ORIGINAL ARTICLE

AL-ANBAR MEDICAL JOURNAL
Anb. Med. J. 21(2): 104–109, 2025

Prevalence of Cytotoxin-producing *Klebsiella oxytoca* Isolated from Medical City Complex, Baghdad, Iraq

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(Received : 1 October 2024; Accepted : 10 January 2025; First published online: 18 February 2025)

ABSTRACT

Background: *Klebsiella oxytoca* (*K. oxytoca*) is a progressively noted pathogen in healthcare services. *K. oxytoca* can produce cytotoxins, which play a role in the pathogenesis of bacteria and are the main virulence factors. *K. oxytoca* can make cytotoxin, a biofilm, resistance, and a hypermucoviscous phenotype, all of which are important for the virus's ability to infect others.
Objectives: To investigate the occurrence of the toxin-producing *K. oxytoca* (*pehX, npsA*, and *npsB*) genes from different Iraqi isolates that were multi-drug resistant, could produce a biofilm, and had a hypermucoviscous phenotype.
Materials and methods: A total of 100 *K. oxytoca* isolates were collected from hospitals in Baghdad, Iraq. The isolates were identified by manual and biochemical tests. The string test was used to determine the hypermucoviscousity. The antibiotic sensitivity test was uncovered using the Kirby-Bauer method. Biofilm formation was detected by the qualitative Congo red agar method and the quantitative microtiter plate method (MTP). *K. oxytoca* galacturonase-specific gene (*pehX*) and the cytotoxin marker genes (*npsA* and *npsB*) were detected using real-time polymerase chain reaction.
Results: *K. oxytoca* was isolated from 62/100 inpatients and 38/100 outpatients. Antibiotic sensitivity tests showed different levels of resistance. The *pehX* gene was positive in 35/50 of the isolates. The *npsA/npsB* PCR targets were positive in 12/35 isolates. Of these 12 isolates, 3/12 (25%) were from urine and 2/12 (16.7%) from blood.
Conclusion: Cytotoxin-producing *K. oxytoca* was a common emerging pathogen in Iraqi patients. Biofilm formation, hypermucoviscous, and antibiotic resistance increase the virulence of this bacterium.

Keywords: Biofilm; Cytotoxin; Hypermucoviscous; *K. oxytoca*; *npsA* gene; *npsB* gene; *pehX* gene.

DOI: 10.33091/amj.2025.154811.1977

INTRODUCTION

Klebsiella oxytoca (*K. oxytoca*), a new emerging pathogen in Iraq, poses potential virulence to both communities and health sectors. Very few studies have been carried out to investigate the prevalence and antibiotics-resistant profile of *K. oxytoca* in Iraq and other close regions. An Iraqi study by Emad *et al.*, in 2024, found that *K. oxytoca* was isolated from 136 samples of different sources. From the isolated bacteria, about 13 isolates were designated as multi-drug resistant (MDR) phenotypes [1]. Additionally, a study conducted in Iran by Ghasemian *et al.* reported that *K. oxytoca* was isolated in each of the 50 samples under investigation [2]. Nowadays, *K. oxytoca* is considered one of the second most common causes of *Klebsiella* clinical infections in humans [3].

K. oxytoca is a complex of nine species *K. grimontii*, *K. huaxiensis*, *K. michiganensis*, *K. oxytoca*, *K. pasteurii*, *K. spallanzanii*, and three unnamed novel species [4].

Interestingly, the organism has developed resistance to various antibiotics, primarily used for treating bacterial infections, due to various mechanisms, including aminoglycoside-

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http://doi.org/10.33091/amj.2025.154811.1977

modifying enzymes, *Klebsiella pneumonia* carbapenemase (KPC), and extended-spectrum β-lactamases (ESBLs) [5].

The hypermucoviscosity (HMV) phenotype is a pathogenic factor of *K. pneumoniae* and *K. oxytoca*. This phenotype enhances bacterial resistance to host defenses and antibiotics and enhances pathogenicity, making it a critical factor in severe infections [6].

