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Original Research Article

Studying the Prevalence of Multidrug Resistant Klebsiella pneumoniae in Kirkuk City

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Abstract

Klebsiella pneumonia is an opportunistic pathogen causes several diseases including sepsis, pneumonia, and wound infections. There are two pathotypes of *Klebsiella pneumonia*: classical *K. pneumoniae* (cKp) and hypervirulent *K. pneumonia* (hvkp), which is an emerging variant of (ckp), clinically distinguished by invasive and multiple site infections. *K.pneumoniae* is also responsible for majority of human infections, and can infected healthy members of the community and hospitalized patients. A total 150 samples were collected from different hospitals in Kirkuk city during the period between November 2021 to June 2022. The age of patients ranged between (1– 60) years old with both sexes. These samples were highly recovered from females with a rate 66.67% compared to the males 33.33%. Thirty *K. pneumonia* (20%) was recovered from different clinical specimens including urine, sputum, burn and wound swabs. The current study reported that females are more likely to be infected with *K. pneumoniae* than man. Likewise, the most common age group infected with *K.pneumoniae* was between (20-40) with a rate of 63.33%. *K.pneumoniae* was commonly recovered from inpatients 53.33% compared with outpatients (46.67%). Antibiotic susceptibility test was conducted to all the isolates by using disc diffusion test towards 8 antimicrobial agents. *Klebsiella pneumoniae* isolates showed multiple resistance against 3 or more of different antibiotic groups such as gentamicin 93%, ampicillin 96% and amoxicillin –clavulanate 90%, cefotaxime 83%, ceftazidime 96%, meropenem 36%, levofloxacin 76 %and gentamicin 93 %. K. pneumoniae isolated from inpatients and from sputum samples were more resistance to various kinds of antibiotics.

Keywords: *Klebsiella pneumoniae*, Antimicrobial resistance, Risk factors.

INTRODUCTION

Klebsiella pneumoniae is a non-motile Gram-negative bacterium that belongs to the Enterobacteriaceae family (José *et al*., 2019). *K. pneumoniae* is considered as one of the opportunistic nosocomial pathogens (Ranjbar *et al*., 2019). This microorganism causes a variety of diseases such as bacteremia, pneumonia and urinary tract infections. In recent years, *K. pneumoniae* has attracted the attention of researchers around the world due to its disease severity, resistance against several antibiotics and the difficulty of the treatment (Ranjbar *et al*., 2019). Increased the prevalence of multidrug-resistant (MDR) *K. pneumoniae* strains in recent years might be due to overuse and uncontrolled use of conventional antimicrobial agents (Fuzi *et al*., 2020). *K. pneumoniae* has developed several mechanisms for resistance towards different antimicrobials (Wanjiang *et al*., 2019). One of the most important mechanisms for developing the MDR is efflux pump systems and biofilm formation capacity (A. S *et al*., 2022). Efflux pumps are protein-based structures that are capable to extrude the different toxic substances out of cells (Montazeri *et al*., 2020). The biofilm-forming ability in *K. pneumoniae* allows the protection of strains from the host immune response and antibiotics in MDR isolates (Sundaramoorthy *et al*., 2021). Subramanian *et al*., (2012) found that 80% of biofilm-forming isolates from 100 clinical samples showed an MDR phenotype (Subramanian *et al*., 2012). Resistance of pathogenic bacteria to different antibiotics has become a serious worldwide problem because of the fatal outcome of defective treatment and the difficulty to find treatment options (TJ *et al*., 2017). *Klebsiella pneumoniae* has been revealed to have the ability to acquire resistance to many antibiotics, especially third generation cephalosporins. Multidrug resistance of *K. pneumoniae* against the antibiotics showed virulence potential of *Klebsiella*. Beta - lactam antibiotics are one of the most commonly used antibiotics in the treatment

of bacterial infections and the production of B - lactamase enzymes are the most common bacterial resistance mechanisms (Vasaikar *et al*., 2017). In the recent years, Extended Spectrum Beta Lactamase (ESBL) producer *K. pneumoniae* have increased over the world. The ESBLs are divided to several groups; the main groups are TEM, CTX, and SHV derivatives (Manoharan *et al*., 2011). In this study we focused on isolation of the *Klebsiella pneumoniae* from different clinical samples and studying their antibiotic resistance profile against different groups of antibiotics.

MATERIALS AND METHODS

Bacterial Strains

One hundred and fifty clinical samples (urine, sputum, blood, wound and burn swabs) were collected from patients attending hospitals in Kirkuk city during the period (November 2021 to March 2022). K. pneumoniae isolates were identified by Gram staining, culture and biochemical tests, and further confirmed by using VITEK-R2 compact system.

