

Hypervirulent and the Multi-Drug Resistant *Klebsiella oxytoca*: A New Emerging Pathogen in Baghdad Hospitals, Iraq

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ABSTRACT

Background: The human microbiota, *K. oxytoca*, is resistant to multiple drugs, endangering the lives of patients. Hypervirulent strains evolved into multidrug-resistant strains due to the acquisition of mobile genetic elements.

Objectives: To detect the antibiotic-resistant profile and the related virulence genes in the hypervirulent and non-hypervirulent strains isolated from clinical specimens.

Materials and methods: A total of 136 clinical samples were collected from patients at Baghdad City Hospitals, Iraq. The isolates were identified, and an antibiotics sensitivity test was carried out. The polymerase chain reaction typing method was used to detect the virulence genes.

Results: The most frequent source of *K. oxytoca* was urine samples (36.03%), then blood samples (15.44%), particularly inpatient samples. About 12.5% of isolates were positive for the hypervirulent test (the string test). Isolates showed variable levels of resistance towards antibiotic groups. The *bla_{CTX-M}* and *aac(6')-Ib-cr* genes were revealed in 88% of isolates, and the *bla_{OXA-48}* gene was in 44%. All the tested isolates were negative for the *rmpA* gene.

Conclusion: *K. oxytoca* is recognized as one of the leading causes of hospital-acquired infections. The rapid identification of antibiotics-resistant, hypervirulent isolates that present a considerable threat to human health is highly recommended in the local hospitals.

Keywords: Antibiotic resistant genes; Hypervirulent; *K. oxytoca*; Multiple drugs resistance; Non-hypervirulent.

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INTRODUCTION

There are at least four species in the genus *Klebsiella*. The two most significant human pathogens in terms of disease severity and frequency are *K. pneumoniae* and *K. oxytoca*. *K. pneumoniae* is an opportunistic pathogen that can colonize mucosal surfaces in humans and cause serious nosocomial and community-acquired infections [1].

Significantly, *K. oxytoca* is being isolated more frequently after *K. pneumoniae* species, *K. oxytoca* is the second most frequent *Klebsiella* group identified as the source of clinical infections in humans. The Asia-Western Pacific region, North America, and Western Europe have seen the majority of *K.*

oxytoca infections, with Africa and South America seeing less of them. All types of infections caused by *K. oxytoca* are observed in Western Pacific Asia, Europe, and North America, whereas antibiotic-associated hemorrhagic colitis is more common in Western Pacific Asia, especially in Iran and Japan [2]. In Iraq, few studies were available discussing the prevalence of *K. oxytoca* in clinical samples. A study conducted by Ahmed *et al.*, in 2022 found that from 250 collected clinical samples, *K. oxytoca* was recorded in 32 (12.8%) cases, in which the main prevalence was detected in throat swabs, wound swabs, and vaginal swabs, respectively [3]. Moreover, another study done by Jasim *et al.*, in 2020 found that out of 100 clinical samples, *K. oxytoca* was detected in 50 samples, mainly from urine samples [1]. Like its sister, *K. oxytoca* can survive in a variety of environments, such as moist environments, hostile environments like hand-washing soaps, prosthetic materials like central venous catheters, and within gastrointestinal flora, which all support its capacity to pro-

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duce opportunistic infections in medical settings, It is closely related to *K. pneumoniae*, but may be identified by being indole-positive [4].

K. oxytoca was isolated from a variety of clinical specimens, primarily from the blood and secretions of the respiratory system, with a clear clinical impact on patients admitted to intensive care units who are immunocompromised and weak. Natural habitats for *K. oxytoca* include the mouth, nose, and gastrointestinal tract. It is a gut bacterium inside the human intestine, but it can lead to life-threatening infections outside the gut [5].

The hypermucoviscosity (HMV) phenotype is an identified virulent factor of *K. pneumoniae*, which can be established by a simple technique: the colony on an agar plate is stretched using a loop, and a positive string test is characterized as the development of viscous threads that are > 5 mm long. Current studies show that hypervirulence and hypermucoviscosity are different phenotypes. Therefore, hypervirulence can be defined as the power of bacteria to generate invasive infections following the primary source of infection (metastatic transmission) in apparently healthy adults. The hypermucoviscous phenotype of *K. oxytoca* was not previously documented in studies, and it is also noted that its virulence nature was not thoroughly investigated [6].

