Annals of R.S.C.B., ISSN:1583-6258, Vol. 27, Issue 1, 2023, Pages. 242-248
Received 25 December 2022; Accepted 01 January 2023

Isolation and Identifection of Klebsiella Pneumoniae Isolated from Iraqi Patients

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Abstract
This study focused on the isolation and identification of *K. pneumoniae* isolates from patients with urinary tract infections. 62 isolates (32 women, 30 men) of *K. pneumoniae* clinical specimens of mid-stream urine were collected from three hospitals in Baghdad. The diagnosis of bacteria was done by culture, gram stain, biochemical tests,. A string test was done to detect the hypermucoviscosity strains. The result was, 4 (6.45%) isolates were HV+
Keywords: Klebsiella pneumoniae**,** biochemical tests

Introductıon
K. pneumonia is a gram-negative, encapsulated, rod-shaped, non-motile bacteria. It is a member of the *Enterobacteriaceae* family that colonises the mucosal surfaces of mammals. It can also be found in the sputum of the patient who has pneumoniae, as well as in the vegetation, water, and soil. In the case of human beings, these bacteria are an example of an opportunistic microorganism**,** This bacterium inhabits the oesophagus and the gastrointestinal system, from which it can easily enter the blood stream and other tissues and cause infections such as wounds, UTI, bacteremia, septicemia, hospital acquired pneumonia, and ventilator-associated pneumonia (Mirzaie and Ranjbar 2021, Riwu *et al.* 2020).). *K. pneumoniae* strains are resistant to a wide range of beta-lactams (penicillins, third-and fourth-generation cephalosporins, carbapenems, and monobactams) due to plasmid-encoded ESBLs and carbapenemases. This resistance additionally extends to other groups of antibiotics, including sulphonamides, fluoroquinolones, and aminoglycosides (Vuotto *et al.* 2017). The WHO has added *Klebsiella* spp. to the critical list of bacteria. This is due to the high rate of multidrug-resistant *Klebsiella* spp., which was detected in 2017 (Stojowska-swędrzyńska *et al.* 2022). Which means new therapeutics are definitely required (Bengoechea and Sa Pessoa 2019).
There are factors behind the colonization of *K. pneumoniae* in a community in addition to a hospital setting. There is a difference in the infections caused by these bacteria between countries. In a study by Ling reported that, infections caused by *K. pneumoniae* can be region-specific although there can be some form of intercontinental comparison (Ling *et al.* 2015).
Urinary tract infections are defined into two categories: complicated and uncomplicated. A complicated UTI is distinguished by the presence of factors that compromise the urinary tract or the host's defences. These factors include urinary obstruction, immunosuppression, urinary retention caused by neurological disease, renal failure, renal transplantation, kidney stones, catheters, and pregnancy. And the uncomplicated UTI, which affects people who are healthy and have no physical or neural urinary tract anomalies, and complicated, which is distinct

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from uncomplicated UTI because it is associated with factors that compromise the urinary tract or the host's (Priya 2019). The bladder (cystitis) and the urethra, are the first sites of the UTI. From these sites, the infection rises into the kidney (pyelonephritis) (Al-Badr and Al-Shaikh 2013).

Materıals And Methods

Clinical samples collection

The specimens were collected during the period from December 2021 to April 2022. From Ibin- Albalady hospital, Al-Imam Ali hospital, and Al- Zaafaranya hospital at Al-Resafa in Baghdad. 250 clinical specimens of mid-stream urine were collected from 100 women and 150 men suffering from urinary tract infections. 62 samples of Klebsiella pneumoniae were isolated from 30 men and 32 women at different ages. The identification of Klebsiella pneumoniae depends on the morphology and culture features on blood and MacConkey agar, biochemical tests, and Vitik2 (Biomerieux, France).

The preparation of culture media

The preparation of the culture media was achieved according to the manufacturing company

1. The MacConkey agar, Muller Hinton agar, brain heart infusion broth, Kligler iron agar, Indol, urea, Simon citrate, and semi-solid mannitol were prepared according to the manufacture company and were autoclaved at 121°C for 15 minutes.
2. Blood agar base 5–10 percent of sheep's blood was additionally added to a sterilised blood agar base that was cooled to 50 °C.

The bacteriology dignosis

MacConkey agar: The samples were cultured on MacConkey agar and incubated for 24 hours at (35± 2) °C to identify bacterial growth patterns on MacConkey agar media (Patel *et al.* 2017).

Blood agar: To identify the morphology of the colonies on blood agar media, the samples were cultured on blood agar and incubated at (35± 2) °C for 24 hours (Mahon *et al.* 2018).

String test (hypermucoviscosity test): If the colony that had been grown overnight on an agar plate at (35 ± 2) °C formed a mucoviscous colony with a string that was longer than 5 mm, the colony was declared positive for the test. *K. pneumoniae* strains that were classed as hypermucoviscosity had a positive string test result (Zheng *et al.* 2018).

