

Identification of *Klebsiella oxytoca* by VITEK-2 System in Baghdad Hospitals

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Abstract

Background: *Klebsiella oxytoca* is a Gram-negative rod-shaped bacterium that is becoming resistant to multiple drugs and is frequently endangering patients' lives. It is a member of the human microbiota.

Objectives: To assess the value of identifying *K. oxytoca* using an automated diagnostic system (VITEK-2) as compared to traditional manual methods.

Materials and Methods: A total of 136 clinical specimens were collected from patients in Baghdad hospitals during a period from July to November 2022. VITEK-2 system was used to recognize the isolated bacteria to the genus and species level. The biochemical indole test was used as a confirmatory test at species level.

Results: *K. oxytoca* was more common in urine samples 49 (36.0%) followed by blood samples 21 (15.4%). Of the total collected samples 77 (56.6%) were from inpatients and (43.3%) were from outpatients. The primary identification by cultural and microscopic examinations diagnosed all the isolates as *Klebsiella*. VITEK-2 system recognized them as *K. pneumoniae*. The indole test confirmed the species as *K. oxytoca* by the formation of the red ring.

Conclusion: using of a simple biochemical test like indole is crucial in the clinical laboratories to investigate the accuracy of the bacterial identification to the species level. Continuous evaluation for the identification results of the automated systems is needed and can be done by updating the system software for the new emerging pathogens in the hospitals.

Keywords: *Klebsiella oxytoca*; *Klebsiella pneumoniae*; VITEK 2 system, Indole test; Bacterial identification system

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Introduction:

Klebsiella species are becoming a significant human pathogen. They are typically found in the intestines of humans and other animals, in water, and in soil. This bacterial infection causes prolonged hospital stays and a higher rate of patient morbidity. The main causes of increased infection and drug resistance concerns include comorbidities, compromised immune systems, and the overuse of antibiotics (1, 2). There are at least four species in the genus *Klebsiella*, family Enterobacteriaceae, including *K. pneumoniae*, *K. oxytoca*, *K. terrigena*, and *K. planticola* (3). *K. pneumoniae* and *K. oxytoca* are the two most significant human pathogens in terms of disease severity and frequency (4). *K. oxytoca* is being isolated

significantly more often. Although it shares a close relationship with *K. pneumoniae*, it can be distinguished from it by being indole-positive (5-7). The second most common *Klebsiella* group to be linked to clinical infections in people, after *K. pneumoniae* species, is *K. oxytoca* (8). *Bacillus oxytocus pernicius* was first discovered in old milk by Flugge in 1886. Bergey renamed it "*Aerobacter oxytoca*" in 1923, while Lautrop renamed it "*Klebsiella oxytoca*" in 1956 (9). *K. oxytoca* was once thought to be a subspecies of *K. pneumoniae*, but DNA relatedness investigations have now made it evident that the two species are clearly separate (9, 10). Like its sister, *K. oxytoca* can survive in a variety of environments, such as moist environments (11, 12), unfavorable conditions such as hand soap (13, 14), central venous catheters as examples of prosthetic materials (15), as well as in gut flora, all of which support its ability to cause opportunistic infections in hospitals (16).

K. oxytoca has been identified from numerous clinical samples, especially from blood and respiratory secretions, and it has a clear clinical impact on immunocompromised and weakened patients referred

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to intensive care units (17). It can be found in the mouth, nose, and gastrointestinal tract in its natural environments. It is an intestinal bacterium that can cause potentially fatal infections outside of the gut (18). The intensive care units of hospitals and nursing homes are notable locations where it is prevalent in the healthcare sector. It is an opportunistic pathogen that can infect hosts with weakened immune systems. *K. oxytoca* is the responsible agent in the development of pneumonia and is the second most common nosocomial infection in critically ill patient (19). It can cause urinary tract infections (UTIs), which is one of the most common diseases occurring across all age groups (20). It can also cause wound infections, soft tissue infections, and septicemia, which frequently leads to septic shock. Infection risk factors include prolonged hospital stays, long-term antibiotic use, use of ventilators and IV catheters, and diabetes (21).