Bacteria create biofilms for survival, increasing resistance to antimicrobial agents and external stresses and increasing pathogen tenacity on surfaces, which is linked to infections [7].

K. oxytoca is recognized for its ability to produce cytotoxin and form biofilms, both of which are critical contributors to its pathogenicity and virulence. These factors are particularly significant in gastrointestinal infections and antibiotic-associated complications such as hemorrhagic colitis [8].

Two separate cytotoxins are synthesized by *K. oxytoca*: Tilimycin (also called kleboxymycin or carbinolamine) and tilivalline (which is formed when free indole attacks tilimycin through a nucleophilic reaction). The disease-relevant cytotoxins tilivalline and tilimycine produced by certain *K. oxytoca* isolates are encoded by the non-ribosomal peptide synthetase genes A (*npsAnpsA*) and B (*npsB*) [9].

Thus, the current study aimed to determine the incidence of toxin-producing *K. oxytoca* (*pehX*, *npsA*, and *npsB* genes) among a variety of MDR Iraqi isolates that were capable of developing a biofilm and hypermucoviscous phenotype.

MATERIALS AND METHODS

Bacterial isolation and phenotypic characterization

From November 2023 to February 2024, 100 samples were taken from patients at the Medical City Teaching Hospitals complex in Baghdad, Iraq, as part of a cross-sectional study. Hospital-acquired infections were authorized by doctors, and the samples were separated into inpatient and outpatient infection samples. Isolates that tested positive for *K. oxytoca* in culture and biochemical assays were included in the study; isolates that tested positive for other genera or species were excluded.

The study was conducted following the Helsinki Declaration Guidelines and approved by the Ethics Committee of the Department of Microbiology, College of Medicine, University of Baghdad, Baghdad, Iraq (Reference number 0234 on 27-11-2023).

The samples were streaked on MacConkeys' agar for phenotype characteristics and suspected bacterial isolates were identified to the genus and species level using the VITEK-2 Compact (bioMerieux, Marcy l'Etoile, France). Analyzing various substrates, the GN Colorimetric Identification Card reagent cards regulated metabolic activities. For every product, there were quality control strains and their results (ATCC 700323). An indole test was used to further confirm the bacterial species, evaluating the precision of the results at the species level [10]. The hypermucoviscous feature of the isolates was recorded using the string test method. In which a mucus thread of $\geq$5 mm was detected [6].

Antibiotic sensitivity tests were done using the Kirby Bauer method for five antibiotics (ciprofloxacin 10 µg/disc, imipenem 10 µg/disc, gentamicin 10 µg/disc, tetracycline 10 µg/disc, and cefotaxime 10 µg/disc). MDR was referred to as having acquired resistance to at least one drug from three or more antibiotic classes [11]. The chosen antimicrobial agents depended on local susceptibility patterns of *K. oxytoca* isolates as detected by a previous study [1].

The qualitative Congo red agar)CRA) the technique was used to detect biofilm development [12]. To measure the biofilm development strength quantitatively, microtiter plates (MTP) were utilized [13].

Genetic investigation

DNA Extraction

To detect the specific *K. oxytoca* gene (*pehX*) and the cytotoxin genes (*nspA* and *nspB*), genomic deoxyribonucleic acid (DNA) was isolated from 50 isolates that were MDR, with strong biofilm formation, and with/without hypermucoviscous features. The manufacturer's instructions were followed using a Mericon DNA bacterial kit (Catalogue No. 69534, Qiagen, Germany). All the molecular techniques in the experiments were carried out under biosafety guidelines.

Real-time polymerase reaction (PCR) setup

PCR reactions were prepared in accordance with optimized protocols using the GoTaq®qPCR Master Mix kit (Catalogue No. A6001, Promega, USA). The primers' sequences for the targeted genes are shown in (Table 1). VeritiPro™ Thermal Cycler (Thermo Fisher Scientific Inc., USA) was used with the cycling conditions in which 1 cycle at 95°C for 2 minutes for polymerase activation, 40 cycles for denaturation at 95°C for 15 seconds, and annealing and extension at 60°C for 1 minute.