Antimicrobial susceptibility test

Aantibiotic susceptibility test (Kirby- Bauer disk diffusion) was used for detect of *K. pneumoniae* strains resistance towards selected antibiotics according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (M. A *et al*., 2023). Antimicrobial susceptibility assays to 8 antibiotics were performed using commercially available antibiotics including ceftazidime (30 µg), cefotaxime (30 µg), gentamicin (10 µg), Amoxicillin/Clavulanic acid (AMC, 20/10 µg), meropenem (10 µg). By selecting three or four colonies, pure inoculum was formed, A 0.9% saline solution is used for inoculation, and the culture develops active growth when the broth becomes visibly turbid. This takes 3 to 5 hours for the majority of organisms. After incubation, the bacterial suspension's turbidity was compared to McFarland turbidity standards corresponds to 1.5 × 108 CFU/ml. Using a swab dipped in a bacterial suspension standardized to match the McFarland turbidity standard, then swabbing the plate's surface in three directions ensures that the inoculum is evenly distributed across the entire surface after that we incubated the plates 15 min. Then Sterile forceps was used to apply antibiotic discs, after applying antibiotic discs the plates are turned over for incubation (at 37 °C for 24 h) to prevent the formation of moisture on the agar surface, which would make it difficult to explain the test results. Following the incubation, the inhibition zone diameters were recorded in millimeter and the interpretation was carried out based on CLSI. Moreover, multidrug-resistant (MDR) isolates were detected based on their resistance to at least to three or more antimicrobial classes.

RESULTS AND DISCUSSIONS

prevalence of *Klebsiella pneumonia* among clinical samples

Out of 150 clinical samples, 30 (20%) were positive for *K. pneumoniae*. Bacterial isolates were identify by depending on cultural and microscopical examination, after that the purity of the bacterial strains was confirmed by using vitek2-compact system (S. S *et al*., 2017) as seen in Figure 1.

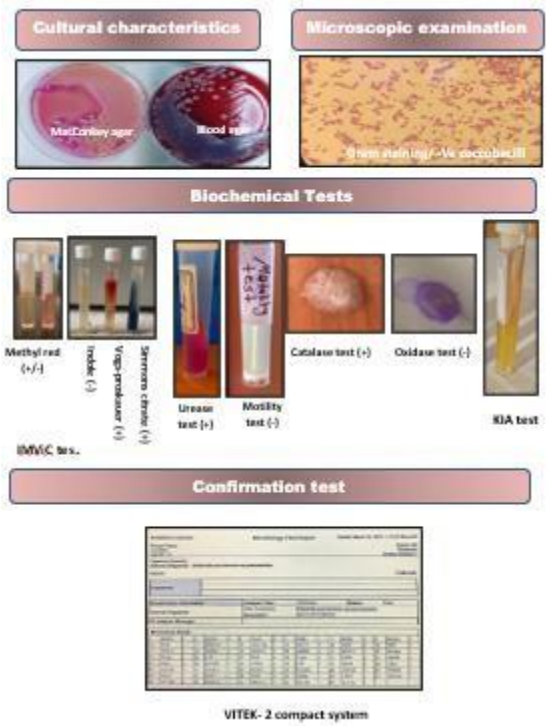

Figure 1: Diagram showing cultural and biochemical profile of *Klebisella pneumoniae*

These findings agree with Al-Obadi (2014) reported that out of 268 clinical samples, 36 (13.4%) *K. pneumoniae* isolates were recovered. In contrast, Omar-Zahid, (2009) found that *Klebsiella* spp. formed 54.16% of total isolates from clinical specimens and 79.12 % was identified as *K. pneumoniae.* Jasim *et al*., (2018) also revealed the highest percentage of *K. pneumoniae* (74.4%), which is higher than that reported in the current study.

According to the results of present study, the prevalence of *K. pneumoniae* among sputum samples 10(33.33%) and urine samples 9(30%) were found to be considerably higher than that in other clinical samples (wound and burn swabs). Similar to our findings, Ajimud *et al*., (2022) reported that *Klebsiella pneumoniae* was dominant among all clinical samples isolated from urine. Similarly, Vandhana *et al*., (2022) showed that *K. pneumoniae* isolated from urinary tract infection cases were found to yield the highest number when compared to other cases.

Another study done by Hasani *et al*., (2021) out of 468 different clinical samples, 61 (13.03%) isolates were identified as *K. pneumoniae* and urine showed the highest percentage (50.8%) followed by wound swab (24.6%) and (13.1%) of blood samples. These results are supported by a local study done by Al-aajem (2020) in Diyala province reported that *K. pneumonia* (42.9%) was the dominant among urine samples. Similarly, Zaki *et al*., (2016) also showed that 44% of *K. pneumoniae* strains were isolated from urine. The latter study also recorded that 8% of *K. pneumoniae* recovered from sputum ,8% burn swabs and 4% wound swabs, these numbers however are lower than that reported in the recent study. In contrast, Al-Hilfi (2009) reported the lowest percentage of *Klebsiella* spp. isolated from the UTI (2.1%).

