Research Article

In Vitro Evaluation of Inhibitory Activity of Immunoglobulins Y on the Growth of a Multidrug-Resistant Strain of *Klebsiella* Pneumoniae

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Abstract

Background: Multidrug-resistant (MDR) Klebsiella pneumoniae poses a significant global health threat, causing severe infections like pneumonia and bacteremia with high mortality rates, particularly in vulnerable populations. The rise of resistance to conventional antibiotics necessitates alternative antimicrobial strategies. Immunoglobulin Y (IgY) offers a promising, non-antibiotic approach due to its targeted action, high yield, and low risk of inducing resistance, as demonstrated in prior studies against other pathogens. This study aimed to evaluate the in vitro efficacy of IgY against an MDR K. pneumoniae strain to address gaps in data for resistant isolates.

Methods: An MDR K. pneumoniae strain, confirmed via API 20E and EUCAST 2022 antibiogram, was inactivated with 70% ethanol and used to immunize laying hens intramuscularly with Freund's adjuvant over six weeks. IgY was extracted from egg yolks and purified. Specificity and cross-reactivity were assessed by indirect ELISA against K. pneumoniae, Staphylococcus aureus, and Escherichia coli. Growth inhibition was evaluated in Muller Hinton Broth at IgY concentrations of 50, 100, and 150 µg/mL, measuring viable CFU counts at 0, 2, 4, and 8 hours.

Results: The MDR strain showed resistance to seven antibiotic classes but sensitivity to chloramphenicol and norfloxacin. Extracted IgY reached peak concentrations at week 7 (210.41 ± 80.72 mg/mL), with SDS-PAGE confirming purity (~186 kDa native, 65 and 27 kDa chains). ELISA revealed high specificity for K. pneumoniae with minimal cross-reactivity. Specific IgY exhibited dose- and time-dependent bacteriostatic effects, significantly reducing CFU ($p<0.001$) at 100 and 150 µg/mL, achieving up to 92.75% bacterial load reduction after 8 hours, while non-specific IgY had no effect.

Conclusion: These results highlight IgY's potential as a safe, scalable alternative for combating MDR K. pneumoniae, offering possible biotechnology development for antibody drugs to combat this bacterium.

Keywords: Immunoglobulin Y; Klebsiella pneumoniae; Multidrug-resistant; In vitro inhibition; Bacteriostatic effect

List of Abbreviations

- AMX: Amoxicillin + clavulanic acid
- API 20E: Analytical Profile Index 20E
- BSA: Bovine serum albumin

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- CEF: Cefixime
- CFU: Colony-forming units
- CHL: Chloramphenicol
- CI: Confidence interval
- CLED: Cystine Lactose Electrolyte-Deficient (medium)
- ELISA: Enzyme-Linked Immunosorbent Assay
- ERY: Erythromycin
- ESBL: Extended-spectrum beta-lactamase
- EUCAST: European Committee on Antimicrobial Susceptibility Testing
- GEN: Gentamicin
- HCl: Hydrochloric acid
- HRP: Horseradish Peroxidase
- IgY: Immunoglobulin Y
- kDa: Kilodalton (unit of molecular weight)
- MDR: Multidrug-resistant
- MHB: Muller Hinton Broth
- NaCl: Sodium chloride
- NOR: Norfloxacin
- OFL: Ofloxacin
- PBS: Phosphate-buffered saline
- PBST: PBS + 0.05% Tween 20 (v/v)
- SDS-PAGE: Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
- TET: Tetracycline
- TMB: 3,3',5,5'-Tetramethylbenzidine
- v/v: Volume/volume
- w/v: Weight/volume
- WHO: World Health Organization

