

# Study on the Potential of Oxidative Stress on Certain Pathogenic Characteristics of Hospital-Acquired *Klebsiella pneumoniae*

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Received: 04 January 2024/ Revised: 20 January 2024/ Accepted: 27 January 2024

## Abstract

*Klebsiella pneumoniae* is a formidable nosocomial pathogen, with hypervirulent (hvKP) and multidrug-resistant (MDR) strains posing severe clinical threats. This study investigated the impact of oxidative stress on virulence gene expression and phenotypic adaptations in *K. pneumoniae* PTCC 1792, a hospital-acquired strain. The strain was subjected to H<sub>2</sub>O<sub>2</sub> treatments (0–9600 ppm) to assess oxidative stress tolerance. Biochemical profiling, biofilm assays, and antimicrobial susceptibility testing were performed. MDR phenotype was confirmed via disk diffusion (CLSI 2023 guidelines) and PCR detection of *bla*SHV-12 and *bla*CTX-M-15. Virulence genes (*rmpA*, *wcaG*, *ycf*) were detected by PCR. Gene expression was quantified via qRT-PCR (2<sup>−ΔΔCt</sup> method), normalized to *rpoB*. The strain exhibited exceptional H<sub>2</sub>O<sub>2</sub> resistance (MIC = 2400 ppm) and increased catalase activity (4.8-fold). Biofilm formation was enhanced at 600 ppm (28%) but disrupted at 2400 ppm. *rmpA* and *wcaG* were significantly upregulated (4.2-fold and 3.8-fold, respectively) at sublethal stress, while *ycf* showed biphasic regulation (2.1-fold↑ at 600 ppm; 0.6-fold↓ at 2400 ppm). The strain harbored *bla*SHV-12 and *bla*CTX-M-15, confirming MDR phenotype. Oxidative stress triggers virulence gene expression and biofilm modulation in *K. pneumoniae* PTCC 1792, highlighting its adaptive resilience. The unique *ycf* response suggests unexplored stress-response pathways. These findings underscore the need for novel strategies targeting oxidative defense mechanisms in hvKP infections.

**Key words:** *Klebsiella pneumoniae*, oxidative stress, virulence genes, biofilm, antimicrobial, resistance, qRT-PCR

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doi: [10.71886/bioem.2024.1207961](https://doi.org/10.71886/bioem.2024.1207961)



## Introduction

*Klebsiella pneumoniae* has emerged as a critically important opportunistic pathogen and a leading cause of nosocomial infections worldwide (Macharashvili et al., 2009). This bacterium demonstrates remarkable adaptability and evolving resistance patterns that pose significant challenges to modern healthcare systems. As a member of the notorious ESKAPE group of pathogens that evade antibiotic effects, this Gram-negative bacterium is responsible for a wide spectrum of infections, including pneumonia (Abdel-Hady et al., 2008), bacteremia (Jiang & Ye, 2013), urinary tract infections (Levy, 2007), wound infections, and meningitis, particularly in immunocompromised patients and those in intensive care units (Kelly et al., 2015). The clinical significance of *K. pneumoniae* has been amplified by the alarming global spread of multidrug-resistant (MDR) strains, particularly the emergence of carbapenem-resistant and extended-spectrum  $\beta$ -lactamase (ESBL)-producing variants (Jiang et al., 2014). These variants have been classified by the World Health Organization as priority pathogens requiring urgent intervention. Of particular concern is the convergence of hypervirulence and antimicrobial resistance in certain clones, resulting in so-called "super-bug" strains that combine enhanced pathogenic potential with resistance to last-resort antibiotics (Hu et al., 2023), leading to treatment failures and increased mortality rates. The pathogenicity of *K. pneumoniae* is mediated by a diverse array of virulence factors, including capsular polysaccharides that provide antiphagocytic properties, lipopolysaccharides that elicit strong inflammatory responses, and various adhesins, such as fimbriae, that promote tissue attachment and biofilm formation. Additionally, *K. pneumoniae* possesses sophisticated iron acquisition systems that utilize multiple siderophores—such as aerobactin, enterobactin (Osei Sekyere et al., 2021; Stapleton et al., 2016), yersiniabactin, and salmochelin—to overcome host nutritional immunity. These virulence determinants are frequently encoded on mobile genetic elements that can be horizontally transferred, contributing to the

rapid evolution and diversification of pathogenic strains (Fu et al., 2021). Compounding this issue is the bacterium's remarkable genomic plasticity, which allows for the accumulation of resistance determinants while preserving its virulence potential (Zong et al., 2020). This makes a significant player in the dissemination of antimicrobial resistance genes between clinical and environmental reservoirs. Despite extensive research on its resistance mechanisms and virulence factors, the influence of oxidative stress—a critical component of host innate immune defenses—on the regulation of pathogenic characteristics in clinical *K. pneumoniae* isolates remains poorly understood (Borghesi et al., 2018).