Distribution of *K. pneumoniae* isolates according to the age and gender:

The results of recent study showed that 63.33% of patients with *K. pneumonia* infection were in the age group (20- 40), 26.67% from patients within the age group 40-60 years while the lowest rate was < 60 age group as show in Table (1). The current results disagreed with those demonstrated by Khan *et al*., (2010) stated higher rate of *K. pneumoniae* infections among elderly patients, mainly in the age group ≥ 60 years. They referred that male patients above the age of 60 years were subjected to greater frequency of *K. pneumoniae* infections. The high percentage of infections was recorded in the age group of 20-40 which may be due to certain reasons comorbidities of some disorders like diabetes or the collection of a significant number of samples in this age group compared to the samples of other age groups. The prevalence of infections in recent study are in disagreement with those recorded by Khan *et al.*, (2010) found that *K. pneumoniae* were mainly recovered from patients with the age group less than 10 years. Our results also demonstrated that 66.67 % of *K. pneumoniae* was female and 33.33% was male. These results referred that female more susceptible to get *K.pneumoniae* infections than male. Similarly, Khan *et al*., (2010) stated that the infection in female was higher (54.5%) than male (45.6 %). In the study of Magliano *et al*., (2012) viewed that 209 cases were males and 995 cases were females. Jasim *et al*., (2020) also showed that out of 50 *K.pneumonia* isolates from UTI, female infections were more than males.

On the other hand, these results were in contrary with those recorded by Vandhana *et al*., (2022) showed that the male was more susceptible to Klebsiella infections than female. Another study done by Parrot *et al*., (2020) also showed that all *K.pneumonia* infections an apparent bias toward male predominance and to affect older adults. Although there is no significant difference in percentage of origin of samples, recent study in agreement with study done by Li *et al*., (2022) showed that the samples were predominantly derived from hospitalized patient(inpatient).

Table 1: Distribution of *K. pneumonia* isolates according to age and gender

Age groups (years)	No.	%
20-40	19	63.33
40-60	8	26.67
60 >	3	10
Total	30	100
Gender		
Male	10	33.33
Female	20	66.67
Total	30	100

Antimicrobial Resistance

Agar disc diffusion test (Kirby-Bauer method) was used in accordance with the Clinical Laboratory Standards Institute (CLSI) standards (2020) to assess antimicrobial susceptibility test for eight antibiotics as seen in Figure (2). The isolates showed a high levels of resistance towards antibiotics used in this study as seen in Figure (3). The 29 (96.67%) of *K. pneumoniae* isolates were resistant to ampicillin, 29 (96.67%) resistant to ceftazidime,26 (93.33%) resistant to gentamicin, 27(90%) resistant to azithromycin, 27(90%) resistant to amoxicillin-clavulanate, 25(83.33%) resistance to cefotaxime and 23(76.67%) resistance to levofloxacin, However, although meropenem is one of most effective antibiotic against *K. pneumoniae*, while these isolates showed moderate resistance 11 (36.67%) to meropenem. Likewise, in recent study 30 (100%) of *K.pneumoniae* isolates showed multidrug resistant (when bacteria showe resistant against 3 or more antibiotic class) against 8 antibiotics used in recent study.

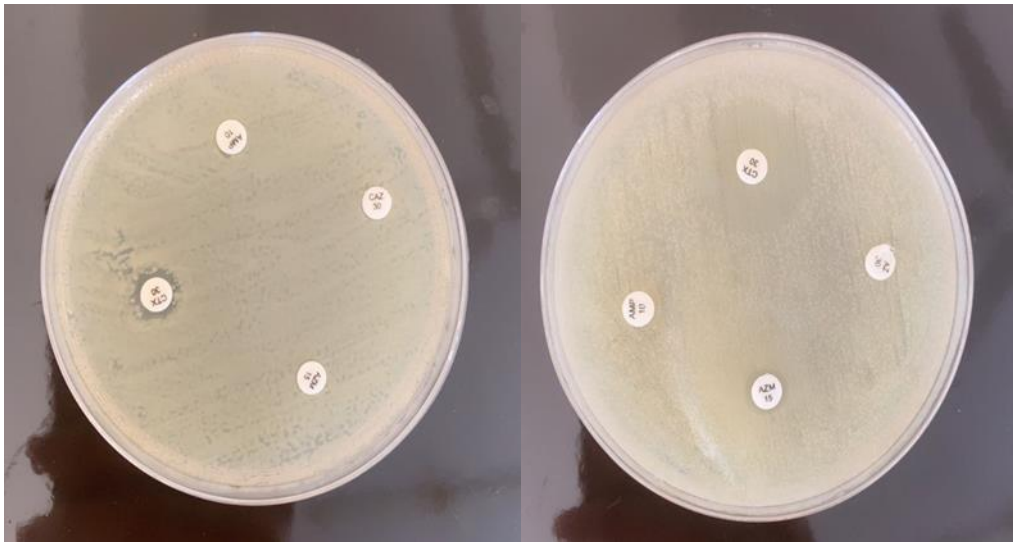

Figure 2: Disc diffusion test of *K. pneumoniae* isolates by using Kirby-Bauer method