Introduction

Klebsiella pneumoniae, a gram-negative bacterium and member of the Enterobacteriaceae family, has become a leading cause of healthcare-associated infections globally, including pneumonia, urinary tract infections, and bacteremia. Its opportunistic nature makes it particularly dangerous for vulnerable populations, such as immunocompromised individuals, the elderly, and neonates, where it can lead to severe outcomes with mortality rates exceeding 50% in some MDR cases. The epidemiology of MDR *K. pneumoniae* highlights its rapid spread in clinical settings; a systematic review in South-Eastern Asia revealed a pooled MDR prevalence of 55% (95% CI: 9-96), with ESBL production at 27% (95% CI: 32-100), often associated with respiratory infections and driven by factors like antibiotic overuse and poor infection control. Molecular studies further indicate the role of plasmids carrying resistance genes in facilitating horizontal gene transfer, contributing to outbreaks and endemicity in hospitals [1,2]. The challenges posed by conventional antibiotics are profound, as MDR *K. pneumoniae* often exhibits resistance to beta-lactams, fluoroquinolones, aminoglycosides, and even last resort agents like colistin, mediated by enzymatic degradation, efflux pumps, and biofilm formation [3]. This has led to treatment failures, and the World Health Organization classified it as a priority pathogen for new antimicrobial development. In response, much research have shifted toward non antibiotic approaches, including bacteriophages, probiotics, and passive immunization strategies like IgY antibodies, which offer targeted action without disrupting the host microbiome or inducing resistance [4]. IgY, the avian equivalent of mammalian IgG, is produced in high yields (up to 100 mg per egg) from immunized hens, making it a cost-effective and ethical alternative to mammalian antibodies. Literature over the past decade demonstrates IgY versatility in combating bacterial infections through multiple mechanisms: binding to surface antigens to prevent host cell adhesion, neutralizing toxins, inhibiting biofilm formation, and promoting opsonization for phagocytic clearance [5]. A review found that IgY is effective against respiratory, skin, and gastrointestinal infections, with survival rates up to 75–100% in mouse models of resistant pathogens like *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, often via dose-dependent effects. IgY stability in acidic environments and synergy with antibiotics further enhance its appeal [6]. Regarding *K. pneumoniae*, studies are sparse but promising. In 2015 (Ref) a small sample size randomized trial tested oral IgY (0.18 mg/ml, 70 ml daily for 28 days) against ESBL-producing strains, showed bacterial elimination in fecal carriers. Other work includes IgY against mastitis-causing *K. pneumoniae*, where antibodies neutralized growth in broth assays and bovine models, with high specificity via ELISA titers up to 1:10,000 (ref). Cross-reactivity with related Enterobacteriaceae like *E. coli* is low, attributed to targeted epitopes on outer membranes. However, gaps persist: most studies use non-MDR strains, lack detailed in vitro growth inhibition data for resistant isolates, and overlook geographic variations in resistance profiles. In vitro assays are crucial for mechanistic insights before advancing to in vivo or clinical applications.

Material and Methods

Bacteria and Culture Conditions

A multidrug-resistant Klebsiella pneumoniae strain was provided by Professor Victor Kuete of the Infectious Diseases

and Antimicrobial Substances Research Unit, Department of Biochemistry, University of Dschang (Cameroon). This strain, documented by Fankam et al., (Fankam et al., 2011) as resistant to ampicillin, ceftazidime, tetracycline, and aztreonam, was cultured in Muller Hinton Broth (MHB) and used for hen immunization [7]. Staphylococcus aureus and Escherichia coli strains were provided by the Microbiology Laboratory of the University of Yaoundé I and were cultivated in nutrient broth.

Identification and Antibiotic Susceptibility Testing of K. pneumoniae

The K. pneumoniae bacterial strain was re-identified using the API 20E miniaturized test system [8]. Its multidrug resistance was confirmed by testing antibiotics including AMX, CEF, NOR, ERY, GEN, OFL, TET, and CHL via the swab method on Muller Hinton agar in 90 mm Petri dishes (see Table 1). Following incubation at 37°C, results were interpreted according to the EUCAST 2022 (European Committee on Antimicrobial Susceptibility Testing) standards, version 12.0 [9].

Table 1: List of antibiotics used with mechanisms.

Abbreviation	Full Name	Class	Mechanism of Action
AMX	Amoxicillin	Aminopenicillin (Beta-lactam)	Inhibits bacterial cell wall synthesis by binding to penicillin-binding proteins.
CEF	Cefepime	4th-generation Cephalosporin (Beta-lactam)	Broad-spectrum cell wall disruption, effective against Gram-positive and Gram-negative bacteria, including Pseudomonas.
NOR	Norfloxacin	Fluoroquinolone	DNA gyrase and topoisomerase IV, preventing bacterial DNA replication.
ERY	Erythromycin	Macrolide	Binds to the 50S ribosomal subunit, inhibiting protein synthesis.
GEN	Gentamicin	Aminoglycoside	Binds to the 30S ribosomal subunit, disrupting protein synthesis; bactericidal.
OFL	Ofloxacin	Fluoroquinolone	Like NOR, targets DNA enzymes to halt replication.
TET	Tetracycline	Tetracycline	Inhibits protein synthesis by binding to the 30S ribosomal subunit.
CHL	Chloramphenicol	Phenicol	Inhibits protein synthesis at the 50S ribosomal subunit; broad-spectrum but toxic.