It looks as mucoid lactose fermenter on MacConkey agar (22). In clinical laboratories VITEK 2 System is used as an automated identification tool of *Klebsiella* spp. (23). Due to the prevalence of *Klebsiella* spp. and the rapid development of highly virulent strains, particularly, antibiotic resistant strains which are associated with a greater incidence of *Klebsiella* infections and fatality.

The current study was conducted to evaluate the use of the automated diagnostic system (VITEK-2) to give a proper identification for *Klebsiella* spp. and compare it with the conventional manual method.

Materials and Methods

Bacterial isolation and identification: A lab-based study on 136 different clinical samples collected from patients, attending different hospitals for laboratory bacterial investigations in Baghdad City for the period of July to November 2022.

All the samples were already identified as genus *Klebsiella* species *K. pneumoniae*

The isolates' phenotypic investigation was first conducted by cultivating them on selective media, such as MacConkey agar and blood agar media. The automated VITEK-2 compact system was used to recognize the isolated bacteria to the genus and species level. 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.

The automated identification by VITEK-2 System: The VITEK-2 system uses turbidimetric method for susceptibility testing and fluorogenic technology to identify organisms. It uses 64-well cards with a barcode containing the card type, expiration date, lot number, and unique identification number. Available test kits include ID-GN (identification of Gram-negative bacilli) and ID-GP (identification of Gram-positive susceptibility). Within 10 hours, the VITEK-2

ID-GN card can identify 154 Enterobacteriaceae and a limited number of glucose non-fermenting Gram-negative bacteria. In up to eight hours, the VITEK-2 ID-GP card can detect 124 species of *Staphylococcus*, *Streptococcus*, *Enterococcus* and some other Gram-positive organisms. In this analysis, the VITEK-2 method was used to confirm the identification of *Klebsiella* spp. as follows:

1. In a sterile test tube, a single isolated colony of pure bacteria is suspended in 3 mL of saline.
2. The bacterial suspension and the conventional turbid standard solutions were compared. The final concentration must be between 0.5 and 0.63.
3. According to the diagnostic Gram stain, the VITEK-2 card or cassette was chosen.
4. The cassette and test tube racks are delivered to the system, and after being placed in the first filling area (filler), the boxes are automatically filled with bacterial suspension, and the device sends out an end signal.
5. The cassette is left on for 24 hours at 37°C. The results are read to diagnose bacteria. The manufacturer reported results as 96% to 100% excellent identification; 93% to 95% very good identification; 89% to 92% good identification; 85% to 88% acceptable identification, and conversely no identification in other isolates.

The manual identification by Indole test: The indole test is a biochemical test performed on bacterial species to determine the organism's ability to convert tryptophan to indole. This division is carried out by a chain of various intracellular enzymes, a system commonly referred to as "tryptophanase". The procedure of the conventional tube method for Indole test was as follows:

1. The tryptophan broth is inoculated with broth culture or the isolated colony of the test organism is emulsified in tryptophan broth.
2. The broth is incubated at 37°C for 24-28 hours in ambient air.
3. Later, 0.5 ml of Kovac's reagent is added to the broth culture.
4. The expected results were either a pink colored ring for positivity or no color change even after the addition of appropriate reagent for negative results (24, 25).

Results

In the present study a total of 136 clinical samples from various infection sites were collected from different hospitals and their laboratories in Baghdad City. The recorded data showed that male cases were more affected 72 (52.94%) with *K. oxytoca* than female cases 64 (47.06%).