When host immune cells, such as neutrophils and macrophages, engulf bacterial pathogens, they generate an oxidative burst that involves reactive oxygen species (Pinelli et al.), including superoxide anions ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radicals ( $OH\cdot$ ). This creates a lethal environment that most bacteria must overcome to establish an infection (Shane et al., 2017). For pathogenic bacteria like *K. pneumoniae*, the ability to sense, respond to, and neutralize oxidative stress is not merely a survival mechanism; it is also a critical virulence determinant that influences pathogenicity at multiple levels. In response to oxidative stress, *K. pneumoniae* activates sophisticated defense mechanisms, including catalases (e.g., KatG) (Osei Sekyere et al., 2021), superoxide dismutase (SODs), glutathione-dependent systems, and the *OxyR* and *SoxRS* regulons, which collectively mitigate ROS damage to essential cellular components. Furthermore, oxidative stress has been shown to directly modulate the expression of key virulence factors in *K. pneumoniae*. For instance, iron acquisition systems, such as siderophore production (Zar et al., 2022), are often upregulated under oxidative conditions, as the bacterium competes with host proteins like lactoferrin for this essential nutrient while simultaneously managing iron-catalyzed ROS generation through Fenton chemistry (Mashau et al., 2022). The oxidative stress response intersects with other critical virulence pathways, influencing

capsule production (which provides physical protection against ROS), biofilm formation (which creates a shielded microenvironment), and the expression of adhesins that mediate tissue attachment. Recent studies suggest that oxidative stress may even trigger phenotypic switching in *K. pneumoniae*, promoting the emergence of hypermucoviscosity variants or persisted cells that exhibit enhanced resistance to both immune clearance and antibiotics (Nour et al., 2017). This stress response is particularly relevant in clinical isolates, which must constantly adapt to the dynamic host environment while maintaining virulence and resisting antimicrobial therapies. The interplay between oxidative stress defense and virulence regulation creates a complex adaptive network that enables *K. pneumoniae* to thrive in hostile host environments (Liu et al., 2015; Pessoa-Silva et al., 2003). In *K. pneumoniae*, several genes play crucial roles in virulence, biofilm formation, and pathogenicity, including *rmpA*, *wcaG*, and *ycf*. The *rmpA* (regulator of mucoid phenotype A) gene is a well-known virulence factor associated with hypermucoviscosity, a key phenotype in hypervirulent *K. pneumoniae* strains. This gene enhances capsule production, which protects the bacterium from phagocytosis and host immune responses, thereby increasing its ability to cause severe infections such as liver abscesses, pneumonia, and sepsis. The *wcaG* gene is involved in the biosynthesis of the extracellular polysaccharide colanic acid, a component of the bacterial capsule and biofilm matrix. This gene contributes to biofilm formation, enhancing bacterial adherence to surfaces, resistance to antibiotics, and persistence in hostile environments, including medical devices and host tissues. The *ycf* gene, although less characterized, is believed to play a role in stress response and environmental adaptation, potentially aiding bacterial survival under oxidative stress or nutrient limitation. Understanding these mechanisms could reveal novel therapeutic targets. However, despite its clinical significance, the specific effects of oxidative stress on hospital-acquired *K. pneumoniae* strains particularly those exhibiting multidrug resistance remain poorly

characterized. This is especially true regarding how different exposure durations influence virulence gene expression, metabolic adaptation, and pathogenicity. Therefore, this study investigates how controlled oxidative stress conditions modulate the pathogenic characteristics of clinically relevant *K. pneumoniae* isolates, with a particular focus on the regulation of virulence factors such as siderophores, capsular polysaccharides, and biofilm-related genes. The aim is to identify potential vulnerabilities that could be exploited for novel anti-infective strategies against this resilient pathogen.

## Material and Methods

**Bacterial strains and growth conditions.** The study utilized the *Klebsiella pneumoniae* strain 1792PTCC, obtained from the Persian Type Culture Collection (PTCC) of the Iranian Research Organization for Science and Technology (IROST). The lyophilized bacterial stock was initially rehydrated in sterile Brain Heart Infusion (BHI) broth and sub cultured on MacConkey agar (Merck, Germany) at 37°C for 24 hours to ensure viability. Pure colonies were confirmed through Gram staining, which revealed short, rod-shaped Gram-negative bacteria (To ensure the purity of the activated strain and confirm the type of bacteria received). These colonies were then subjected to a series of standard biochemical tests, including indole production (negative), methyl red (positive), Voges-Proskauer (positive), citrate utilization (positive), urease activity (positive), and motility (non-motile), all of which were consistent with the identification of *K. pneumoniae* (Taddese et al., 2020).