Immunogen Preparation

Bacteria intended for use were inactivated with 70% ethanol and verified by culture on CLED medium and Gram staining microscopy, to ensure no viable growth post-inactivation compared to active controls according to a modified protocol of Taddese et al.,2021 [10]. Specifically, bacteria pre-cultured in liquid medium (MHB: Muller Hinton Broth) were concentrated by centrifugation and then suspended in ethanol at a concentration of 6 mg/mL and incubated at room temperature for 10-15 minutes. The pellet obtained after centrifugation at 3000 × g for 10 minutes was retained. To verify complete bacterial inactivation, the treated bacteria were re-plated on Cystine Lactose Electrolyte-Deficient (CLED) agar and incubated at 37°C for 24 hours; the absence of growth confirmed successful inactivation.

Hen Immunization

Six-month-old laying hens (Gallus gallus domesticus) were obtained from a poultry farm in Foumbot, Noun Department (West Cameroon). The immunization was done according to Jahangiri et al.,2018 protocol [11]. Inactivated bacteria were suspended in PBS, pH 7.4, to a final concentration of 8×10^9 cells/mL and then emulsified with Freund's adjuvant at 1:1 (v/v). A group of hens were immunized intramuscularly with 1 mL of antigen at 4×10^9 cells/mL. For the primary immunization, the inactivated bacterial suspension was emulsified in Complete Freund's Adjuvant, while booster injections, administered every two weeks, were performed using Incomplete Freund's Adjuvant. A total of two booster immunizations were performed.

Extraction and Purification of the Water-Soluble Egg Yolk Fraction Containing IgY

IgY proteins were extracted and purified by precipitation via salting out with sodium chloride following egg yolk delipidation, according to the method of Hodek et al.,2013 prevouisly modify by Nfombouot et Al., 2026 from egg collected two weeks after last immunization when IgY titer is high [12,13]. Briefly, egg yolks were manually separated from the egg whites, washed with distilled water, drained, and diluted with an equal volume of phosphate-buffered

saline (PBS) and stored at +4°C for subsequent extraction. Delipidation was performed as follows: one volume of the yolk solution was diluted with eight volumes of distilled water, the pH was adjusted to 5.0 using HCl 0.5 M, and the mixture was frozen at -20°C. After spontaneous thawing, the sample was centrifuged at 4000 × g for 30 minutes. The supernatant containing the protein was filtered with filter paper 0.22µm, and its volume was recorded. IgY precipitation from the resulting water-soluble filtrate was performed by adding NaCl to a final concentration of 8.8% (w/v) and adjusting the pH to 4.0 with HCl, 0.5 M. The mixture was incubated for 2 hours with constant agitation in an ice bath and then centrifuged at 3700 × g for 30 minutes at 4°C. The resulting pellet was dissolved in PBS containing 0.001% (w/v) sodium azide equivalent to one-tenth of the initial water-soluble fraction volume.

Total Protein Quantification

The Bradford method was used to determine protein concentration in the various obtained water-soluble fractions. To perform the assay, a standard curve was prepared using bovine serum albumin (BSA) as standard [14].

Analysis of protein by SDS-PAGE

The purity of the isolated IgY present in the fractions was assessed by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis at 10%. Proteins were diluted in sample buffer and incubated for 5 minutes at 95°C for denaturing conditions or at room temperature for native conditions. A volume corresponding to 50 µg of protein was loaded into each well, alongside colored protein molecular weight markers loaded into other wells. Electrophoresis was performed at a constant voltage of 200 V for 40 minutes. Finally, the gel was carefully removed from the plates and immersed in a staining solution using Coomassie blue protocol. Excess dye was removed by destaining the gel according to the same protocol.