K. oxytoca is a rapidly developing multidrug-resistance opportunistic pathogen. This organism can cause pneumonia, urinary tract infections, soft tissue infections, and sepsis, often leading to septic shock. In the past, *K. oxytoca* was more commonly isolated in the neonatal intensive care unit, but now it can be isolated from a variety of samples even from adult patients admitted to the intensive care unit. It exhibits multidrug resistance and is more resistant than *K. pneumoniae* [7]. By producing extended-spectrum beta-lactamases, *K. oxytoca* is highly resistant to ampicillin and penicillin. In Europe, 10–20% of these bacteria are also resistant to multiple drugs like ceftazidime and cephalosporins. This is because they have Class A chromosomal beta-lactamase genes and plasmid-borne beta-lactamase genes. Due to the limited efficiency and effectiveness of current antibiotics and the rising tolerance and resistance to multiple drugs, there is an increasing need for novel approaches to combat bacterial resistance and persistence. A thorough understanding of the factors that influence the development of antibiotic-resistant bacteria is crucial [2].

Due to the prevalence of *Klebsiella spp.* and the rapid development of highly virulent strains, particularly, antibiotic-resistant strains, those are associated with greater incidence and fatality. Thus, the current study aimed to characterize the antibiotic-resistant profile of the hypervirulent and non-hypervirulent *K. oxytoca* strains isolated from clinical specimens. In addition, it aimed to investigate the mobile genetic elements that associated with the hypervirulent criteria. Such genes are, the regulator of the mucoid phenotype (*rmpA* gene) found only in strains that possess a hypermucoviscous phenotype. The gene *bla_{OXA-48}* carbapenemase is related to carbapenem resistance. The gene *bla_{CTX-M}* is related to the extended-spectrum beta-lactamase resistance (ESBL). The gene *aac6'-Ib-cr* is related to plasmid-mediated quinolone resistance (PMQR).

MATERIALS AND METHODS

Bacterial isolation

A cross-sectional study was conducted to estimate the prevalence of *K. oxytoca*. The collected samples were 136

patients attending different hospitals and their laboratories in Baghdad City, Iraq, including Baghdad Teaching Hospital (Medical City), Ghazi Al-Hariri Hospital for surgical specialties (Medical City), Teaching Laboratories (Medical City), Ibn Al-Balady Hospital, Yarmouk Teaching Hospital, Al-Imam Al-Kazem Hospital, and Al-shahid Alsadr Hospital, in the period from July to November 2022. Patients were permitted to obtain the sample, keeping the personal information of patients anonymous. Informed consent was obtained from every participant enrolled in the current study.

All the specimens with positive *K. pneumoniae* were included, while specimens with other genera or species of bacteria were excluded.

The samples from patients were divided into two categories: samples from inpatients or hospital-acquired infections, and samples from outpatients or community-acquired infections. A "nosocomial infection" or "healthcare-associated infection" occurs in a patient who is receiving medical care in a hospital or other health care facility and was absent upon admission [8]. Hence, all the samples in the current study which were considered as hospital-acquired infections were approved by clinicians who recorded that patients were admitted to the hospital for another reason and the recent infection wasn't documented at the time of admission.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Department of Microbiology/College of Medicine/University of Baghdad, No. 023 on 19th of June 2023.

Bacterial identification

The phenotypic examination of isolates was first determined by culturing on selective media, including MacConkey agar and blood agar. The VITEK-2 Compact (bioMérieux, Marcy l'Etoile, France) was used to identify the isolated bacteria to the genus and species level. The reagent cards, "Gram Negative (GN) Colorimetric Identification Card", have 64 wells that can each check an individual test substrate. Different metabolic processes, like acidification, alkalization, enzymatic hydrolysis, and growth in the presence of inhibitory substances, are controlled by the substrates. Each product is listed with quality control strains [*Enterobacter hormaechei* (ATCC 700323)] and its expected results, and it was tested according to the procedures described in the instructions found within the VITEK 2 Compact System and OBSERVA Computer System (bioMérieux) manuals [9]. The automated biochemical test was used to ensure the primary identification of *K. pneumoniae*. The indole test was used as a confirmatory test to confirm the species as *K. oxytoca* by the formation of the red ring, which was repeated three times to exclude any error.

The hypermucoviscous phenotype

To detect the hypervirulent (hypermucoviscous) characteristic of the isolates, a string test was conducted on all 136 identified *K. oxytoca*. The bacterial isolates were an overnight culture growing on blood agar plates. The colony on an agar plate is stretched using a loop, and a positive string test is characterized as the development of viscous threads that are > 5 mm long [6].