Statistical analysis

Statistical analysis was performed using the statistical package for the social sciences (SPSS), version 26 (IBM, Chicago, USA) and Microsoft Office Excel 2010. Normally distributed data was expressed as numbers and percentages. Pearson Chi-square test (χ^2) and binomial Z test for comparisons of qualitative variables between studied groups (i.e., Specimens, sex, in or outpatients, and string and biofilm). The statistical significance threshold was accepted at a P-value < 0.05.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

χ^2: Chi-square, $\sum$: Summation, O: Observed number, E: Expected number. The level of confidence is a 95% confidence interval (CI).

RESULTS

Identification of the bacteria

Of 100 isolates, there were 62 from inpatients (hospital-acquired infection), and 38 from outpatients (community-

Table 1. Oligonucleotides used in polymerase reaction [14].

Primers	Sequence 5'–3'
pehX-F	GACGAGCCCGCTCAGT
pehX-R	CCGAAGTACCATCGACGG
npsA-F	GAAGCGGGAATTACCCGGTAT
npsA-R	TTTCCTGTTGAACCAGAGGTCA
npsB-F	AGGGCTTTGCCACACAAAGT
npsB-R	TCATGCTCCAGCCTCACCA

acquired infection) with a significant (P-value = 0.016) correlation.

Of the 100 identified *K. oxytoca* isolates, 14 (14%) were positive for the string test. The isolates' degrees of resistance to the antibiotics utilized in this investigation varied. Cefotaxime and other βeta-lactam antibiotics were extremely resistant (91/100) isolates. About (55/100) of the isolates were susceptible to Imipenem, a member of the carbapenem group (Table 2).

Detection of biofilm among *K. oxytoca* isolates

Out of 100 isolates, 80 were identified as biofilm-forming bacteria after being cultured on CRA. Six out of 80 isolates tested positive after 72 hours, while 74 out of 80 tested positive after 24 hours. The quantitative evaluation for the biofilm-forming isolates was carried out on the 80 isolates that were found to be positive on CRA by the MTP method. The potency to form biofilm by the MTP method proved that 25/80 (31.25%), 24/80 (30%), 18/80 (22.5%), and 13/80 (16.25%) isolates were with strong, moderate, weak, and non-biofilm, respectively.

Molecular identification of *K. oxytoca* using *pehX* gene

From the selected 50 (50%) isolates, only 35 (70%) were positive for *K. oxytoca* galacturonase-specific gene (*pehX*).

Cytotoxin identification

According to Table 3, the gene *npsA* was found to be present in 21 out of 35 cases (60%) and the gene *npsB* in 20 out of 35 cases (57%). Remarkably, 12 out of 35 isolates (34.2%) had positive results for both the *npsA* and *npsB* genes.

Of these 12 isolates, 3 (25%) were from urine and 2 (16.7%) from blood, while the rest were from different sources. Thus, there was no significant correlation (P-value > 0.05) between the source of the isolate and its cytotoxin-producing effectiveness (Table 4).

Seven (58.5%) of the 12 isolates that tested positive for both *npsA* and *npsB* were taken from inpatient samples, whereas 5 (41.7%) were taken from outpatient samples. Furthermore, only two of the 12 isolates (16.7%) were positive for the string test. The aforementioned criteria did not, however, significantly (P-value > 0.05) correlate with the capacity to generate cytotoxins (Table 5).

Distribution of biofilm formation among the cytotoxin-producing isolates

The results of the current study showed that there was no significant association between the ability of *K. oxytoca* to form a biofilm and its ability to produce cytotoxins. Nevertheless, the results found that 11/12 (91.7%) could form a biofilm. The strong biofilm formation was detected in 5/12 (45.5%) isolates. According to the data analysis, the MTP approach was helped in determining the strength of biofilm creation and was substantially correlated (P-value < 0.01) with the capacity to generate toxins (Table 5).

Table 2. Antibiotic-resistant percentage of K. oxytoca isolates.