Evaluation of inhibition activity of extracted IgY on K. pneumoniae growth

The protocol described by Neema et al.,2012 was used for this test [15]. The same K. pneumoniae strain used as the antigen for hen immunization was reactivated on CLED agar and suspended in MHB. The suspension was adjusted to a cell density of approximately 2×10^8 CFU/mL. The solutions of specific isolated IgY (day 49 post-immunization) and non-specific IgY (non-immunized control) were sterilized using a 0.22 µm sterilizing filter. Two-milliliter aliquots of the bacterial suspension were prepared, each containing the IgY solutions at different protein concentrations (50, 100, and 150 µg/mL of culture medium). A control tube containing 2 mL of bacterial suspension without any antibodies served as the negative control. These suspensions were incubated at 37°C and manually shaken every 10-15 minutes. Sample aliquots were taken at 0, 2, 4, and 8 hours of incubation. Viable bacterial counts were performed using the spread plate method. The inoculated plates were incubated at 37°C overnight. The number of colony-forming units (CFU) per plate was counted to determine the total number of CFU per mL of sample.

The number of colony-forming units per mL of culture medium is given by the formula:

CFU/mL = (Number of colonies counted × Dilution Factor) / Volume plated (mL)

Specific Activity and Cross-Reactivity of Produced IgYs

The immunoreactivity of the IgYs and their cross-reactivity with other enterobacteria were evaluated by indirect ELISA. 96-well ELISA plates were coated with 100 µL of antigen solution (inactivated bacteria at a concentration of 10^8 cells/mL in 50 mmol/L carbonate buffer 0.05M pH 9.5) and incubated overnight at 4°C. After three washes with 300 µL of PBST (PBS + 0.05% Tween 20 (v/v)), unoccupied sites were blocked with 100 µL of blocking buffer (PBS + 5% skimmed milk (w/v)) and incubated for one hour at 37°C. After a second wash cycle as previously, 100 µL of a solution containing the IgY anti-Klebsiella pneumoniae prepared at different concentrations was added to the wells and incubated at 37°C for 1 hour. The maximum IgY concentration used was 500 µg/mL. The washing procedure was repeated, and then 100 µL of a solution of rabbit anti-IgY antibody conjugated to Horseradish Peroxidase (HRP) was added to the wells and incubated at 37°C for 1 hour. Following another wash step, 100 µL of TMB substrate was added to each well. After 30 minutes, the reaction was stopped with 100 µL of a 3 M sulfuric acid solution. The absorbance of each well was read at 450nm.

Results

Antibiotic Susceptibility Profile of the K. pneumoniae Strain

The study confirmed the identity of the bacterial strain as Klebsiella pneumoniae using the Api 20E gallery. Antibiogram testing was conducted on ten antibiotics from various families, revealing that the strain is multidrug-resistant and an extended-spectrum beta-lactamase (ESBL) producer. It exhibited resistance to penicillins (amoxicillin + clavulanic acid), cephalosporins (cefixime and ceftazidime), macrolides (erythromycin), monobactams (aztreonam), tetracyclines, and ofloxacin. The strain showed sensitivity to chloramphenicol and norfloxacin, with intermediate sensitivity to gentamicin (Table 2).

Analysis of extracted IgY

Total protein concentrations in water-soluble fractions from egg yolks were measured over time as shown in figure 1. For immunized hens, extractions occurred pre-immunization

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Table 2: Antibiotic susceptibility profile results for K. pneumoniae strain; zone diameters interpreted according to EUCAST 2022.

Family	Antibiotic (Disc Content)	Inhibition Diameter	Susceptibility Profile
Penicillins	Amoxicillin + Clavulanic Acid (30 µg)	15 mm	Resistant
Cephalosporins	Cefixime (5 µg)	14 mm	Resistant
	Ceftazidime (30 µg)	11 mm	Resistant
Fluoroquinolones	Norfloxacin (10 µg)	24 mm	Susceptible
	Ofloxacin (5 µg)	24 mm	Resistant
Aminoglycosides	Gentamicin (50 µg)	23 mm	Intermediate
Macrolides	Erythromycin (15 µg)	6 mm	Resistant
Tetracyclines	Tetracycline (30 µg)	19 mm	Resistant
Monobactams	Aztreonam (30 µg)	20 mm	Resistant
Phenicols	Chloramphenicol (30 µg)	27 mm	Susceptible

(days 1-2) and weekly post-immunization (days 14 to 49), yielding an average of 210.41 ± 80.72 mg/ml. Control hens averaged 214.30 ± 63.39 mg/ml over seven weeks (days 7 to 49). There was no statistical difference in protein level in immunized and non-immunized group (p=0.7970). although, protein level peaking at week 7 (day 49).