Microscopic examination

Gram stain: A few colonies were taken from cultures on glass slides and the gram stain technique was done. The smear was examined under a microscope (oil emersion under 100x).This step is to examine cell morphology, such as the arrangement of cells, figure, size, and reaction (Becerra *et al.* 2016).

Biochemical tests

Cataleas test: On a glass slide, a few drops of H_2O_2 (hydrogen peroxide solution) were mixed with a few colonies of bacterial growth at a concentration of 3%. The production of bubbles, which is induced by the release of oxygen from the H_2O_2, is considered a positive result, while the absence of bubbles indicates the negative result of this test (Procop *et al.* 2020).

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Oxidase test: This test was achieved by adding a few colonies on filter paper containing a few drops of (N,N,N,N-tetramethyl-p-phenylenediamine). The purple blue colour was an indicator that the organism possessed cytochrome c oxidase (Shields and Cathcart 2010).

The bio chemical identification

The culture medium contains supplements such as chemical indicators and specific substrates that detect changes in pH or certain microbial by products. All of the media that were used in this study were prepared in accordance with the manufacturer's instructions.

Kligler iron agar: This test was performed to detect the ability of the isolated bacteria to ferment lactose and glucose (dextrose). A pure colony from culture media was stabbed at the bottom and streaked on the surface of a slant medium and was incubated for 18–24 hours at (35 2) °C.

Urea agar: This test was performed to detect if the bacteria containing the enzyme urease are capable of hydrolyzing urea, and producing ammonia. A pure colony was stabbed at (35 ±2) °C for 18–24 h on urea agar. This reaction causes a rise in the substance's pH that is more than 8.0. If the test is positive, the medium will turn pink.

Indole Test: This test was performed to determine whether or not the organism is capable of hydrolyzing tryptophan to produce indole. The principle of this test is that the combination of indole with the aldehyde group of p-dimethylaminobenzaldehyde results in the formation of a red colour complex. After 18–24 h of inoculation with a pure colony at (35 ± 2) °C of indole broth, Kovac's reagent was added, and the test was considered positive if a red ring formed.

Simon citrate agar: This test was performed to determine if the organism has the ability to utilise sodium citrate as a source of carbon for metabolism and growth. The Simon citrate agar was inoculated with a pure colony. If the media turns blue after 24-48 hours of incubation at (35 ±2) °C, the result is positive.

Semi solid mannitol: This test was performed to determine the motility of the bacteria. A few pure colonies were inoculated into the medium by making a stab and incubated for 18–24 h at (35± 2) °C. If the organism is motile, its growth goes beyond the line of stab (Tang and Stratton 2018).

Results And Dıscussıon

Patients and Samples Collection

A total of 250 clinical samples from patients who were suffering from UTI (150 men and 100 women) were collected, and 62 isolates of *Klebsiella pneumoniae* (30 men and 32 women) from different ages were obtained. The total number and the percentage of isolates were demonstrated in Table 4.1.

Table Error! No text of specified style in document.**.1** The percentage of *K. pneumoniae* isolated from total number of UTI samples

Sex	Number of samples	No. of *Klebsiella pneumoniae* isolates	Percentage of isolation
Male	150	30	20%
Female	100	32	32%
Total	250	62	24.8%

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In this study, the percentage of total isolates was 62 (24.8%), similarly to which recorded that, from all clinical samples isolates of *K. pneumoniae*, the percentage of urine isolates was 21.21%. A local study by Shlash and Tuwaij, found that from all clinical samples of *K. Pneumoniae* that were isolated, the percentage of urine isolates was 14.98% (Shlash and Tuwaij 2018). Recorded the percentage of *K. pneumoniae* that was isolated from urine (48%), another study by (Cruz-Córdova *et al.* 2014). When comparing this current study's isolation percentages of *K. pneumoniae* to other previous studies, it is possible that the differences can be attributed to the number of clinical specimens used in this study, the place, or the isolation period,as well as the conditions that may have contributed to the isolation period.

In this recent study and according to Table 4.1, the results revealed that from 250 urine samples, 100 were from females and 150 were from males to obtain 62 isolates, indicating that the UTI in females is higher than in males. This may be due to the anatomical differences in the urinary systems between males and females. The urethra of females is much shorter than that of males and during pregnancy (Alshahrani *et al.* 2022, Ali *et al.* 2020).

In this study, and according to Table 4.2, the samples were taken from both sexes at different ages. The patients were ranged in age from 15 years old to more than 55 years old. The results showed that there was no statistically significant distinction between women and men in the same age group. But there was a statistically highly significant distinction in women from different age groups of $0.0094 \sim P \leq 0.01$, and there was a statistically significant distinction in men from different age groups of $0.0498 \sim P \leq 0.05$. The age group of 15–35 years old was the highest age group diagnosed by UTI, followed by the age group of more than 55 years old, while the ages of 36–45 years and 46-55 years are the least.

