sul1* genes) Antimicrobial resistance genes in avian pathogenic *E.coli*



Detection of *papC* gene in samples.
Positive samples produce band 501 bp.

Detection of *iutA* gene in samples.
Positive samples produce band 300 bp.

Detection of *iss* gene in samples.
Positive samples produce band 266 bp

Fig. 5. PCR bands for (*papC,iutA and iss* genes) virulence associated genes in avian pathogenic *E.coli*.

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مكافحة الاشيرشية القولونية المسببة للأمراض فى الطيور و المقاومة للأدوية المتعددة و تحديد جينات الضراوة و تأثير جزيئات الكيتوزان النانونية عليها

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الملخص

الإيشريشية القولونية الممرضة للطيور (APEC) تُعد من الأسباب الرئيسية للإصابة بالقولون البكتيري، مما يؤدي إلى خسائر اقتصادية كبيرة في إنتاج الدواجن على مستوى العالم. هدفت هذه الدراسة إلى التحقيق في مدى انتشار جينات الضراوة ومقاومة المضادات الحيوية في عزلات APEC من محافظات مصرية مختلفة، بالإضافة إلى تقييم فعالية جسيمات الكيتوزان النانوية (CNPs) كمُعالج بديل محتمل. تم جمع 180 عينة من الأعضاء الحشوية من دجاج التسمين والبياض والأمهات المشتبه بإصابتها بالقولون البكتيري؛ حيث أظهرت 158 عينة (88%) نموًا بكتيريًا، وتم تأكيد 85 عزلة (54%) على أنها E. coli بواسطة الاختبارات البيوكيميائية، وتم تصنيف 69 عزلة منها (81%) مصليًا. أظهر اختبار الحساسية للمضادات الحيوية مستويات عالية من المقاومة، حيث وُجدت مقاومة كاملة للستربتوميسين وحمض الناليديكسك، بالإضافة إلى مقاومة ملحوظة للأمبيسيلين والتتراسيكلين والدوكسيسيكلين. تم الكشف عن ثلاث جينات للضراوة(papC) ، iss، (iutAوثلاث جينات للمقاومة(blaCTX-M ، qnrA، و sul1)باستخدام تقنيةPCR ، وكانت جينة sul1 موجودة في جميع العزلات. تم تصنيع جسيمات الكيتوزان النانوية باستخدام تقنية التبلور الأيوني، بمتوسط حجم جسيم بلغ 56.6 نانومتر، وأظهرت نشاطًا مضادًا فعالًا ضد البكتيريا عند تركيز مثبط أدنى بلغ 50 ملغم/مل. تُبرز هذه النتائج الانتشار المقلق لـ APEC متعددة المقاومة في الدواجن المصرية، وتشير إلى أن جسيمات الكيتوزان النانوية تُعد بديلًا واعدًا ومستدامًا للمضادات الحيوية.

الكلمات الدالة: الايشيريشية القولونية، مقاومة مضادات الميكروبات، جينات الضراوة، تفاعل البلمرة المتسلسل، جزيئات الكيتوزان النانونية.