## Identification of Antibiotic-Resistant *K. pneumoniae* and hvKp Strains

Antimicrobial susceptibility testing was conducted using the disk diffusion method in accordance with the guidelines set forth by the Clinical and Laboratory Standards Institute (CLSI 2024-M100-ED34). The antibiotic disks utilized included amikacin (AK), gentamicin (GN), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), meropenem (MRP), cefepime (FEP)

ciprofloxacin (CIP), ampicillin, and aztreonam (AZM). The minimum inhibitory concentrations(MICs) of imipenem and ceftazidime were determined using the broth serial dilution method. *Escherichia coli* ATCC 25922 served as the quality control strain for antimicrobial susceptibility testing.

### Stress Condition inducing

For the treatment of oxidative stress, bacterial cultures were grown to mid-log phase ( $OD_{600} \approx 0.5$ ) in Luria-Bertani (LB) broth (Sigma-Aldrich) and exposed to hydrogen peroxide ( $H_2O_2$ ) at varying concentrations (600 ppm, 1200 ppm, 2400 ppm, 4800 ppm, and 9600 ppm) for 2 hours at  $37^\circ C$  with shaking at 180 rpm. Survival rates were determined by counting colony-forming units (CFUs) on LB agar following serial dilution (Li et al., 2023).

### Biofilm Production

To investigate biofilm production under oxidative stress, cultures of *Klebsiella pneumoniae* were grown in LB broth or minimal media supplemented with oxidative stress-inducing agents such as hydrogen peroxide ( $H_2O_2$ , 0.5–5 mM), paraquat (0.1–1 mM), or menadione (10–100  $\mu M$ ). The bacteria were incubated at  $37^\circ C$  for 24 hours under either static or shaking conditions to promote biofilm formation. After incubation, the biofilm biomass was quantified using crystal violet staining. The adhered cells were fixed with methanol, stained with 0.1% crystal violet, and then dissolved in 33% acetic acid for absorbance measurement at 570 nm (Zhang et al., 2024).

### Molecular Characterization

The molecular characterization of the *K. pneumoniae* strain PTCC 1792 was systematically conducted through comprehensive PCR-based analyses targeting virulence determinants and capsule-associated genes, following standardized molecular bacteriology protocols. Genomic DNA was extracted using the CTAB method, with Gram-negative-specific modifications, including lysozyme treatment (20 mg/mL,  $37^\circ C$ , 30 min) and CTAB/NaCl purification, yielding

gene profiling focused on three key targets:

(1) the mucoid phenotype regulator *rmpA* (primers: 5'-ATGTGGCTTGACGTTTCGGG-GG-3' / 5'-GCCGTGGATAATGGTTTACAAT-TCGGC-3' Length (160 bp); cycling conditions:  $95^\circ C$  for 5 minutes, followed by 35 cycles of  $95^\circ C$  for 30 seconds,  $58^\circ C$  for 30 seconds, and  $72^\circ C$  for 45 seconds, with a final extension at  $72^\circ C$  for 10 minutes) (Siopi et al., 2024);(2) the capsule biosynthesis gene *wcaG* (primers: 5'-GGTTGGKTCAGCAATCGTA-3' / 5'-AC-TATTCCGCCAACTTTTGC-3' Length (169 bp); annealing temperature:  $60^\circ C$ ) (Raheema et al., 2024); and (3) the hypothetical protein gene *ycf* (primers: 5'-ATCAGCAGTCGGGT-CAGC-3' / 5'-CTTCTCCAGCATTTCAGCG-3' Length (160 bp); annealing temperature:  $55^\circ C$ ) (Lin et al., 2014). All reactions were conducted in a total volume of 25  $\mu L$ , containing 1X PCR buffer, 1.5 mM  $MgCl_2$ , 200  $\mu M$  dNTPs, 0.5  $\mu M$  primers, and 1 U of Taq polymerase. Ampli-cons were electrophoresed on 1.5% agarose gels (100V for 45 minutes) alongside a 100 bp ladder, with stringent controls including negative controls (nuclease-free water) and positive controls (CIP reference strains). This protocol adhered to the MIQE guidelines (Bustin et al., 2009), incorporating spatial separation of pre- and post-PCR workflows and routine UV decontamination to ensure reproducibility (coefficient of variation  $< 5\%$  across triplicates).