Antibiotics	Sensitive	Resistant	Intermediate
Gentamicin	53 (53%)	46 (46%)	1(1%)
Cefotaxime	3 (3%)	91 (91%)	6 (6%)
Imipenem	55(55%)	37 (37%)	8 (8%)
Tetracycline	22 (22%)	66 (66%)	12 (12%)
Ciprofloxacin	22 (22%)	60 (60%)	18 (18%)

Table 3. Distribution of 35 genes (*nspA* and *nspB*) in *K. oxytoca* isolates.

Gene		NO.%	P-value
nspA	Positive	21(60%)	0.311
	Negative	14(40%)	
nspB	Positive	20(57.1%))	0.5
	Negative	15(42.9%)	

DISCUSSION

K. oxytoca is an opportunistic pathogen that has been recently identified as an actual complex. Studying how *K. oxytoca* makes biofilms and kills cells can help us understand how it infects and survives, as well as how to make antibiotics that can fight its growing resistance [4]. The emergence of a new pathogen in local hospitals with significant virulence factors, as identified in the current study, necessitates further attention from the academic and health sectors regarding the pathogen's origins and control strategies.

The current study found that the majority of the *K. oxytoca* isolates were from inpatients (hospital-acquired infection). This aligns with the study by Alvarez *et al.* (1985), in the United States, who found that 23/44 (52%) were considered nosocomial-acquired infections [15]. According to reports, *K. oxytoca* primarily causes hospital-acquired infections, typically affecting immunocompromised patients or those requiring intensive care [16].

Like other bacterial societies, *K. oxytoca* has also progressed its resistance to a wide range of antibiotics through numerous mechanisms [17]. For individuals who are very sick, agents with high intrinsic activity against *K. pneumoniae* should be chosen. Third-generation cephalosporins (like cefotaxime and ceftriaxone), carbapenems (like meropenem and imipenem/cilastatin), aminoglycosides (like gentamicin and amikacin), and quinolones are a few examples of these drugs. These substances can be used either alone or in combination [17]. Tetracyclines are a group of broad-spectrum antibiotic compounds that are generally used in the treatment of infections of the urinary tract, respiratory tract, and the intestines [18]. Thus, it could be an alternative in seriously ill patients. The results were interpreted in accordance with the recommendations of the Clinical and Laboratory Standards Institute [19]. Cefotaxime, tetracycline, and ciprofloxacin showed the lowest effect on *K. oxytoca* isolates, while imipenem and gentamicin showed the highest effect. Moreover, a recent study in Iraq found that *K. oxytoca* isolates demonstrated a high level of resistance against amoxicillin (98.5%), cefotaxime (92.6%), cefepime (86.7%), and piperacillin (88.2%). Researchers reported a reduced level of resistance (65.4% and 59.5%) to tetracycline and ciprofloxacin, respectively.

Due to variations in healthcare practices, antibiotic usage, and infection control measures, geographic context often affects resistance behaviors [1]. To control these resistance de-

http://doi.org/10.33091/amj.2025.154811.1977

Table 4. Toxicity distribution among *K. oxytoca* isolates from different clinical specimens*.

Specimens type	Toxicity/ Number (%)				P-value
	+ A and + B	+ A and - B	- A and + B	- A and - B	
Urine	3(25%)	3(33.3%)	3(37.5%)	1(16.7%)	0.457
Blood	2(16.7%)	0(0%)	3(37.5%)	0(0%)	
Wound swab	2(16.7%)	1(11.1%)	0(0%)	1(16.7%)	
Ascites fluid	0(0%)	2(22.2%)	0(0%)	1(16.7%)	
Sputum	1(8.3%)	0(0%)	0(0%)	2(33.3%)	
Stool and wound pus cell	1(8.3%)	0(0%)	0(0%)	0(0%)	
Endotracheal tube-aspiration	1(8.3%)	2(22.2%)	1(12.5%)	0(0%)	
Burn swab	1(8.3%)	0(0%)	0(0%)	1(16.7%)	
Indwelling urinary-catheter	0(0%)	1 (11.1%)	1(12.5%)	0(0%)	
Total	12(100%)	9(100%)	8(100%)	6(100%)	

* +A = Positive for *npsA* gene and +B = Positive for *npsB* gene, -A = Negatives for *npsA* gene and -B = Negative for *npsB* gene.

