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Original Article

Bacteriological and Molecular Detection of *Klebsiella oxytoca* and its Resistance to Antibiotics among Clinical Specimens from Kirkuk, Iraq

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Abstract

Klebsiella spp. are gram-negative bacteria that are considered serious public health problems causing urinary tract infections, bloodstream infections, pneumonia infections, and soft tissue infections. This study was designed to investigate the prevalence of *Klebsiella oxytoca (K. oxytoca*) among clinical samples and determine their resistance against various antimicrobial medicines with molecular identification of *K. oxytoca* by polymerase chain reaction (PCR) technique using a specific sequence of pehX gene. A total of 250 clinical samples including throat, wound, and vaginal swabs were obtained. Participants were of both genders and different ages. The samples were streaked on the blood and MacConkey agars. Antibiotic sensitivity test was made by modified Kirby-Bauer disc diffusion technique. Molecular identification of *K. oxytoca* was performed for all isolates. Out of 250 clinical samples, *K. oxytoca* was reported in 32 (12.8%) cases. The highest prevalence was observed in 18(18%) cases of throat swabs, 16 (16%) cases of wound swabs, and 6 (6%) cases of vagina swabs. By the way, female cases were more affected 22 (14.5%) with *K. oxytoca* than male cases 10 (10.10%). Infected participants aged 15-40 years were more affected with *K. oxytoca* (23, 12.73%) compared to patients aged 41-65 years (9, 9.67%). The highest resistance pattern of *K. oxytoca* was 100% against Augmentin, Ampicillin, Cephalothin, Piperacillin, and Rifampin on one hand, and 62.50%, 59.37%, 53.12%, 53.12%, and 50% against Ceftazidime, Cefixime, Cefotaxime, Trimethoprim, and Aztreonam on the other hand, respectively. The highest sensitivity was observed against Amikacin and Imipenem (9.37%) and it was 21.87%, 21.87%, 25%, 25%, 28.12%, 28.12%, and 28.12% against Meropenem, Chloramphenicol, Nalidixic acid, Ciprofloxacin, Tobramycin, Gentamicin, and Doxycycline, respectively. Through molecular identification of *K. oxytoca*, all isolates showed a PCR product with 344-bp specific primer (pehX) that performed the *K. oxytoca*.

Keywords: Antibiotic resistance, Clinical samples, *Klebsiella oxytoca (K. oxytoca)*, PCR, pehX Gene

1. Introduction

Klebsiella spp. are gram-negative bacteria that are considered serious public health problems causing urinary tract, bloodstream, and soft tissue infections and also pneumonia (1). *Klebsiella oxytoca (K. oxytoca)* is highly resistant to ampicillin and penicillin by producing extended-spectrum beta-lactamase, which in Europe, approximately 10-20% of them are multi-drug resistant against ceftazidime or cephalosporins because of carrying class A chromosomal beta-lactamase and plasmid-borne beta-lactamases genes (2).

Since an accurate diagnosis of *Klebsiella* species by conventional biochemical tests is difficult, *K. pneumoniae* and *K. oxytoca* are only differentiated by indole reaction which is positive in *K. oxytoca* and negative in *K. pneumoniae* (3). The polymerase chain reaction (PCR) is an accurate, sensitive, and specific technique to detect *K. oxytoca* in which the specific

sequence of the *pehX* gene was used to encode a specific enzyme called polygalacturonase that cleaves a polygalacturonic chain of demethoxylated pectin. On the other hand, the *pehX* gene was used to provide accurate identification of *K. oxytoca*, and the PCR method was applied for a PCR amplicon of 344-bp which is typical for *K. oxytoca*. This gene was considered a specific, sensitive, and rapid method for the identification of *K. oxytoca* from other *Klebsiella* spp. (3, 4).

This study was designed to investigate the prevalence of *Klebsiella oxytoca* among clinical samples and determine their resistance against various antimicrobial medicines with molecular identification of *K. oxytoca* by PCR technique and using a specific sequence of *pehX* gene.

2. Materials and Methods

A total of 250 clinical samples including throat, wound, and vaginal swabs were obtained from Azadi Teaching Hospital in Kirkuk, Iraq, from January to May 2019. Participants in this study were of both genders and different ages.