SDS-PAGE analysis under native and denaturing conditions confirmed the purity of IgY extracts from immunized and non-immunized groups. Native conditions revealed a ~186 kDa band representative of intact IgY, while denaturing conditions showed ~65 kDa (heavy chain) and ~27 kDa (light chain) bands (Figure 2). These molecular weights align with expected IgY structures, indicating successful isolation of high-purity IgY without significant contaminants, which is essential for reliable downstream functional assays

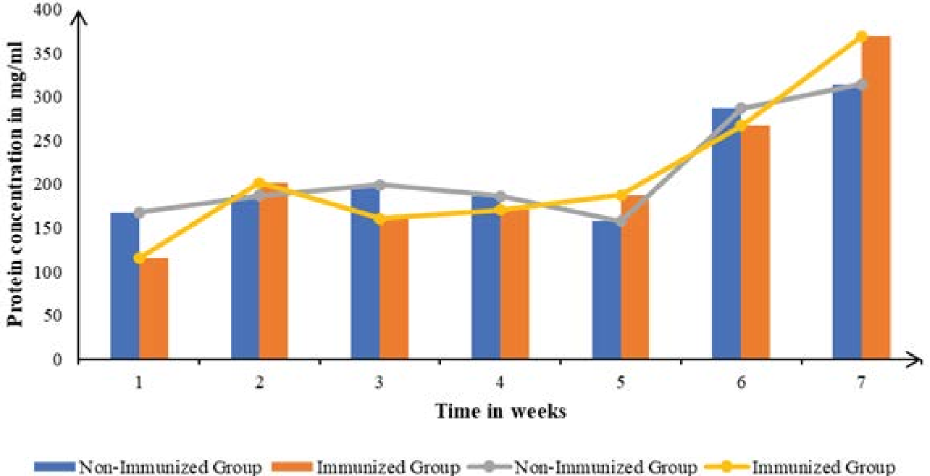

Figure 1: Total protein concentration in the water-soluble fractions of egg yolks from immunized and non-immunized hens. The highest concentration was reached 7 weeks after the first immunization.

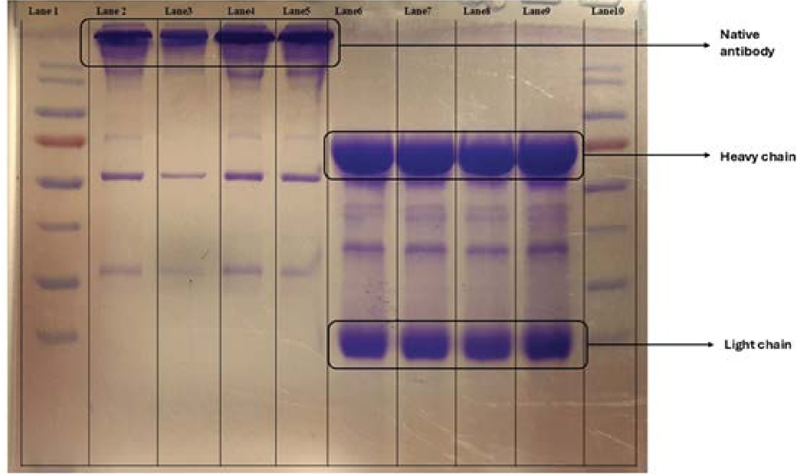

Figure 2: Representative photograph of IgY extracts on a polyacrylamide gel via SDS-PAGE. Molecular weight markers (Lanes 1 and 10), IgY extract under native conditions (Lanes 2, 3 for non-immunized group and lanes 4, 5 for immunized group). IgY extract under denaturing conditions (Lanes 6, 7 for non-immunized group and lanes 8, 9 for immunized group).