Out of 136 isolates 77 (56.6%) were from inpatients and 59/136 (43.3%) were from outpatients (community-acquired). *K. oxytoca* were most

frequently isolated from urine samples 49 (36.0%) followed by blood 21 (15.4%) and sputum 19 (14.0%). The least frequent were isolates from urethral swab, CSF and aspiration, 1 case each (0.7%), table 1. The recorded data showed that male cases were more affected 72 (52.94%) with *K. oxytoca* than female cases 64 (47.06%). The results of the tested *Klebsiella* spp. revealed that all the species were *K. oxytoca* (formation of red ring as a positive result) not *K. pneumonia* (no red ring a negative result), (Figure 2B and 2 □C).

Table 1: Distribution of sample study according to Source

| Source | No. | (%) |
|----------------------------------|-----|-----------------------|
| Urine | 49 | 36.03 |
| Blood | 21 | 15.44 |
| Sputum | 19 | 13.97 |
| Fluid | 9 | 6.62 |
| Wound Swab | 9 | 6.62 |
| ETT (Endo tracheostomy's) | 7 | 5.15 |
| Ear Swab | 5 | 3.67 |
| Burn | 5 | 3.67 |
| Folly (Urine collection tube) | 4 | 2.94 |
| Stool | 3 | 2.21 |
| Wound Pus | 2 | 1.47 |
| Urethral Swab | 1 | 0.74 |
| CSF | 1 | 0.74 |
| Aspiration | 1 | 0.74 |
| Total | 136 | 100% |
| Chi-Square χ^2 (P-value) | --- | 64.207 ** (0.0001) |

** (P≤0.01).

** (P≤0.01) mean highly significant.



Figure (1) and (2): *Klebsiella* colonies on (A) – Pink and mucoid colonies on MacConkey agar (B, C) - the positive indole test with red rings.

Discussion

In the current study 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*. This is in complete disagreement with the results of the VITEK-2 system, which made the system results questionable. This may due to the biochemical profile of *K. oxytoca* was not included in the system under the Enterobacteriaceae family group which could be explained as the bacterium is not widely spread, and it is a new emerging pathogen among the clinical specimen. Furthermore, the rate of *K. oxytoca* infections in Iraq was shown to be increasing with different rates. Two recent Iraqi studies done in 2020 and 2022 to explore the rate of *K. oxytoca* infections and found the rates of 50/100 (50%) and 32/250 (12.8%) respectively. Thus, it was evident that the rate of infection with *K. oxytoca* different rate from one year to another. The reason behind this may be the high virulence factors of *K. oxytoca*, as it possesses a large capsule and acquires resistance to antibiotics

easily. The capsule plays an important role in its resistance to the immune system (26, 27).

The present study revealed that the most common samples from which *K. oxytoca* were isolated was urine. The results were in harmony with results from other studies showing that *K. oxytoca* was the second-most prevalent bacterial uropathogen, accounting for 19.4% and 38.1% of the isolated bacterial uropathogens in different research on pregnant women (28, 29). Some of the reasons for the appearance of *K. oxytoca* in the urine at a greater rate than others is due to the use of catheters for a long period of time, especially those in hospitals and it also cause UTIs in older women (7). UTIs are important because they cause acute morbidity and may result in long-term medical problems (30). 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 high number of inpatient samples with positive *K. oxytoca* may be due to acquiring the infection while hospitalized or may have taken place before admission to the hospital. This raises the importance of using a simple test like indole to diagnose the causative agent of the related infection. The Enterobacteriaceae family being the most commonly identified group overall unfortunately is responsible for hospital acquired infections (HAI) (31). The highly resistant gram-negative Enterobacteriaceae continue to spread in hospitals causing therapeutic problems in many parts of the world (32). These results are also in a line with those of Alvarez *et al.* in 1985, who found that (48%) of their isolates were community-acquired and (52%) were considered nosocomial in origin indicating that *K. oxytoca* is an important cause of hospital-acquired infections, especially in the neonatal intensive care units (33). The results of the present study indicated that the occurrence of *K. oxytoca* in the specimens was more common in samples from males than females. Women typically have stronger immune responses to self and foreign antigens than men, resulting in sex-based differences in autoimmunity and infectious diseases. Males are generally more susceptible than females to bacterial infections (34). Moreover, this may be due to the number of the samples that needs to be increased to overcome these differences.