The samples were streaked on the blood and MacConkey agars and then incubated at 37°C for 24 h. Gram stain and biochemical tests, such as oxidase, IMViC, urea hydrolysis, lactose fermentation, H2S production, coagulase, lysine decarboxylase, catalase, and gas production were done for *K. oxytoca* identification (5).

2.1. Antibiotic Sensitivity Test

Kirby-Bauer disc diffusion method was used to detect the sensitivity of isolates to Rifampin (5 µg), Gentamicin (10 µg), Imipenem (10 µg), Cefotaxime (30 µg), Aztreonam (30 µg), Meropenem (10 µg), Ciprofloxacin(5µg), Tobramycin (10 µg), Amikacin (30 µg), Doxycycline (10 µg), Ampicillin (10 µg), Ceftazidime (30 µg), Tobramycin (30 µg), Amoxicillin (25 µg), Cephalothin (30 µg) Cefixime (5 µg), Nalidixic (30 µg), Trimethoprim/Sulfamethoxazole (1.25/23.75µg), and Chloramphenicol (30 µg) (5).

2.2. Polymerase Chain Reaction

DNA extraction made by Bioneer kit PCR test was performed with species-specific primers forward primer 5- GAT ACG GAG TAT GCC TTT ACG GTG -3 and reverse primer 5- TAG CCT TTA TCA AGC GGA TAC TGG -3 were used for the amplification of the *K. oxytoca* target genes (*pehX* gene*)* with a molecular weight of 344-bp in size. The reaction was made by adding 20 µl of volume, 3 µl of a ready master mix, 2 µl of each primer, and 5 µl of DNA, while nuclease-free water was used to complete the volume. The PCR programming was as following: initial denaturation in one cycle for 5min at 95ºC, amplification in 35 cycles each for 30 sec at 94ºC, 30 sec at 55ºC, and 30 sec at 72ºC, followed by a final extension cycle for 7min at 72ºC (1, 6, 7).

2.3. Statistical Analysis

The data were analyzed in SPSS software (version 16.0) and a *P*-value less than 0.05 was considered statistically significant.

3. Results

Out of 250 clinical samples from the patients, *K. oxytoca* was reported in 32 (12.8%) cases through standard cultural and biochemical tests (Figure 1). The results showed that the highest prevalence was observed in 18 (18%) cases of throat swabs, 16 (16%) cases of wound swabs, and 6 (6%) cases of vagina swabs (Figure 2). The recorded data showed that female cases were more affected 22 (14.5%) with *K. oxytoca* than male cases 10 (10.10%) (Figure 3). On the other hand, the results revealed that patients aged 15-40 were more affected with *K. oxytoca* 23 (12.73%) than patients aged 41-65, 9 (9.67%) (Figure 4). The highest antibiotic resistance pattern of *K. oxytoca* was 100% against Augmentin, Ampicillin, Cephalothin, Piperacillin, and Rifampin on one hand and 62.50%, 59.37%, 53.12%, and 50% against Ceftazidime, Cefixime, Cefotaxime, Trimethoprim, and Aztreonam on the other hand, respectively (Figure 5).

The recorded data revealed that the highest sensitivity was observed against Amikacin and Imipenem (9.37%) on one hand and 21.87%, 21.87%, 25, 25%, 28.12%, 28.12%, and 28.12% against Meropenem, Chloramphenicol, Nalidixic acid, Ciprofloxacin, Tobramycin, Gentamycin, and Doxycycline on the other hand, respectively. Furthermore, according to the molecular identification of *K. oxytoca*, all isolates showed PCR product with 344-bp specific primer (pehX) which approved that the samples were affected by *K. oxytoca* (Figure 6).