Table 5. The association between cytotoxin production by *K. oxytoca* isolates and different parameters*.

Parameters		Toxicity/ Number (%)				P-value
		+ A and + B N = 12	+ A and - B N = 9	- A and + B N = 8	- A and - B N = 6	
Patients	In	7(58.3%)	7(77.8%)	5(62.5%)	3(50%)	0.708
	Out	5(41.7%)	2(22.2%)	3(37.5%)	3(50%)	
String test	Positive	2(16.7%)	1(11.1%)	2(25%)	0(0%)	0.598
	Negative	10(83.3%)	8(88.9%)	6(75%)	6(100%)	
Biofilm	Positive	11(91.7%)	9(100%)	8(100%)	6(100%)	0.578
	Negative	1(8.3%)	0(0%)	0(0.0%)	0(0.0%)	
Biofilm by MTP	Week	5(45.5%)	0(0%)	1(12.5%)	1(16.7%)	0.033
	Medium	1(9.1%)	2(22.2%)	4(50.0%)	4(66.7%)	
	Strong	5(45.5%)	7(77.8%)	3(37.5%)	1(16.7%)	

* +A = Positive for *npsA* gene and +B = Positive for *npsB* gene, -A = Negatives for *npsA* gene and -B = Negative for *npsB* gene, MTP: microtiter plate method.

velopments, careful antibiotic management and ongoing monitoring are essential.

The string test revealed 14% of the 100 *K. oxytoca* isolates to be positive. This was inconsistent with a study conducted in India (2020) that found 9% of *K. oxytoca* had positive string tests. This was consistent with a study conducted in India (2020) that found 9% of *K. oxytoca* had positive string test. The hypermucoviscous phenotype was believed to be a sign of hypervirulence of the bacteria and related to severe illness, like invasive infections, particularly community-acquired infections [6].

Biofilm formation is crucial for bacteria's pathogenicity as it enhances the bacteria's ability to adhere to the surface, resist antimicrobial agents, and evade host immune responses contributing to the progress of various diseases [7]. Out of 100 tested *K. oxytoca* isolates, 80% formed a biofilm, with 74/80 forming after 24 hours and 6/80 after 72 hours. Slime production was examined depending on colony morphology produced on CRA. The current isolates demonstrated significant potential in forming a biofilm after 24 hours of incubation. In comparison to a study conducted by Rajivgandhi *et al.* (2021) who recorded that the ability of *K. pneumoniae* to form black color production of colonies on CRA plates was increased after 3, 6, 12, and 24 hours of incubation [20]. Biofilm development probably occurs in discrete stages that vary with time. Bacterial cells first attach themselves to a surface, then multiply and release extracellular polymeric substances (EPS), which form the matrix of the biofilm. The biofilm becomes stronger and thicker over time, improving its ability to protect and adapt [20]. Thus, it may indicate that the ability to develop biofilms by *K. oxytoca* was a time-dependent manner. In which the capacity of *K. oxytoca* to create biofilms grows with time.

The polygalacturonase-encoding gene *pehX* was found to be unique to *K. oxytoca*. *PehX* gene was detected in (70%) of the isolates. *PehX* gene provides accurate identification and a sensitive, rapid method for separating *K. oxytoca* from other *Klebsiella* spp. [21]. The current genetic analysis of 50 *K. oxytoca* isolates found that 30% were negative for the *pehX* gene. Although the *K. oxytoca* complex was 99% similar in the 16S rRNA gene sequence, a study done by Saha *et al* in 2013 explained this phenomenon in which *K. michiganensis* is negative for the *pehX* gene despite its positivity to the indole test [22]. This result highlighted the importance of whole genome sequencing for the current isolates to give insight into the frequency of the newly developing *K. oxytoca* complex in Iraqi hospitals.