The sensitivity of the bacterial isolates

The test was performed on Mueller Hinton agar using the disc diffusion method (Kirby Bauer) to determine the

resistance of *K. oxytoca* against 10 antibiotics, including gentamicin (10 µg/disc), piperacillin (100 µg/disc), cefotaxime (30 µg/disc), cefepime (10 µg/disc), imipenem (10 µg/disc), meropenem (10 µg/disc), tetracycline (10 µg/disc), and ciprofloxacin (10 µg/disc). The plates were incubated at 37°C for 18 hours under aerobic conditions, and inhibition zone diameter around the antibiotic discs was performed and compared with the tables of international measurements according to the Clinical and Laboratory Standards Institute (CLSI, 2022). Multidrug-resistant (MDR) was referred to as having acquired resistance to at least one drug from three or more antibiotic classes. Extensively drug-resistant (XDR) was referred to as being resistant to all but one or two antibiotic classes in at least one instance [10].

DNA extraction

Whole genome extraction and purification were done on 25/136 fresh bacterial colonies using the ABIOPure protocol (ABIOPure™ Total DNA ABIOPure, USA). Polymerase chain reaction (PCR) was done to investigate the presence of *rmpA* gene (regulator of mucoid phenotype A), *bla_{CTX-M}* beta-lactamases gene, *bla_{OXA-48}* beta-lactamases, and *aac(6′)-Ib-cr* gene using specific primers (Table 1). All the primers were designed by Primer Blast NCBI. For a PCR reaction volume of 25 µl, a total of 12.5 µl PCR Master Mix 2× (Promega/USA), 2 µl of template DNA, 1 µl of both the forward and reverse primers (10 µM) and 8.5 µl of nuclease free water were added. The mixes are placed in the thermocycler after vortex mixing. After adjusting the thermocycler program, 32 cycles in total were run. As follows: 95°C pre denaturation for 5 minutes, 95°C denaturation for 30 seconds, *bla_{CTX-M}* 61.5°C, *rmpA* 61.3°C, *bla_{OXA-48}* 63°C, and *aac6-lb-cs* 66.4°C annealing temperatures for 30 seconds, 72°C extension for 30 seconds, and 72°C final extension for 7 minutes. The PCR product was analyzed on a 1.5% agarose gel using Agarose Gel Electrophoresis to confirm the presence of the amplified genes [11].

Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences System (SPSS) version 22. The effect of different factors in the studied parameters was detected by the program. The significant comparison between percentages was calculated using Chi-square (0.05 and 0.01 probability).

$$(O - E)^2$$

Table 1. Primers' names and sequences with their product sizes.

Primer Name	Oligo Sequence 5'→3'	Product Size (bp)
<i>rmpA</i>	F-ATGGCCTAAAGCAGTTAACTG	560
	R-CTAAATACTTGGCATGAGCCA	
<i>bla_{OXA-48}</i>	F-TTGGTGGCATCGATTATCGG	738
	R-GAGCACTTCTTTTGTGATGGC	
<i>bla_{CTX-M}</i>	F-CGCTTTGCGATGTGCAG	551
	R- ACCGCGATATCGTTGGT	
<i>aac6′-Ib-cr</i>	F-TTGGGATGCTCTATGAGTGGCTA	482
	R-CTCGAATGCCTGGCGTGTTT	

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

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

RESULTS

The recorded data found that 77 (56.6%) out of 136 isolates were from inpatients (hospital-acquired infections), while 59/136 (43.3%) were from outpatients (community-acquired infections). The bacterial isolates were diagnosed as *K. pneumoniae* by the manual and automated VITEK 2 Compact System. The species was confirmed as *K. oxytoca* using the biochemical indole test. *K. oxytoca* was significantly isolated from urine samples (n = 49, 36.03%) followed by blood samples (n = 21, 15.44%), and then sputum samples (n = 19, 13.97%). While the isolates from urethral swabs and cerebral spinal fluid were the less prevalent source 1 (0.74%), as shown in (Table 2). The source of the samples and the incidence of *K. oxytoca* infection were significantly associated. The recorded data showed that male cases were more affected (n = 72, 52.94%), by *K. oxytoca* than female cases (n = 64, 47.06%); however, there were no significant associations (P-value = 0.492) between both sexes.