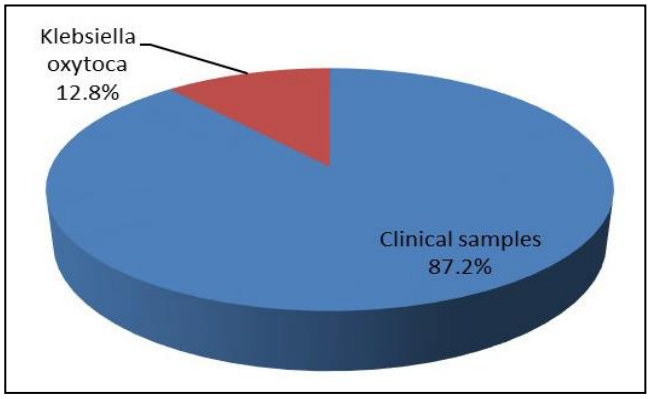

Figure 1. Prevalence of *Klebsiella oxytoca* among clinical samples

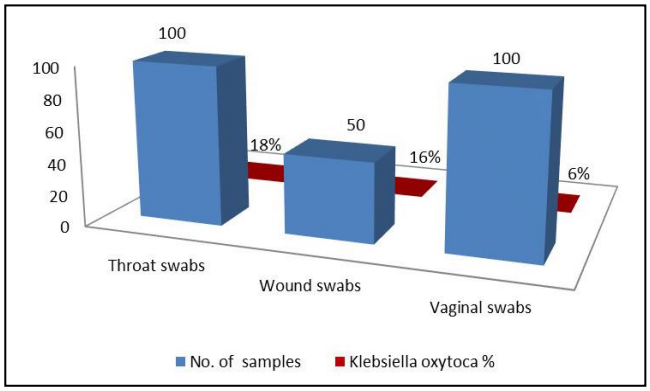

Figure 2. Distribution of *Klebsiella oxytoca* among different clinical samples

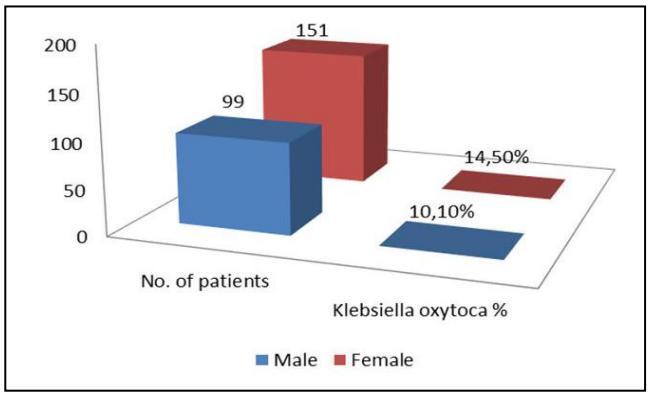

Figure 3. Prevalence of *Klebsiella oxytoca* depending on the patients' genders

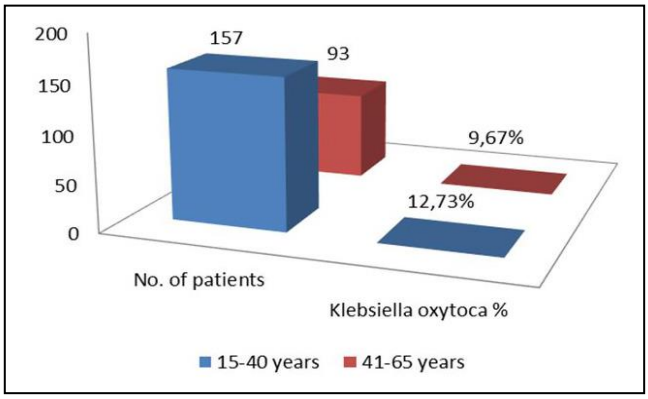

Figure 4. Prevalence of *Klebsiella oxytoca* depending on the patients' ages

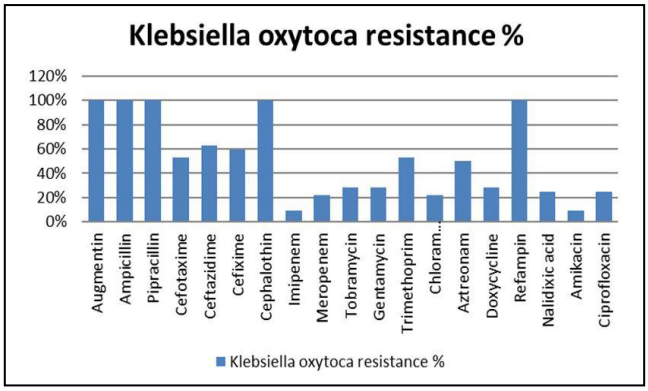

Figure 5. Resistance pattern of the clinical isolates

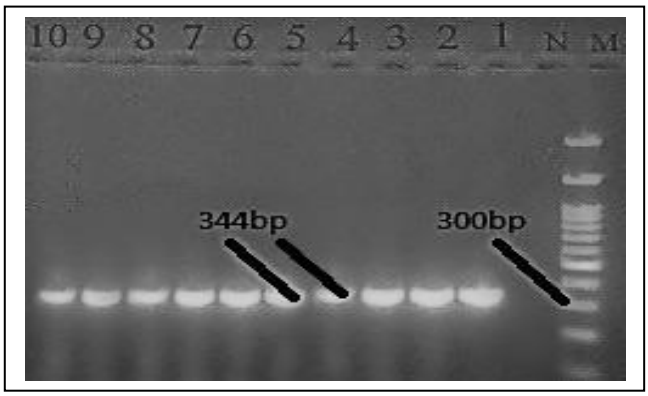

Figure 6. PCR product of (*pehX*) gene for *Klebsiella oxytoca* by gel electrophoresis

4. Discussion

In recent years, the increasing prevalence rate of *Klebsiella spp.* among patients has become a serious global issue. In this present study, the prevalence rate of K. *oxytoca* was reported 12.8% in the patients while a lesser prevalence rate (10.83%) was observed in a study by Younis, Elbialy (8)., 2017. However, studies by Chakraborty, Mohsina (5) 2016, and Zedan (1), showed a prevalence of 2% and 1.1%, respectively. These variations in prevalence rate could be due to the different geographical locations of the studies (6, 9-12).

The highest rate of *K. oxytoca* was isolated among female patients aged 15-45 years because the highest number of the collected samples in this study were from female cases and this age range was considered the reproductive age which was more associated with *Klebsiella* infections. This result agreed with Chakraborty, Mohsina (5) study in 2016.

Augmentin, Ampicillin, Cephalothin, Piperacillin, and Rifampin showed the lowest effect on K. oxytoca isolates while Amikacin and Imipenem showed the highest effect. These results were supported by many studies, such as Al-Khikani (13) in 2020, and AL-Khikani, Abadi (14) in 2020.