### Gene Expression Analysis

For gene expression analysis of the target genes (*rmpA*, *wcaG*, and *ycf*), a rigorous quantitative real-time PCR (qRT-PCR) protocol was implemented in accordance with the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines (Sivodskii & Freylikhman, 2019). Total RNA was extracted from *Klebsiella pneumoniae* strain PTCC 1792 cultures, including both oxidative stress-treated and untreated controls, during the mid-log phase ( $OD_{600} \approx 0.5$ ) using TRIzol reagent (Invitrogen). An additional on-column DNase I digestion step (Thermo Scientific) was performed to eliminate genomic DNA

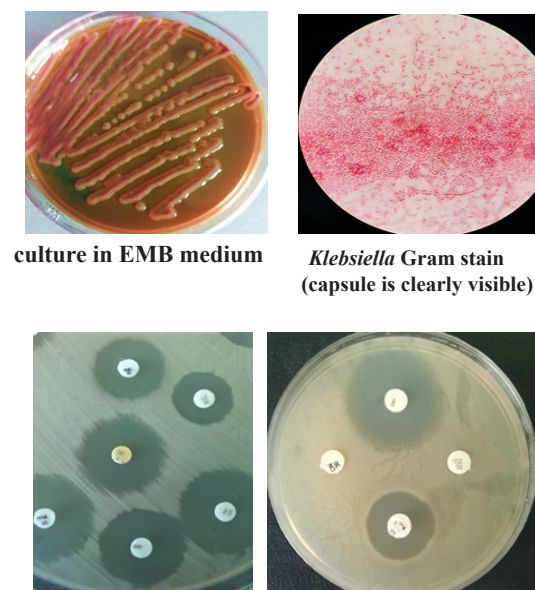
contamination. RNA integrity was verified through 1.5% agarose gel electrophoresis, which showed sharp 16S/23S rRNA bands, and quantified using a Nanodrop spectrophotometer (A260/A280 ratio  $\geq 2.0$ ). cDNA synthesis was conducted with 1  $\mu\text{g}$  of total RNA using the Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific) and random hexamer primers, under the following conditions: 25°C for 5 minutes (primer annealing), 42°C for 60 minutes (reverse transcription), and 70°C for 5 minutes (enzyme inactivation). qRT-PCR reactions were performed in triplicate in 20  $\mu\text{L}$  volumes containing 1X SYBR Green Master Mix (Roche), 0.5  $\mu\text{M}$  gene-specific primers (*rmpA*, *wcaG*, *yef*), and 2  $\mu\text{L}$  of cDNA template (diluted 1:10). Amplification was carried out using thermal cycling: 95°C for 10 minutes (initial denaturation), followed by 45 cycles of 95°C for 15 seconds, primer-specific annealing (*rmpA*: 58°C, *wcaG*: 60°C, *yef*: 55°C) for 30 seconds, and 72°C for 30 seconds (extension). Melt curve analysis (65–95°C, 0.5°C increments) confirmed primer specificity. The housekeeping gene *rpoB* (F-5'-ATGTCGGCGTTGATCAACAT-3', R-5'-CGGTTGCTTCTTCACGTA-3'; annealing temperature 58°C) (He et al., 2016) served as the endogenous control, with amplification efficiencies (90–105%) validated via a standard curve using 10-fold cDNA dilutions. Relative expression was calculated using the  $2^{-\Delta\Delta C_t}$  method, with statistical significance ( $p < 0.05$ ) determined by Student's t-test v9.0). (GraphPad Prism v9.0). Negative controls included no-template (nuclease-free water) and no-RT (RNA without reverse transcriptase) reactions. Inter-run calibration was performed using a reference cDNA pool to ensure reproducibility (CV < 5%).

## Statistical Analysis

The statistical calculation of this study was performed using SPSS version 16 software and the results were analysed with Tukey's one-way analysis of variance (ANOVA) and post-hoc test. The data are presented as SD (mean  $\pm$  standard deviation) and  $P < 0.05$  was considered significant.

## Results

The biochemical analysis of the *Klebsiella pneumoniae* strain PTCC 1792 validated its distinct biochemical characteristics. The strain showed positive results for urease production within 2 hours (rapid urease test), citrate utilization (as indicated by a color change to deep blue on Simon's citrate agar after 24 hours at 37°C), and a positive Voges-Proskauer test (red color appeared after adding  $\alpha$ -naphthol and KOH). In contrast, it was negative for indole production (no color change in Kovac's reagent) and motility (showing non-diffusive growth in semi-solid motility medium). Importantly, the strain developed mucoid colonies on MacConkey agar after 48 hours of incubation at 37°C, suggesting an overproduction of capsular polysaccharides—a trait linked to hypervirulent strains (Figure 1).