Figure 1 shows the results of the string test in this study. It showed that 17 out of 136 isolates (12.5%) were string test positive and were therefore likely to be highly infectious *K. oxytoca* isolates. Whereas, 119/136 (87.5%) of them were identified as non- hypervirulent *K. oxytoca*. The sample source and the string test positivity were significantly correlated (Table 3).

The antibiotic resistance prototype of *K. oxytoca* was 98.5%, 92.6%, 86.7%, and 88.2% against amoxicillin, cefotaxime, cefepime, and piperacillin on the one hand, and 65.4%, and 59.5% against tetracycline and ciprofloxacin on the other hand, respectively. The analyzed data revealed that the sensitivity was detected against imipenem at 40.4%, gentamicin at 51.4%, and meropenem at 55.1%.

Table 2. Primers' names and sequences with their product sizes *.

Source	Number	%
Urine	49	36.03
Blood	21	15.44
Sputum	19	13.97
Ascites fluid	9	6.62
Wound swab	9	6.62
Endotracheal tube aspiration	8	6.15
Ear swab	5	3.67
Burn	5	3.67
Indwelling urinary catheters	4	2.94
Stool	3	2.21
Wound pus	2	1.47
Urethral swab	1	0.74
Cerebral spinal fluid	1	0.74
Total	136	100%
Chi-Square $-\chi^2$		64.207*
(P-value)		(0.0001)

* P-value ≤ 0.01) highly significant.

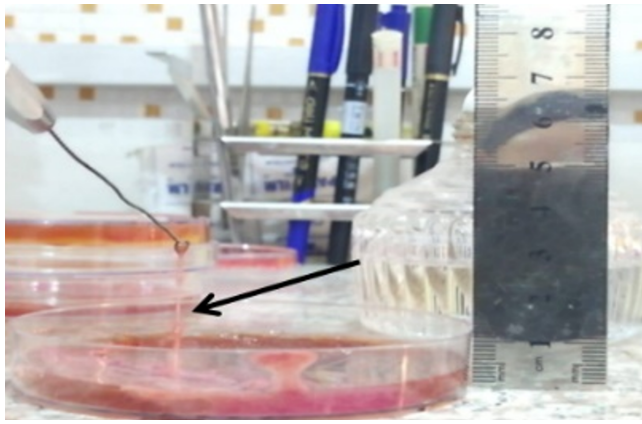


Figure 1. The black arrow shows the positive string test thread that is > 5 mm long for colonies of *K. oxytoca*.

Table 3. The distribution of the studied sample according to source vs. string test positivity *.

Source	String test positive	Percentage (%)
Urine	6	35.29
Blood	3	17.65
Sputum	2	11.76
Fluid	1	5.88
Wound Swab	3	17.65
Ear Swab	1	5.88
Burn	1	5.88
Total	17	100%
Chi-Square $-\chi^2$		4.791*
(P-value)		(0.0437)

* P-value < 0.05).

The results showed that 13 out of 136 (0.09%) of the isolates were designated as having an MDR phenotype, while 67/136 (49%) of the isolates were categorized as having an XDR phenotype, with highly significant association. Based on the PCR results, 25 out of 136 *K. oxytoca* isolates were investigated to harbor the virulence genes. Results showed that out of 25 isolates, there were 22 (88%) isolates positive for *bla_{CTX-M}* and *aac(6')-Ib-cr* genes, with product sizes of 551bp and 482bp, respectively, as shown in Figures 2 and 3. Moreover, 11 isolates (44%) were positive for the *bla_{OXA-48}* gene with a product size of 738bp (Figure 4). Unlike, all the tested 25 isolates, all were negative for the *rmpA* gene (Figure 5).

DISCUSSION

In this study, Klebsiella isolates were mostly identified by the characteristics of the colonies on MacConkey and blood agars, as well as features seen under a microscope. Interestingly, when the indole test was done for all 136 isolates, the results of the indole test were positive. Thus, based on this confirmatory test and its results, the isolates were determined to be *K. oxytoca*. It was completely disagreeing with the results of the VITEK-2 system, which made the system's results questionable. The rate of *K. oxytoca* infections in Iraq

was shown to be increasing at different rates. Thus, it was evident that the rate of infection with *K. oxytoca* increased from one year to another. The reason behind this may be due to the high virulence factors of *K. oxytoca*, as it possesses a large capsule and is resistant to antibiotics easily. The capsule plays an important role in its resistance to the immune system [12, 13].