In the present study, 12/35 (34.8%) of *K. oxytoca* isolates that were positive for the *pehX* gene were toxigenic by the amplification of two *npsA* and *npsB* genes. It had been found

that 6/35(17.1%) isolates were negative for the two genes, 9/35 (25.7%) of isolates were only positive for *npsA*, while 8/35 (22.8%) of isolates were only positive for *npsB*. Similarly, a study conducted by Leitner *et al.* (2022) explored that the toxin marker gene *npsA* was detected in 139/291(47.8%) of the isolates and *npsB* in 137/291(43.6%) of the isolates [23]. Toxigenic *K. oxytoca* strains carry a gene cluster that codes for proteins that synthesize a cytotoxin known as tilivalline (TV), which is largely responsible for bacterial toxicity. The cytotoxin biosynthetic gene cluster is a part of a pathogenicity island (PAI). One possible explanation is that certain strains may have merely acquired the PAI involved in toxin production, or they may have lost one or both of the genes [24].

Despite the absence of cytotoxic strains in isolates from normal *K. oxytoca* infection locations, evidence suggests a higher prevalence of cytotoxin-producing strains in skin and stool samples compared to bloodstream infections [3]. According to the current results, isolates that tested positive for both toxin marker genes were more common in urine samples (25%) and blood samples (16.7%). By disrupting the intestinal barrier, *K. oxytoca* induces hemorrhagic colitis, which in turn causes bacteremia. Bacteria can enter the bloodstream as a result of ulceration and breaches brought on by this damage [25].

Among 12 (34.2%) cytotoxin-producing isolates, 2 (16.7%) were positive for the string test, developing hypermucoviscous exhibited hypervirulent isolates that are a considerable threat to human health.

Beyond 12 of positive-cytotoxin *K. oxytoca*, 7 were isolated from hospitalized patients. Studies suggest that *K. oxytoca* outbreaks in intensive care units and newborn intensive care units may be linked to hand-washing sinks and sink drains, acting as environmental reservoirs. Healthcare institutions' washing machines may harbor *K. oxytoca*, a pathogen that can be transmitted to newborns. These areas may serve as possible pathways for the horizontal transfer of resistance genes such as ESBLs, as well as for cross-contamination with other resistant gram-negative microorganisms [25].

Our study found that 45.5% of cytotoxin-producing isolates formed strong biofilms, crucial for intestine colonization and toxin production [2]. Biofilms protect bacteria from harm and allow them to make large amounts of cytotoxin, which can make the cytotoxic effect on host tissues stronger and help the infection spread and last longer [26]. When *K. oxytoca* learns to make toxins and other virulence factors, it changes from an opportunistic pathogen to a potentially dangerous one [27], necessitating immediate attention to infection control, surveillance, and antibiotic stewardship in Iraqi hospitals and is considered a big threat to the health sector.

The rise of MDR strains of bacteria to various antibiotics and cytotoxicity is a cause for concern. The need for new antimicrobial products has risen due to antibiotic resistance. In addition, some patients treated with antibiotics can develop *K. oxytoca* infections due to the overgrowth of toxin-producing bacteria in the gut [3].

The study's limitation lies in its small sample size, necessitating a larger study to better understand the prevalence of a new emerging pathogen complex in local hospitals. Further genetic analysis and whole genome sequencing are needed to identify the origin and complexity of *K. oxytoca* complex. Further studies on the *pehX* gene in combination with other housekeeping genes, such as *infB*, to differentiate and identify the *K. oxytoca* complex are required. It is also important to study the cytotoxicity assay using the cell culture method to find out if *K. oxytoca* can produce cytotoxin in order to connect the genetic and phenotypic traits of bacteria that produce cytotoxin.

CONCLUSION

The specific *pehX* gene for *K. oxytoca* detection was negative among some of the isolates, despite its positivity for the indole test. Antibiotic resistance was found to be quite high among the present isolates. These isolates showed additional virulence characteristics, such as the ability to form strong biofilm and the hypermucoviscous phenotype, in addition to the MDR isolates that had been recorded. The important finding was the potential to produce cytotoxin, which increases the threat of the prevalence of these isolates in Iraqi hospitals. Cytotoxin production by drug-resistant *K. oxytoca* prevents the complete eradication of associated infection, necessitating rapid identification and control measures as chief requirements.