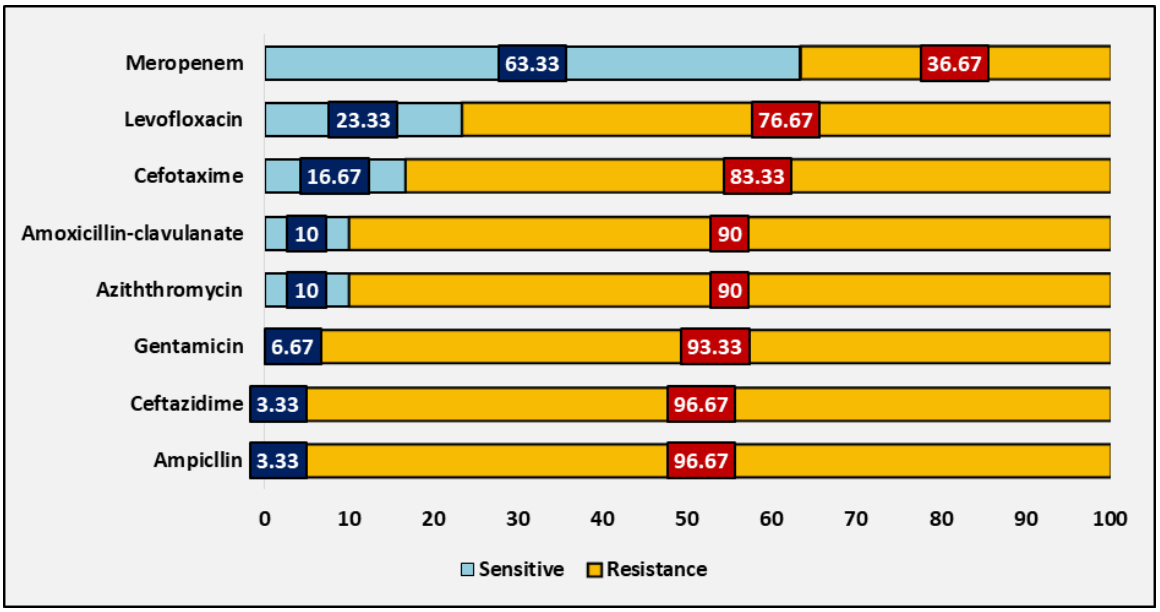

Figure 3: Antibiotic susceptibility test of *K. pneumonia* isolates

Through the possess mechanisms of resistance to carbapenems include production of lactamases and mutations that alter the expression and/or function of porins and PBPs (Walsh, 2010; Bleriot *et al*., 2020). Combinations of these mechanisms can cause high levels of resistance to carbapenems in *K. pneumoniae* (Suay-García and Pérez-Gracia, 2019). It is very important for public healthcare to monitor and report the changes in antimicrobial-resistant isolates. (Effah *et al*., 2020). The current study agreed with a study done by Namikawa *et al*., (2019) who mentioned that *Klebsiella pneumoniae* was highly resistant to ampicillin by producing β-lactamases that render these isolates resistant to most β-lactam antibiotics. Recent results were in agreement with the results of N. R. *et al*., (2023) who noticed that the percentage of resistance to ampicillin, was (92%). The results were also consists with those of Rastegar *et al*., (2019) who found that resistance percentage of *K.pneumoniae* against β-lactam antibiotics include ampicillin (97.6%) and cefotaxime (66.1%).

A Study done by Kareem *et al*., (2021) revealed the emergence of efflux pump-mediated drug resistance in MDR K. pneumoniae bacteria in Iraq. Another study done by Fatima *et al*., (2021) for a total (107) clinical *K. pneumoniae* isolates showed that all isolates were MDR to minimum 6 and maximum 14 antibiotics out of 17.

CONCLUSION

In this study we demonstrated prevalence of *Klebsiella pneumoniae* in Kirkuk city with percentage 20% among 150 samples and most isolates were recovered from young people their age ranged (20-40) years, the most isolates were recovered from sputum samples followed by urine, wound and burn swabs, likewise the female were more susceptible to *Klebsiella*

pneumoniae than male. We also demonstrated that k.pneumoniae mostly infect hospitalized patient (inpatient).

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