The current study found that urine was the most frequent source of *K. oxytoca*, accounting for 49/136 (36.03%), with blood coming in second at 21/136 (15.44%). The results were in line with those of other studies that found *K. oxytoca* to be the most common bacterial cause of UTIs in pregnant women (19.4% to 38.1% of all isolated bacterial uropathogens; second only to *Escherichia coli* [14]). It was proven that some of the reasons for the appearance of *K. oxytoca* in the urine at a greater rate than others are due to the use of catheters for a long time, especially those in hospitals, and that it also causes UTIs in older women [4].

The present study results found that *K. oxytoca* had emerged as a major cause of hospital-acquired infections. The collected samples found that 77/136 (56.6%) were from inpatients (hospitalized), while 59/136 (43.3%) were from outpatients (community-acquired). The highly resistant gram-negative Enterobacteriales continue to disseminate in hospitals, causing therapy problems in many parts of the world [15]. These results were also in line with Alvarez *et al.* (1985), who found that 21/44 (48%) isolates were community-acquired and 23/44 (52%) were considered nosocomial in origin. Thus, it is reported that *K. oxytoca* is an important cause of hospital-acquired infections, especially in neonatal intensive care units [16].

The sex-wise occurrence of *K. oxytoca* among the isolated clinical specimens found that the isolates in the present study were more common in samples from males than females. This may be due to the number of samples that need to be increased to overcome these differences.

The results of the string test in the present study revealed that 17/136 (12.5%) isolates were found to be string test positive and designated as probable hypervirulent *K. oxytoca* isolates. Whereas, 119/136 (87.5%) of them were identified as non-hypervirulent *K. oxytoca*. The hypermucoviscosity of the isolates usually seems to be related to an aggressive type of infection, and therefore, these isolates are reflected as hypervirulent [6]. The effective virulence factor of the hypervirulent is the *rmpA* gene (regular mucoid phenotype) which was investigated in the current study and tried to be linked with the positive hypermucoviscosity phenotype. This gene mediates capsule production and hypermucoviscosity, which is a critical virulence factor [17]. According to the antibiotic sensitivity test results, multiresistant isolates were categorized into MDR and XDR [10]. Although only 13 out of 136 isolates showed MDR activity, there was no significant association. Infections caused by MDR *K. pneumoniae* has been frequently reported in Iraqi hospitals [18]. As fewer or possibly no effective medications are available to treat those extremely drug-resistant isolates, this could make it difficult to treat the infections linked to the isolates. However, the isolates were responsive to imipenem, gentamicin, and meropenem in some way in 40.4%, 51.4%, and 55.1% of the cases, respectively. It was not known what the MDR or XDR activities of *K. oxytoca* were at the time of this study because *K. pneumoniae* and *K. oxytoca* are very similar, which is why they are called sisters [19].