Table.2 The age groups and the percentage of males and females infected by UTI

Age group (year)	**No**	**Female No. (%)**	**Male No. (%)**	**P-value**
15-25	25	15 (24.19%)	10 (16.13%)	0.094 NS
26-35	11	7 (11.29%)	4 (6.45%)	0.278 NS
36-45	7	2 (3.23%)	5 (8.06%)	0.197 NS
46-55	6	2 (3.23%)	4 (6.45%)	0.422 NS
> 55 yr.	13	6 (9.68%)	7 (11.29%)	0.702 NS
Total	62	32	30	---
P-value	--	0.0094 **	0.0498 *	---
* (P≤0.05), ** (P≤0.01).				

According to (Tan and Chlebicki 2016), 40% of women are infected with UTIs during their lives. The group that has the greatest rates is young women who are sexually active or may be during pregnancy. Other groups that are at risk include postmenopausal women and adults due to atrophic vaginitis induced by oestrogen depletion and urinary catheterization. UTIs occur mostly between 15–55 years of age, according to a study by (Kurniawati and Auliyanah 2021). This may be due to sexual relations at an advanced age of activity and at the age of over 55 due to an enlarged prostate in males. and may be attributed to the natural ageing process and the decline in the body's immune system response.

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Phenotypic Identification of *K. Pneumoniae*

Culture characteristics and the biochemical tests

The growth on blood and MacConkey agar was shown in Figure 1.4.

All *K. pneumoniae* isolates were examined by the biochemical tests (Tang and Stratton 2018). The culture characteristics, the gram stain, and biochemical standard tests were demonstrated in Table 4.3.

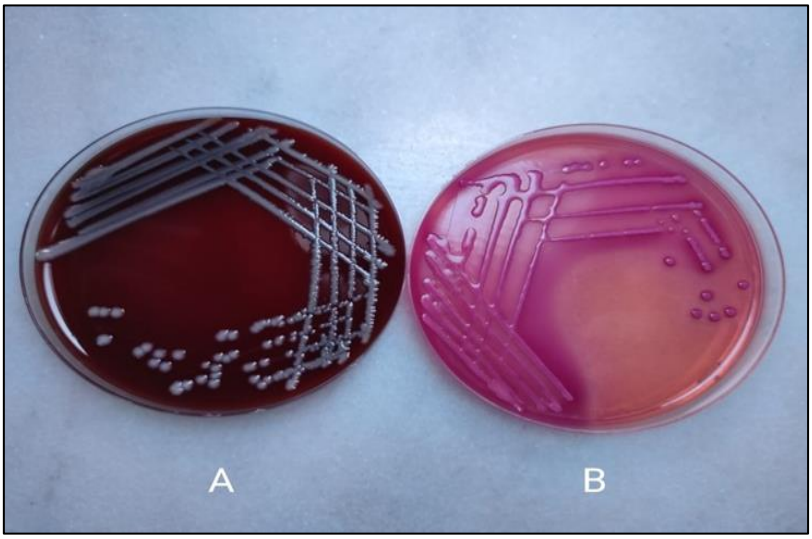

Figure 1 The growth of *K. pneumoniae* on culture media
(A) The growth on blood agar, (B) The growth on MacConkey agar

1.2.1 String test (hypermucoviscosity test)

The mucoviscosity characterization test from 62 isolates, 4 (6.45%) of the isolates were HV+. The hypermucoviscosity test was used to distinguish the HV+ phenotype : if the colonies of *K. pneumoniae* form a viscous filament ≥ 5 mm in length by a loop, this is considered HV+ (Zheng *et al.* 2018). The positive string test is shown in Figure.2.

Figure Error! No text of specified style in document**..1** The string test of *K. pneumoniae* strains show more than 10 cm

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Table Error! No text of specified style in document.**.3** The growth characteristics and biochemical tests of *K. pneumoniae*

The test	**Result**
Growth on Blood agar	A grey-mucoid non-heamolytic colony
Growth on MacConkey agar	large round pinkish mucoid lactose fermenter colonies
Gram stain	Short gram-negative rod
Catalase	positive
Oxidase	negative
Kligler iron agar	A/A (Acid/Acid)
Urea	positive
Indole	negative
Motility	negative
Citrate utilization	positive

Conclusion and **Recommendations**

1. The isolation of *K. pneumoniae* from UTI patients is more frequent in women than in men in the age range of 15–35 years.
2. The hypermucoviscosity in *K. pneumoniae* strains is of low frequency. From 62 isolates, only 4 (6.45%) gave the string test as positive.
3. Increasing the number of samples used in the study, and they are isolated from different sites in the body, such as burns, wounds, and the eye

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