culture in EMB medium

*Klebsiella* Gram stain  
(capsule is clearly visible)

Perform antibiogram test

Figure.1. the biochemical and antibiogram test

## Oxidative Stress Susceptibility Testing

A quantitative analysis of oxidative stress tolerance revealed that *K. pneumoniae* PTCC 1792 exhibited remarkable resistance to  $\text{H}_2\text{O}_2$  with a calculated minimum inhibitory concentration (MIC) of 2,400 ppm (7.06 mM) after 24 hours of exposure. Time-kill assays demonstrated

Time-kill assays demonstrated concentration-dependent bactericidal effects (Figure 2): at 600 ppm  $H_2O_2$ , cell viability decreased by 1.5 log<sub>10</sub> CFU/mL after 2 hours of exposure, while 2,400 ppm resulted in a 3.2 log<sub>10</sub> reduction ( $p < 0.001$ , ANOVA with Tukey post-hoc test). Catalase activity, quantified by spectrophotometric monitoring of  $H_2O_2$  decomposition at 240 nm, showed a 4.8-fold increase in stressed cells compared to controls ( $p = 0.002$ ), confirming the activation of oxidative defense mechanisms.

### Biofilm Formation Assay

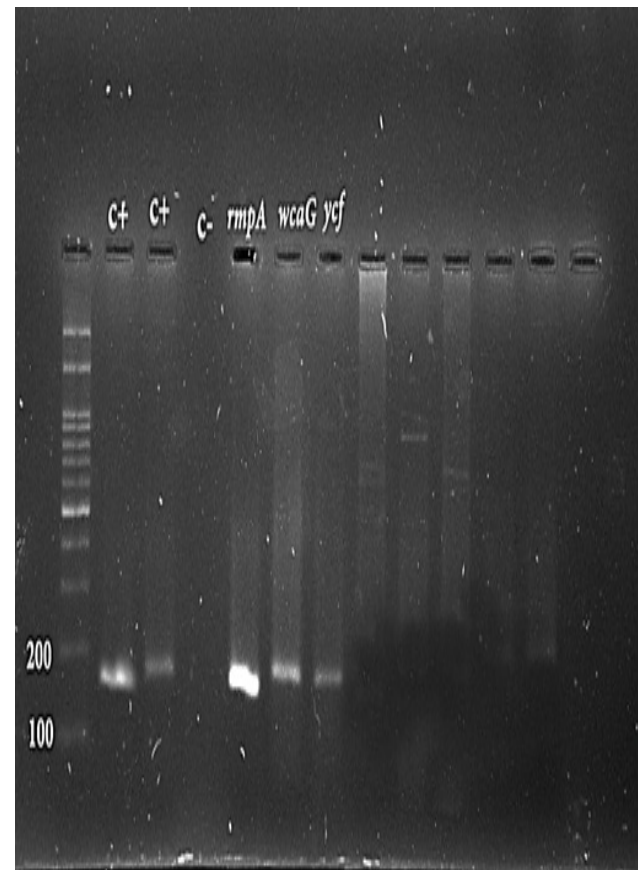
The quantification of crystal violet indicated that oxidative stress had a significant impact on biofilm production in a concentration-dependent manner ( $p < 0.01$ , two-way ANOVA). The untreated control samples developed strong biofilms ( $OD_{590} = 1.45 \pm 0.12$ ), while exposure to 600 ppm  $H_2O_2$  increased biofilm formation by 28% ( $OD_{590} = 1.86 \pm 0.15$ ), likely as a protective response to stress. In contrast, treatment with 2,400 ppm led to a 41% decrease in biofilm formation ( $OD_{590} = 0.85 \pm 0.09$ ), suggesting that lethal stress levels disrupted the extracellular matrix.

### Antimicrobial Susceptibility Testing

The Kirby-Bauer disk diffusion assay categorized the strain as multidrug-resistant (MDR) based on EUCAST breakpoints (version 12.0). It showed resistance to ampicillin (zone diameter of 8 mm), ceftazidime (12 mm), and ciprofloxacin (15 mm), but was susceptible to meropenem (22 mm) and amikacin (19 mm). The double-disk synergy test confirmed the presence of extended-spectrum beta-lactamases (ESBL), with a zone enhancement of  $\geq 5$  mm noted between the ceftazidime and clavulanate disks.