The extracapsular polysaccharide synthesis regulator gene

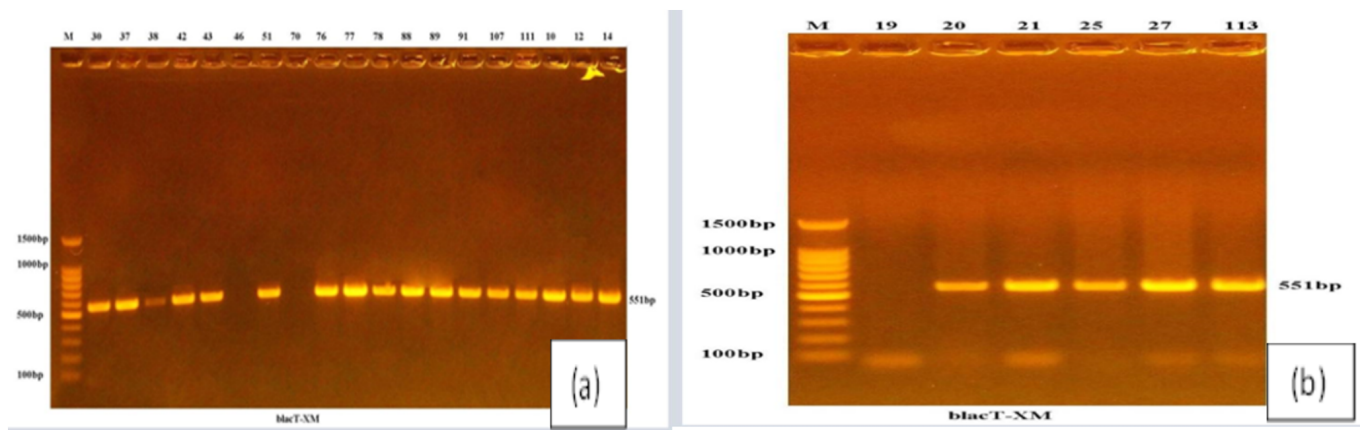


Figure 2. (a) The picture shows how the *bla_{OXA-48}* gene was amplified in different types of bacteria that were separated on a 1.5% agarose gel and stained with ethidium bromide. M: 100bp ladder marker. Lanes 25–136 resemble 738bp the PCR products; (b) It shows results of the amplification of the *bla_{OXA-48}* gene of the 25 selected bacterial isolates which were separated on a 1.5% agarose gel and ethidium bromide stained. M: 100bp ladder marker. Lanes 25–136 resemble 738bp the PCR products.

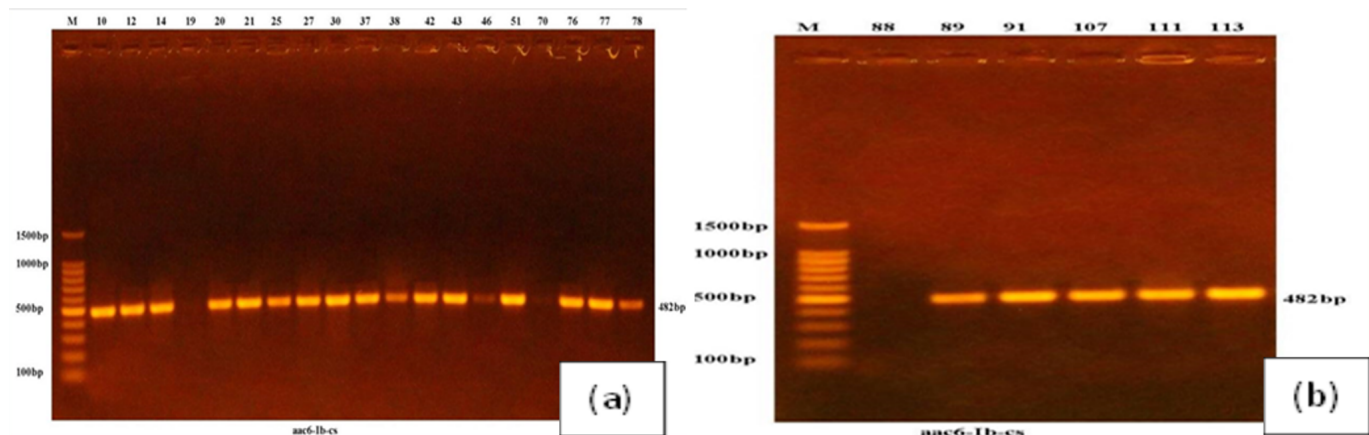


Figure 3. (a) It shows the amplification results of the *aac(6')-Ib-cr* gene of the 25 selected bacterial isolates that were separated on a 1.5% agarose gel and ethidium bromide-stained M: 100bp ladder marker. Lanes 25–136 resemble 482bp the PCR products; (b) It shows the amplification results of *aac(6')-Ib-cr* gene of the 25 selected bacterial isolates which were separated on a 1.5% agarose gel and ethidium bromide-stained M: 100bp ladder marker. Lanes 25–136 resemble 482bp PCR products.

(*rmpA*), is connected to the hypermucoviscosity phenotype [20]. All 25 *K. oxytoca* isolates, which were subjected to the amplification in the current study were found to be negative for the *rmpA* gene. This gene was previously reported in *K. pneumoniae* isolates studied in Iraq. This gene was recorded in *K. pneumoniae* local isolates; the chance of being transferred to *K. oxytoca* is high. The isolates with negative *rmpA* gene results were positive for the hypermucoviscosity phenotype. Hence, other regulatory genes may be connected to the expression of the hypermucoviscosity phenotype. This means that the strains were positive for the hypermucoviscosity phenotype but negative for the *rmpA* gene may be explained by the presence of another gene responsible for this phenomenon, such as the *magA*-associated K1 serotype of the *K. oxytoca* [21]. Overall, more experiments are needed to figure out the complicated regulatory pathway that manages the expression of the hypermucoviscosity phenotype.