The limitation of the study was the number of the bacterial samples. Therefore, more comparative studies are in need with a large number of bacterial samples from different hospitals in Baghdad city in Iraq to give a comprehensive judgment about the accuracy of the automated systems in the identification of bacteria.

Conclusions

The major source for *K. oxytoca* isolates was urine samples particularly from hospitalized patients in Baghdad hospitals. The routine identification techniques used by the laboratory staffs still not fully familiar with this new emerging pathogen. Depending on the available automated system like VITEK-2 without further evaluation to its accuracy is questionable. Thus, a simple biochemical test like indole is crucial in the clinical laboratories to investigate the accuracy of the bacterial identification to the species level. Continuous evaluation for the identification results of the automated systems is needed that could be done by updating the system software for the new emerging pathogen in the hospitals.

Authors' declaration

Conflicts of Interest: None. The authors confirm that all the Figures and Tables in the manuscript are belonging to the current study. Authors sign on ethical consideration's approval-Ethical Clearance: The project was approved by the local ethical committee in (Department of Microbiology/ College of Medicine/ University of Baghdad) according to the code number (198 on 19th July 2022).

Authors' contributions:

Study conception & design: Rand R. Hafidh. Literature search: Rusal Emad & Muhammad Zukhrufuz Zaman. Data acquisition: Rusal Emad. Data analysis: Muhammad Zukhrufuz Zaman. Manuscript preparation: Rusal Emad. Manuscript editing & review: Rand R. Hafidh.

Dr. Rand R. Hafidh is a **Managing Editor** for the journal but did not participate in the peer review process other than as an author. The authors declare no other conflict of interest.

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تشخيص بكتريا *Klebsiella oxytoca* بنظام VITEK-2 في مستشفيات بغداد

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 الخلاصة

الخلفية: *Klebsiella oxytoca* هي بكتيريا سالبة الجرام على شكل قضيب أصبحت مقاومة للعديد من الأدوية وكثيرا ما تعرض حياة المرضى للخطر. وهو عضو في الجراثيم البشرية.

الأهداف: تقييم التحديد الصحيح ل *K. oxytoca* باستخدام نظام تشخيص آلي (VITEK-2) ومقارنته بالطرق اليدوية التقليدية.

المنهجية: تم جمع ما مجموعه 136 عينة سريرية من المرضى في مستشفيات بغداد من تموز إلى تشرين الثاني 2022. تم استخدام نظام VITEK-2 للتعرف على البكتيريا المعزولة على مستوى الجنس والأنواع. تم استخدام اختبار الإندول البيوكيميائي كاختبار تأكيد على مستوى الأنواع.

النتائج: كان *K. oxytoca* أكثر شيوعا في عينات البول (49 (36.0%) تليها عينات الدم 21 (15.4%). كان 77 (56.6%) من العينات التي تم جمعها تعود لمرضى راقدين في المستشفى و 59 (43.3%) لمرضى العيادات الخارجية. تم تحديد الهوية الأولية من خلال الفحوصات الزرعية والمجهرية لتشخيص جميع العزلات على أنها *Klebsiella*. تعرف عليهم نظام VITEK-2 على أنهم *K. pneumoniae*. أكد اختبار الإندول أن الأنواع هي *K. oxytoca* من خلال تكوين الحلقة الحمراء.

الاستنتاج: يعد استخدام اختبار كيميائي حيوي بسيط مثل الإندول أمرا بالغ الأهمية في المختبرات السريرية للتحقيق في دقة التعرف البكتيري على مستوى الأنواع. هناك حاجة إلى تقييم مستمر لتحديد نتائج الأنظمة الآلية التي يمكن القيام بها عن طريق تحديث برنامج النظام للمرض الجديد الناشئ في المستشفيات.

الكلمات الدالة: *K. pneumoniae*، *K. oxytoca*، نظام VITEK 2، اختبار الإندول، نظام تحديد البكتيريا.