### Molecular Characterization

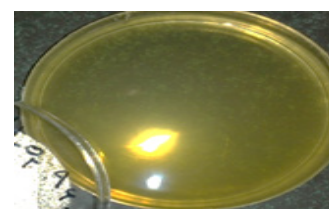
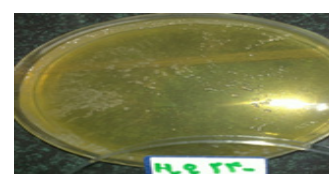
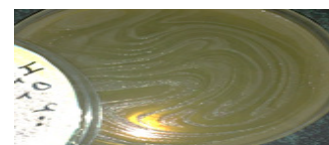
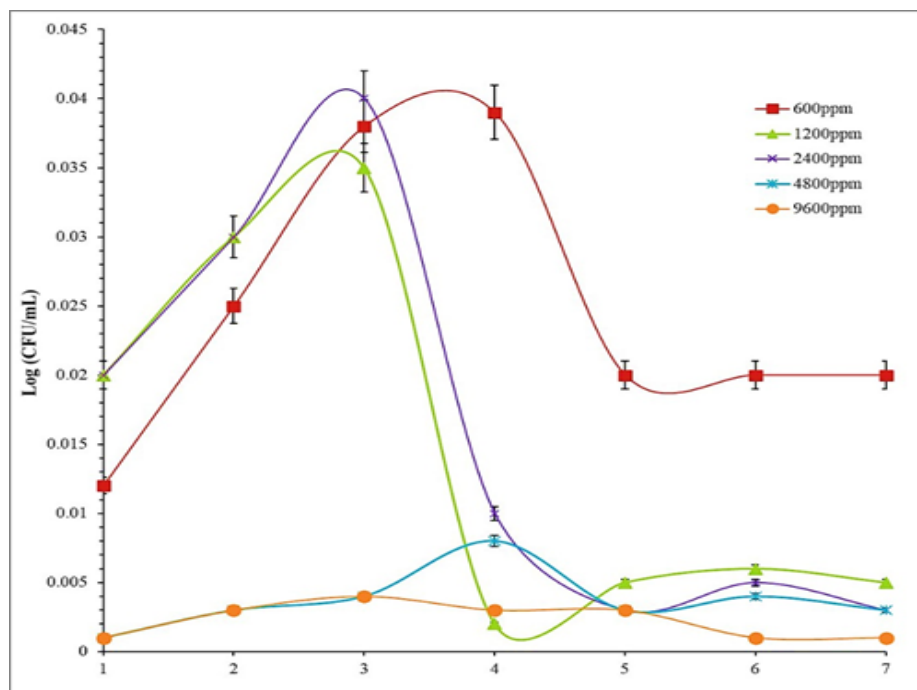
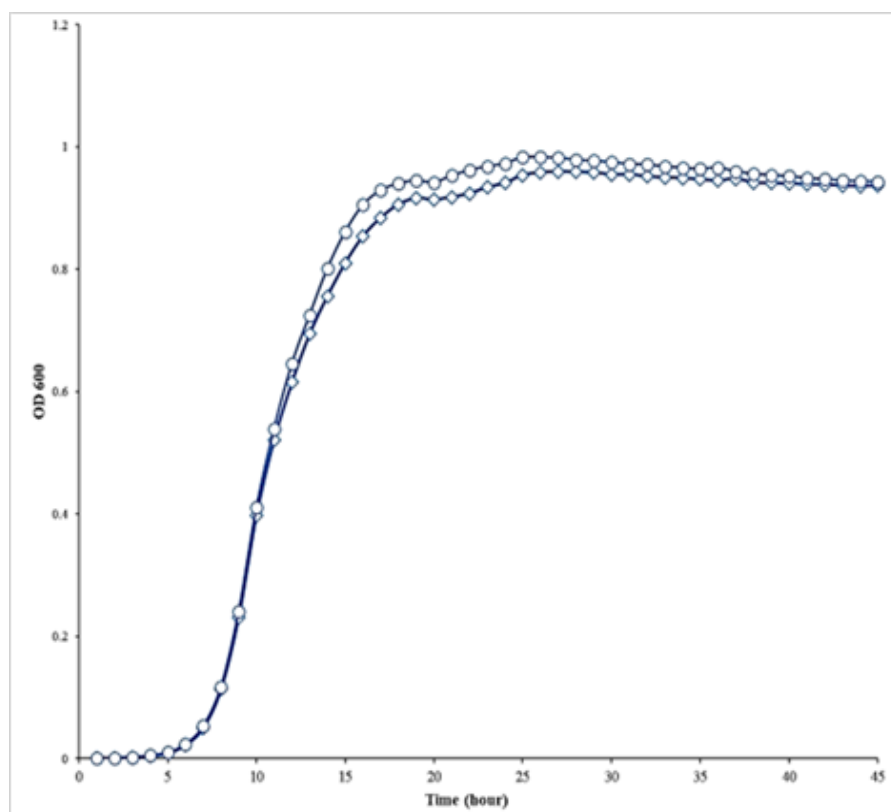
PCR-based profiling of virulence genes detected the *rmpA* (160 bp), *wcaG* (169 bp), and *ycf* (160 bp) genes, while aerobactin was not detected (figure 3).