The results revealed that 22 (88%) were positive for the *bla_{CTX-M}* gene. In industrialized countries such as Canada,

France, and the United Kingdom, the spread of CTX-M has also been described through prospective studies [22]. Antibiotic-resistant outbreaks have dramatically increased as a result of *K. pneumoniae* isolates acquiring extended-spectrum-lactamase plasmids, Enhancing surveillance and control strategies for hospital-acquired infections is necessary due to the growing number of Klebsiella strains developing ESBLs, which limits therapy options [8]. This study explains how the widespread use of antibiotics has helped to develop a remarkable type of resistance in *K. oxytoca*. The production of β lactamases is expressed among Enterobacterales members as resistance to β -lactam antibiotics. In addition, the mechanism of resistance involved is the transferable plasmid mediated β -lactamases that have been described in multi-resistant *K. pneumoniae* [23].

The gene *bla_{OXA-48}* was detected in 11/25 isolates; the *bla_{OXA-48}* enzyme was first reported from a *K. pneumoniae* clinical isolate in Turkey in 2003. Subsequently, *bla_{OXA-48}* producing strains have been highly stated as sources of hospi-

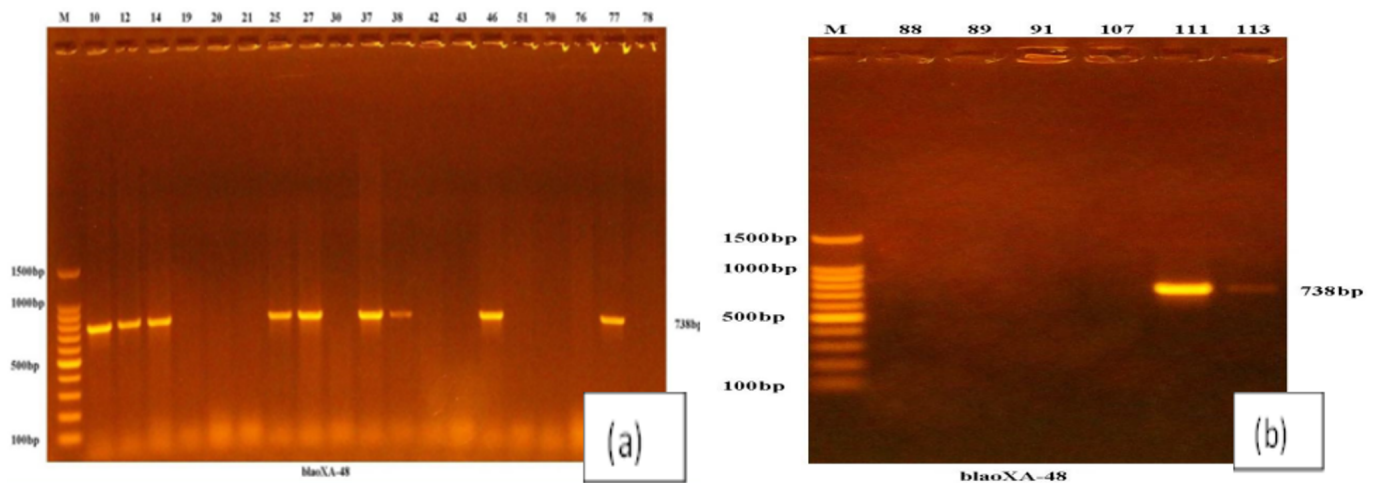


Figure 4. (a) The picture shows how the *bla_{OXA-48}* gene was amplified in different types of bacteria that were separated on a 1.5% agarose gel and stained with ethidium bromide. M: 100bp ladder marker. Lanes 25–136 resemble 738bp the PCR products; (b) It shows results of the amplification of the *bla_{OXA-48}* gene of the 25 selected bacterial isolates which were separated on a 1.5% agarose gel and ethidium bromide stained. M: 100bp ladder marker. Lanes 25–136 resemble 738bp the PCR products.