Furthermore, prolonged and inappropriate use of these antibiotics prescribed by doctors and the consumption of incomplete course of antibiotics by the patients will not eradicate the pathogen but also encouraged it to develop resistance against these medicines (13, 14). However, false diagnosis and misuse/overuse of these medicines are significant factors that increase the spread of antibiotic resistance (12).

All studied isolates demonstrated PCR product with 344-bp by *K. oxytoca* specific primer (*pehX*) that performed *K. oxytoca*. These results were similar to the results of the studies by Kovtunovych, Lytvynenko (15), (2003) and Zedan (1), (2017) in a way that all *K. oxytoca* isolates expressed *pehX* with 344bp. So *pehX* gene demonstrated specific identification of *K. oxytoca* because *K. oxytoca* is closely associated with *K. pneumoniae* in biochemical variation, so PCR was considered a specific, sensitive, and rapid method for identification of *K. oxytoca* from other *Klebsiella* spp. (15-19).

5. Conclusion

Since the identification of *Klebsiella* species by conventional biochemical methods was difficult, PCR was used for the identification of *Klebsiella* species from clinical samples in Kirkuk city, Iraq. By the way, the pehX gene was unique for *K. oxytoca* and could be used as a routine protocol for accurate identification in public laboratories in Iraq.

Authors' Contribution

Study concept and design: S. A. H.
Ahmed Hasan, S1 *, Mohammed Bakr, M1
Acquisition of data: S. A. H.
Analysis and interpretation of data: M. M. B.
Drafting of the manuscript: M. M. B.
Critical revision of the manuscript for important intellectual content: S. A. H. and M. M. B.
Statistical analysis: S. A. H.
Administrative, technical, and material support: S. A. H. and M. M. B.

Ethics

All procedures were approved by the ethics committee of the University of Kirkuk, Kirkuk, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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