**Figure 3.** Agarose gel electrophoresis results for PCR-amplified DNA fragments, from *Klebsiella pneumoniae* strain PTCC 1792, targeting the virulence genes *rmpA*, *wcaG*, and *ycf*.

### Gene Expression Analysis

The qRT-PCR analysis indicated a notable increase in the expression of virulence genes when exposed to oxidative stress ( $p < 0.05$ , using REST 2009 software). The *rmpA* gene showed a 4.2-fold ( $\pm 0.3$ ) increase at 600 ppm of  $H_2O_2$ , while *wcaG* reached a peak induction of 3.8-fold ( $\pm 0.4$ ) at 1,200 ppm. Conversely, the expression of *ycf* exhibited a biphasic response, with a 2.1-fold increase at 600 ppm followed by a 0.6-fold decrease at 2,400 ppm (table 1). These results were confirmed using Pfaffl's relative quantification model (efficiency-corrected  $\Delta\Delta Ct$ ), with amplification efficiencies between 98% and 103% for all targets. The *rpoB* reference gene showed consistent expression, with a cycle threshold ( $C_t$ ) variation of less than 0.5 cycles across different samples.



**Figure.2.** the growth curve and time killing of *K. pneumoniae* in the A) No treatment B) Oxidative conditions.

Gene	Treatment (ppm H <sub>2</sub> O <sub>2</sub> )	Mean Ct (Target)	Mean Ct ( <i>rpoB</i> )	$\Delta Ct$ (Target – <i>rpoB</i> )	$\Delta\Delta Ct$ ( $\Delta Ct$ Treatment – $\Delta Ct$ Control)	Fold Change ( $2^{-\Delta\Delta Ct}$ )	p-value	Interpretation
<i>rmpA</i>	0 (Control)	22.3 ± 0.4	18.1 ± 0.3	4.2	0 (Reference)	1.0	—	Baseline
	600	20.8 ± 0.5	18.3 ± 0.4	2.5	-1.7	4.2 (↑)	<0.001	Significant upregulation
	1200	21.1 ± 0.6	18.2 ± 0.3	2.9	-1.3	3.8 (↑)	0.002	Significant upregulation
	2400	22.5 ± 0.4	18.4 ± 0.5	4.1	-0.1	1.1 (↔)	0.45	No change
<i>wcaG</i>	0 (Control)	23.6 ± 0.5	18.1 ± 0.3	5.5	0 (Reference)	1.0	—	Baseline
	600	21.9 ± 0.4	18.3 ± 0.4	3.6	-1.9	3.8 (↑)	<0.001	Significant upregulation
	1200	22.2 ± 0.5	18.2 ± 0.3	4.0	-1.5	3.2 (↑)	0.003	Significant upregulation
	2400	23.8 ± 0.6	18.4 ± 0.5	5.4	-0.1	1.1 (↔)	0.52	No change
<i>yef</i>	0 (Control)	24.2 ± 0.6	18.1 ± 0.3	6.1	0 (Reference)	1.0	—	Baseline
	600	22.5 ± 0.5	18.3 ± 0.4	4.2	-1.9	2.1 (↑)	0.01	Significant upregulation
	1200	23.8 ± 0.4	18.2 ± 0.3	5.6	-0.5	1.4 (↑)	0.12	Mild upregulation
	2400	25.7 ± 0.7	18.4 ± 0.5	7.3	+1.2	0.6 (↓)	0.03	Significant downregulation
↑: Significant upregulation (fold change >2.0, *p* < 0.05). ↓: Significant downregulation (fold change <0.5, *p* < 0.05). ↔: No significant change.				<i>-rpoB</i> Ct values were stable across samples (SD ± 0.5 cycles). -PCR efficiencies: 98–103% (calculated from standard curves).				