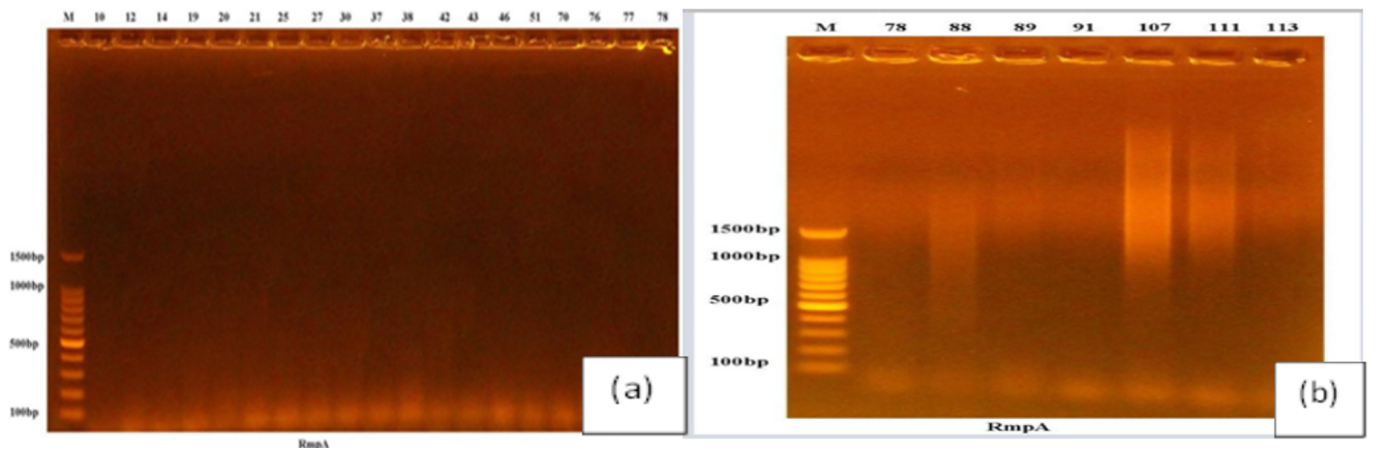


Figure 5. (a) It shows the amplification results of the *rmpA* gene of the 25 selected bacterial isolates, which were separated on a 1.5% agarose gel and ethidium-bromide stained M: 100bp ladder marker. Lanes 25–136 are comparable to the PCR products; (b) It shows the amplification results of the *rmpA* gene of the 25 selected bacterial isolates, which were separated on a 1.5% agarose gel and ethidium bromide stained. M: 100bp ladder marker. Lanes 25–136 resemble the PCR products.

tal acquired infections in Turkey [24]. Since 2003, the endemic spread of these bacteria has been reported in many countries, such as Kuwait, China, and Germany [25]. The present study found that the *bla_{CTX-M}* gene was more predominant than the *bla_{OXA-48}* gene in *K. oxytoca*. Thus, it was in agreement with another study, which recorded that the *bla_{CTX-M}* gene was the principal gene (98.6%) in *K. pneumoniae* compared to other ESBLs genes and is mainly encoded by IncFII; the conjugative epidemic plasmids, which has a critical role in their successful distribution and their prevalence [26].

One goal of this study was to find out how common the *aac (6')-Ib-cr* gene was in *K. oxytoca* clinical isolates that were less likely to be killed by ciprofloxacin in this study. One more reason for the high prevalence of *aac (6')-Ib-cr* could be that PMQR and ESBL genes are located on the same plasmid, which has been reported before [27]. These allelic variants of *aac (6')-Ib-cr* were found to be linked with *bla_{CTX-M}* genes

in isolates from many countries. Additionally, the association of *aac(6')-Ib-cr* with the *aac(6')-Ib-cr* ESBL genes has been widely reported in Uruguay and Argentina [28].

The current study faced some financial limitations in its investigation of other genes associated with the hyperviscosity phenotype, such as the *magA* gene. Furthermore, the gene that differentiates clinical isolates of *K. oxytoca* and *K. pneumoniae*.

CONCLUSION

Blood samples isolated from nosocomial infections were the most frequent source of *K. oxytoca* isolates, with urine samples—especially those from males—having the highest percentage. Despite the string test positivity of some of these isolates, the *rmpA* gene wasn't detected in the tested *K. oxytoca* isolates. The tested *K. oxytoca* isolates were found to contain a high percentage of the antibiotic resistant genes *bla_{CTX-M}*

and *aac6' Ib-cr* in comparison to the low percentage of the *bla_{OXA-48}* gene. Because they are both very virus-like and resistant to antibiotics, the hypervirulent antibiotic-resistant *K. oxytoca* isolates are a major health risk to humans.

ETHICAL DECLARATIONS

Acknowledgments

None.

Ethics Approval and Consent to Participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Department of Microbiology, College of Medicine, University of Baghdad, Baghdad, Iraq (Reference number 023 on 19th of June 2023). Informed consent was obtained from each participant.

Consent for Publication

No personal data is 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

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Authors' Contributions

Both authors have made significant, direct, and intellectual contributions to the work and have approved it for publication.

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