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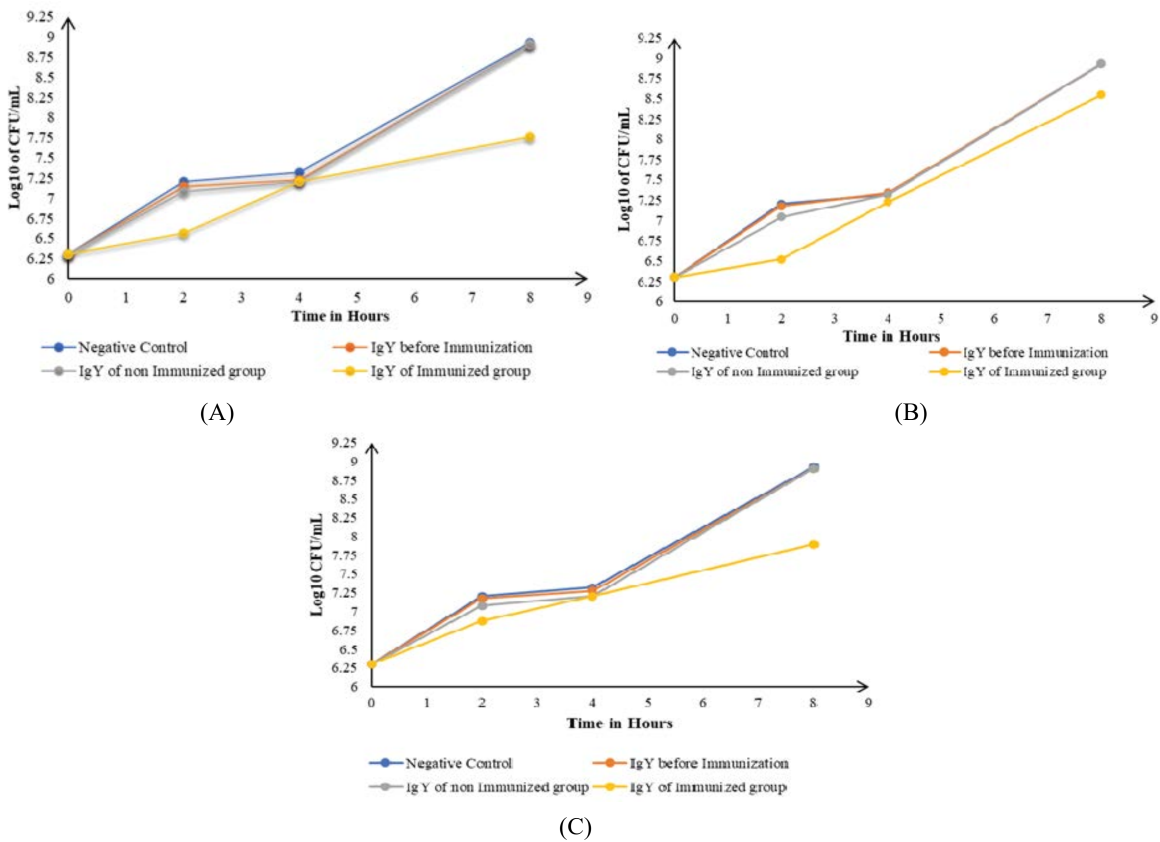

Figure 3: Immunoreactivity and immunospecificity of IgY by indirect ELISA. Optical density of Immunoglobulins Y as a function of the total protein concentration of the extract obtained at day 49 for each bacterial species used.

Specific Activity and Cross-Reactivity of IgY

The immunoreactivity and specificity of the anti-Klebsiella pneumoniae Immunoglobulins Y contained in the day 49 post-immunization egg water-soluble fractions were tested against K. pneumoniae to assess immunoreactivity and against S. aureus and E. coli to assess bacterial specificity (Figure 3). The results obtained show a high affinity of the anti-K. pneumoniae IgY for the bacterium compared to the other bacterial species, for which immunoreactivity was lower.