ETHICAL DECLARATIONS

Acknowledgments

The laboratory staff of Baghdad Teaching Hospital Laboratories and the staff of the College of Medicine, University of Baghdad, Microbiology Department are much appreciated by the authors.

Ethics Approval and Consent to Participate

The project was approved by the Ethical Committee of the Department of Microbiology/College of Medicine/University of Baghdad (No. 0234 on 27th Nov. 2023). Informed consent was obtained from each participant.

Consent for Publication

Not applicable (no individual personal data included).

Availability of Data and Material

The datasets produced and/or analyzed during the present study can be obtained from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

Funding

No funding.

Authors' Contributions

Hafidh RR and Alsodani HM contributed to the design and implementation of the research. Mustaf HA contributed to collecting the isolates, all the laboratory work, the data analysis, and the writing of the manuscript. Hafidh RR contributed to reviewing and proofreading of the manuscript. All authors read and approved the final version of the manuscript.

http://doi.org/10.33091/amj.2025.154811.1977

REFERENCES

[1] R. Emad and R. R. Hafidh. Hypervirulent and the multi-drug resistant klebsiella oxytoca: A new emerging pathogen in baghdad hospitals, iraq. *Al-Anbar Medical Journal*, 20(1):86–93, 2024.

[2] A. Ghasemian, A. M. Mobarez, S. N. Peerayeh, and A. B. Abadi. The association of surface adhesin genes and the biofilm formation among klebsiella oxytoca clinical isolates. *New microbes and new infections*, 27:36–39, 2019.

[3] N. Neog, U. Phukan, M. Puzari, M. Sharma, and P. Chetia. Klebsiella oxytoca and emerging nosocomial infections. *Current Microbiology*, 78(4):1115–1123, 2021.

[4] J. Yang, H. Long, Y. Hu, Y. Feng, A. McNally, and Z. Zong. Klebsiella oxytoca complex: Update on taxonomy, antimicrobial resistance, and virulence. *Clin Microbiol Rev*, 35(1):e0000621, 2022.

[5] R. A. Hussein, S. H. AL-Kubaisy, and M. T.S. Al-Ouqaili. The influence of efflux pump, outer membrane permeability and β-lactamase production on the resistance profile of multi, extensively and pandrug resistant klebsiella pneumoniae. *Journal of Infection and Public Health*, 17(11):102544, 2024.

[6] T. Shanthini and N. Ramesh. Phenotypic detection of hypermucoviscous phenotype in colistin-resistant klebsiella oxytoca from tamil nadu. *Research Journal of Biotechnology Vol*, 15:10, 2020.

[7] M. Aqeel. Biofilm development and resistance to antibiotics in klebsiella pneumoniae isolated from clinical specimens in samawwa city, iraq. *Journal of College of Education for Pure Science*, 13(3), 2023.

[8] C. TOMULESCU, M. MOSCOVICI, I. LUPESCU, R. M. STOICA, and A. VAMANU. A review: Klebsiella pneumoniae, klebsiella oxytoca. *Rom. Biotechnol. Lett*, 26:2567–2586, 2021.

[9] E. M. Alexander *et al.* Biosynthesis, mechanism of action, and inhibition of the enterotoxin tilimycin produced by the opportunistic pathogen klebsiella oxytoca. *ACS infectious diseases*, 6(7):1976–1997, 2020.

[10] R. Emad, R. R. Hafidh, and M. Z. Zaman. Identification of klebsiella oxytoca by vitek-2 system in baghdad hospitals. *Journal of the Faculty of Medicine Baghdad*, 65(4):374–380, 2023.

[11] R. M. Murshid, M. T. S. Al-Ouqaili, and B. A. J. Kanaan. Microbial vaginosis and its relation to single or multi-species biofilm in iraqi women: Clinical and microbiological study. *Pakistan journal of biological sciences: PJBS*, 27(8):404–412, 2024.