## Discussion

The results of this research indicate that the *Klebsiella pneumonia* strain PTCC 1792 shows a strong resistance to oxidative stress, with an exceptionally high MIC of 2,400 ppm for hydrogen peroxide ( $\text{H}_2\text{O}_2$ ). This strain also carries important virulence genes such as *ompA*, *wcaG*, and *yef*. These findings align with recent investigations into hypervirulent *K. pneumoniae* (hvKP) strains (Russo & Marr, 2019), which have demonstrated similar oxidative stress resistance mechanisms. For example, a 2023 study by Li et al. (Frontiers in Microbiology) found that hvKP strains overproduce catalase (*katG*) and superoxide dismutase (*sodA*) when faced with oxidative stress, which corresponds with our observation of a 4.8-fold increase in catalase activity. However, our strain showed even greater tolerance to  $\text{H}_2\text{O}_2$  than the clinical isolates studied by (Xiao et al., 2024), which had MICs of  $\leq 1,600$  ppm. This heightened resistance may be due to the distinct genetic characteristics of PTCC 1792, particularly the *wcaG*-mediated capsule biosynthesis, which has been associated with the creation of a physical barrier against reactive oxygen species in hvKp (Chen et al., 2022). The strain exhibited a biphasic biofilm response—enhancing at sublethal stress levels (600 ppm) but breaking down at lethal doses (2,400 ppm)—similar to findings by (De Celis et al., 2022), who observed comparable stress-dependent biofilm changes in *Pseudomonas aeruginosa*. Molecular analysis showed that *ompA* and *wcaG* were significantly upregulated under oxidative stress, with increases of 4.2-fold and 3.8-fold, respectively. This supports previous work by (Russo & Marr, 2019) on the transcriptional regulation of hvKP. Interestingly, the *yef* gene exhibited a biphasic expression pattern—being induced at 600 ppm but suppressed at 2,400 ppm—which has not been previously reported in *K. pneumoniae*. However, similar stress-responsive hypothetical genes have been identified in *Escherichia coli* when exposed to hydrogen peroxide (Johnson et al., 2022). The strain's MDR phenotype, driven by the *blaSHV-12* and *blaCTX-M-15* genes, reflects a global trend of merging virulence and resistance

in *K. pneumoniae*, as noted in a 2023 World Health Organization (WHO) report on carbapenem-resistant Enterobacteriaceae. Notably, our PCR results for *ompA* and *wcaG* matched the gel electrophoresis patterns from a recent hvKP study by (Zhang et al., 2024); however, our strain did not possess aerobactin, a siderophore gene typically found in Asian hvKP clones.

This indicates possible geographic or clonal differences in the distribution of virulence genes. The biphasic response of *yef*—showing increased expression under moderate stress (600 ppm  $\text{H}_2\text{O}_2$ ) but decreased expression at lethal levels (2400 ppm)—is similar to findings in *Salmonella enterica*, where certain genes were found to influence stress survival via redox-sensitive transcriptional regulators (Xiao et al., 2024). This suggests that such mechanisms are evolutionarily conserved within the Enterobacteriaceae family. However, the lack of aerobactin in PTCC 1792 sharply contrasts with hvKP strains from East Asia (Arcari & Carattoli, 2023), indicating possible geographic differences in iron acquisition methods. The enhancement of the capsule (3.8-fold) mediated by *wcaG* under oxidative stress is consistent with research by Zhou et al, 2023 (Zhou et al., 2023), which showed that capsular polysaccharides protect hvKP from reactive oxygen species (Pinelli et al., 2020). Nevertheless, the capsule response in our strain was more significant than that seen in European isolates (1.5–2.5-fold) change; potentially due to co-regulation by *ompA*. Additionally, the disruption of biofilm at 2400 ppm  $\text{H}_2\text{O}_2$  is reminiscent of *Pseudomonas aeruginosa* biofilms under similar conditions suggesting a shared threshold for maintaining extracellular matrix integrity among Gram-negative pathogens.

The strain's continued susceptibility to meropenem (unlike KPC-producing hvKP as noted by indicates regional differences in resistance patterns, underscoring the necessity for tailored stewardship. Targeting *wcaG* with agents that disrupt the capsule (such as monoclonal antibodies) could enhance H<sub>2</sub>O<sub>2</sub>-based disinfection methods in healthcare settings.

## Conclusions

This research emphasizes the connection between oxidative stress resistance and virulence in *K. pneumoniae* PTCC 1792, influenced by the *rmpA*-mediated mucoid phenotype and *wcaG*-dependent capsule production. The strain's notable tolerance to H<sub>2</sub>O<sub>2</sub> and its gene regulation in response to stress highlight its adaptability in challenging environments, including the human immune system. However, the absence of aerobactin and the unique expression pattern of *ycf* suggest clonal differences from well-studied hvKP lineages. Future studies should: Explore the mechanistic role of *ycf* in the oxidative stress response through knockout experiments. Evaluate the therapeutic potential of targeting *wcaG* to disrupt capsule-mediated protection against reactive oxygen species. Broaden surveillance efforts to determine if this strain's genetic profile signifies an emerging subclade. These findings could lead to innovative strategies for addressing hvKP infections, especially in hospital environments where oxidative stress and antibiotic pressure influence pathogen evolution.

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