Inhibitory Effect of IgY

The growth inhibitory test evaluates the ability of specific IgY antibodies extracted from egg yolks of immunized hens to inhibit the growth of Klebsiella pneumoniae in a liquid medium. Viable cell counts, measured as log_{10} colony-forming units (CFU), were tracked at 2, 4, and 8 hours across concentrations of 50, 100, and 150 µg/ml. Specific IgY at 100 and 150 µg/ml led to lower CFU counts compared to controls, with significant reductions at 2 and 8 hours ($p<0.001$), indicating bacteriostatic effects where bacterial replication is slowed but not necessarily halted entirely (Figure 4-6). No IgY, including pre-immune samples from the case group and IgY from non-immunized hens, exhibited no inhibitory effects, mirroring the negative control (absence of IgY). These results demonstrate dose- and time-dependent bacteriostatic activity of specific IgY, implying that the antibodies target K. pneumoniae epitopes.

Discussion

Klebsiella pneumoniae stands as a leading cause of nosocomial infections globally, primarily due to its production of extended-spectrum beta-lactamases (ESBLs), which contribute to multidrug resistance and limited treatment options. The results from this study on a multidrug-resistant *Klebsiella pneumoniae* strain and the development of specific IgY antibodies from immunized hens provide valuable insights into alternative antimicrobial strategies, particularly in the context of rising antibiotic resistance. The antibiotic susceptibility testing, conducted using the API 20E gallery and interpreted by EUCAST 2022 guidelines, identified the strain as an ESBL producer with resistance to seven major antibiotic classes: penicillins (amoxicillin + clavulanic acid), cephalosporins (cefixime and ceftazidime), macrolides (erythromycin), monobactams (aztreonam), tetracyclines, and the fluoroquinolone ofloxacin. Sensitivity was observed to chloramphenicol and norfloxacin, with intermediate results for gentamicin. This profile exemplifies the challenges in

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treating ESBL producing *K. pneumoniae* infections, as beta-lactam resistance is mediated by enzymes that hydrolyze the antibiotic's core structure, often leading to cross-resistance across related classes. In comparison to previous studies, this resistance pattern is largely consistent with global trends; for instance, a 2024 investigation of ESBL *K. pneumoniae* from diabetic foot ulcers reported similarly high resistance rates to ampicillin (92%), amoxicillin-clavulanic acid (85%), and cephalosporins (65-77%), attributing these to frequent antibiotic exposure in chronic wounds. However, the sensitivity to chloramphenicol here is somewhat atypical, as many ESBL strains exhibit broader resistance, including to this agent, in regions with high MDR prevalence, suggesting possible geographic or strain-specific variations that warrant further genomic analysis [16]. Amid rising antimicrobial resistance, research has pivoted toward novel agents like specific IgY antibodies, as conventional antibiotics face therapeutic dead-ends. This study represents an exploration of IgY against MDR *K. pneumoniae*, as literature often evaluates IgY on strains without specified susceptibility profiles, highlighting a gap in data for resistant isolates. To generate specific IgY, laying hens were immunized whole, inactivated MDR *K. pneumoniae* using 70% alcohol, following protocols like that of Taddese et al. (2021), which achieved 100% non-viability while preserving bacterial morphology and antigens. This approach is prevalent in IgY research because inactivated whole bacteria expose numerous cell walls and outer membrane proteins, comprising thousands of antigens that elicit a broad polyclonal response. Eggs from immunized and control hens were collected weekly over seven weeks, with IgY extracted via delipidation and NaCl precipitation. Total protein concentrations did not differ for immunized hens and for controls the lack of statistical difference between groups suggested that immunization does not disrupt baseline egg protein production, a reassuring finding echoed in veterinary applications where hens maintain productivity during antibody generation [10]. SDS-PAGE analysis under native and denaturing conditions verified IgY purity, with a prominent ~186 kDa band for intact molecules and ~65 kDa (heavy chain) plus ~27 kDa (light chain) under denaturation, matching the expected avian IgY structure reported in some study like reported in studies like. This purity is essential for accurate functional testing and mirrors purification outcomes in previous work, such as a 2012 study purifying IgY against mastitis-causing K. pneumoniae via polyethylene glycol and ion-exchange chromatography, yielding similar band patterns and confirming minimal contaminants. Such consistency underscores the reliability of egg yolk as a source for scalable antibody production [15,17]. Indirect ELISA assessed IgY specificity, showing high immunoreactivity toward the inducing K. pneumoniae strain, but lower affinity for Staphylococcus aureus and Escherichia coli. This specificity is advantageous for targeted therapies, aligning with findings in studies on IgY against K. pneumoniae and P. aeruginosa for bovine mastitis, where titters reached 1:10,000 and selective neutralization occurred without affecting non-targets. Low cross-reactivity to E. coli may stem from shared antigenic determinants in Enterobacteriaceae outer membranes, or prior hen exposure to common microbes at 6 months old. Similar patterns appear in broader IgY reviews, emphasizing minimal microbiota disruption compared to antibiotics (18).