[12] D. Freeman, F. Falkiner, and C. Keane. New method for detecting slime production by coagulase negative staphylococci. *Journal of clinical pathology*, 42(8):872–874, 1989.

[13] S. Er. Comparison of the microtiter plate method and the congo red agar technique in the determination of staphylococcal biofilm. *Mugla Journal of Science and Technology*, 10(1):128–132, 2024.

[14] P. Shibu, F. McCuaig, A. L. McCartney, M. Kujawska, L. J. Hall, and L. Hoyles. Improved molecular characterization of the klebsiella oxytoca complex reveals the prevalence of the kleboxymycin biosynthetic gene cluster. *Microbial Genomics*, 7(6):000592, 2021.

[15] S. Alvarez, J. A. Stinnett, C. G. Shell, and S. L. Berk. Klebsiella oxytoca isolates in a general hospital. *Infection Control & Hospital Epidemiology*, 6(8):310–313, 1985.

[16] C. Lowe *et al.* Outbreak of extended-spectrum β-lactamase-producing klebsiella oxytoca infections associated with contaminated handwashing sinks(1). *Emerg Infect Dis*, 18(8):1242–7, 2012.

[17] G. Muteeb, M. T. Rehman, M. Shahwan, and M. Aatif. Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. *Pharmaceuticals*, 16(11):1615, 2023.

[18] A. Ghasemian, A. Mohabati Mobarez, S. Najar Peerayeh, A. Talebi Bezmin Abadi, S. Khodaparast, and S. S. Mahmood. Expression of adhesin genes and biofilm formation among klebsiella oxytoca clinical isolates from patients with antibiotic-associated haemorrhagic colitis. *Journal of medical microbiology*, 68(7):978–985, 2019.

[19] C. a. L. S. I. CLSI. Performance standards for antimicrobial susceptibility testing clsi supplement m100. 2023.

[20] G. N. Rajivgandhi *et al.* Identification of carbapenems resistant genes on biofilm forming k. pneumoniae from urinary tract infection. *Saudi Journal of Biological Sciences*, 28(3):1750–1756, 2021.

[21] M. Validi, M. M. Soltan-Dallal, M. Douraghi, J. Fallah-Mehrabadi, A. Rahimi-Foroushani, and H. Froheshtehrani. Identification of cytotoxin-producing klebsiella oxytoca strains isolated from clinical samples with cell culture assays. *Microbial pathogenesis*, 113:1–4, 2017.

[22] R. Saha, C. E. Farrance, B. Verghese, S. Hong, and R. S. Donofrio. Klebsiella michiganensis sp. nov., a new bacterium isolated from a tooth brush holder. *Current microbiology*, 66:72–78, 2013.

[23] E. Leitner *et al.* Improved diagnosis of antibiotic-associated haemorrhagic colitis (aahc) in faecal specimens by a new qualitative real-time pcr assay detecting relevant toxin genes of klebsiella oxytoca sensu lato. *Clinical Microbiology and Infection*, 28(5):690–694, 2022.

[24] H. Tse *et al.* A tricyclic pyrrolobenzodiazepine produced by klebsiella oxytoca is associated with cytotoxicity in antibiotic-associated hemorrhagic colitis. *Journal of Biological Chemistry*, 292(47):19503–19520, 2017.

[25] R. M. Schmithausen *et al.* The washing machine as a reservoir for transmission of extended-spectrum-beta-lactamase (ctx-m-15)-producing klebsiella oxytoca st201 to newborns. *Applied and environmental microbiology*, 85(22):e01435–19, 2019.

[26] S. P. Cangui-Panchi *et al.* Battle royale: immune response on biofilms–host-pathogen interactions. *Current research in immunology*, 4:100057, 2023.

[27] K. H. P. Riwu, M. H. Effendi, F. A. Rantam, A. R. Khairullah, and A. Widodo. A review: virulence factors of klebsiella pneumonia as emerging infection on the food chain. *Veterinary world*, 15(9):2172, 2022.