Growth inhibition assays revealed dose- and time-dependent bacteriostatic effects, with specific IgY at 100 µg/ml and 150 µg/ml reducing viable counts ($\log_{10}$ CFU) significantly ($p<0.001$) at 2 and 8 hours, achieving 92.75% and 90.25% bacterial load reductions after 8 hours compared to controls. Non-specific IgY showed no effect, mirroring negative controls. This supports in vitro neutralization seen in prior work, such as Neema et al. (2012) where IgY inhibited *K. pneumoniae* at low doses via broth assays. The mechanism remains incompletely understood but likely involves specific binding to surface components like outer membrane proteins, lipopolysaccharides, fimbriae, or flagella, impairing growth functions as hypothesized by some authors (Lee et al. (2002), Neema et al. (2012), and Leiva et al. (2020)). However, a 2015 trial using oral IgY for ESBL *K. pneumoniae* carriage in humans reported mixed efficacy, influenced by spontaneous clearance and delivery challenges. Reviews advocate encapsulation to enhance in vivo performance, positioning IgY as a safe, scalable adjunct amid antibiotic resistance debates. Overall, these outcomes underscore IgY's potential against MDR egastric stability and host immunity [15,17,19]. This study provides a valuable foundation by demonstrating the feasibility of generating anti-MDR K. pneumoniae IgY with in vitro activity. However, its primary weakness lies in the non-specific, polyclonal nature of the antibody product, which limits mechanistic understanding, specificity, and clinical potential. The logical future perspective is to leverage this foundation to discover defined protective antigens and develop monoclonal, engineered antibodies for rigorous preclinical and eventual clinical evaluation as a targeted therapeutic or prophylactic agent in the fight against antimicrobial resistance.

Conclusion

This study pioneered the production of specific IgY antibodies from hens immunized against a multidrug-resistant, *Klebsiella pneumoniae strain*, resistant to key antibiotics like penicillins, cephalosporins, and fluoroquinolones. Key results showcased high-purity IgY with peak yields at week 7, robust target-specific binding via ELISA, and potent dose-dependent bacteriostatic activity by reducing bacterial viability by up to 92.75% at 150 µg/ml after 8 hours while non-specific IgY had no effect. These outcomes illuminate IgY innovative mechanisms: precise antigen targeting that

disrupts bacterial adhesion, biofilms, and growth without fostering resistance, revolutionizing passive immunization amid the MDR crisis. By harnessing nature ingenuity, IgY emerges as a scalable, affordable, and humane beacon of hope against superbugs, empowering treatments for at risk groups in hospitals and beyond. Future studies should progress from the foundational polyclonal proof-of-concept by first identifying the specific protective bacterial antigens through techniques like immunoprecipitation and mass spectrometry, then engineering monoclonal IgY antibodies against these defined targets to ensure reproducibility and a clear mechanism of action.

Ethics Approval and Consent to Participate

This study was approved by the Comite d'Ethique et d'Assurance Qualite de l'Universite des Montagnes (Approval No.2022/192/UdM/PR/CEAQ) and adheres to the ethical standards for animal welfare. Study was done according to University of Yaoundé 1 Animal Ethics Committee recommendations.

Authors Contributions

M.R.J: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing original draft. N.N.P.H: Conceptualization, Data curation, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, writing original draft, Writing review & editing. H.T: Data curation, Formal analysis, Investigation, Software, Supervision, Visualization, Writing original draft. Y.F: Formal analysis, Supervision, Resources, Validation, Writing – review & editing. A.F.K: Formal analysis, Supervision, Resources, Validation, Writing – review & editing. S.N.F: Data curation, Formal analysis, Supervision, Writing – review & editing. F.N.N: Formal analysis, Resources, Supervision, Visualization, Writing – review & editing. P.F.M: Conceptualization, Resources, Supervision, Visualization, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

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