https://doi.org/10.26682/sjuod.2022.25.2.43
Journal of University of Duhok., Vol. 25, No.2(Pure and Engineering Sciences), Pp473-481 , 2022

DETECTION OF MULTI-DRUG RESISTANT KLEBSIELLA PNEUMONIAE FROM SPUTUM SAMPLES AMONG ICU PATIENTS UTILIZING PCR AND VITEK2 SYSTEM

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(Received: October 9, 2022; Accepted for Publication: December 1, 2022)

ABSTRACT

The study aimed to detect the rate of *K. pneumoniae* in sputum samples among patients with pneumoniae. It also strives to determine Klebsiella pneumonia isolates among ICU patients and the frequency of multidrug-resistant (MDR) *K. pneumonia*e isolates. In the current study, 150 samples were collected from hospitalized adult male and female patients in the ICU unit. Growth of bacteria on MacConkey agar, chocolate agar and blood agar followed by gram staining were used for detection of the bacteria and confirmed by PCR. Out of the 150 study samples, only 39 (26%) samples of *K. pneumoniae* were identified, the remaining 111 samples were variants of bacteria mainly Acinetobacter baumannii. Frequency tabulations and the Chi-square test testing for statistical significance were performed using SPSS version 24. Consequently, the susceptibility rate of *Klebsiella pneumoniae* to antibiotics was determined by using the VITEK2 system. The results revealed that *K. pneumonia* isolates were less sensitive to Cefixime, Ceftazidime, Ceftriaxone, and Cefepem, and completely resistant to Ampicillin and Piperacillin/Tazobactam. Furthermore, the *K. pneumoniae* isolates were highly resistant to Cefixime, Ceftazidime, Ceftriaxone, and Cefuroxime followed by Amoxicillin/clavulanic acid and Cefepem which showed equal rates of resistance. The study concludes that ICU patients are more vulnerable to contracting MDR *Klebsiella pneumonia* because of the high percent of susceptible patients in the ICU units, the frequent use of invasive devices and the excessive consumption of antibiotics exerting selective pressure on bacteria. Amid such observations, the study underscores the importance of (1) determining various resist typing patterns of antibiotics which is vital to produce new and highly effective antibiotics treatment and (2) targeting consumers, prescribers and pharmacy dispensers to reduce OTC antibiotics dispensing.

KEYWORDS: Klebsiella Pneumoniae, Multi-Drug Resistant, PCR System, Vitek2 System

INTRODUCTION

Aminul et al. (2021) defined *Klebsiella pneumoniae* as an opportunistic pathogen that can cause several infections, mainly in hospitalized or immunocompromised individuals. Adding on, Norsigian et al. (2019) outline that this bacterium is a member of the Enterobacteriaceae family which are gram-negative, encapsulated rod-shaped, oxidase-negative and non-motile bacteria. This pathogen is associated with the opportunity could cause several infections such as bacteremia, urinary tract infections, and pneumonia mainly in immunocompromised or hospitalized individuals (Bengoeche et al., 2019).

Though there are possible treatments, it is challenging that the world has observed a substantial increase in multidrug-resistant (MDR) *klebsiella pneumoniae*. The severity of MDR cannot be underestimated and calls for immediate solutions. For instance, Medina and Pieper (2016) contend that MDR strains can prolong the course of the disease, while Sood et al. (2018) echo similar sentiments and highlight that they can hinder antibacterial treatment. Under such circumstances, it, therefore, becomes inevitable that mortality rates together with medical costs will increase rapidly. Hence, the need and significance of this study to explore such issues are of vital theoretical and practical importance as possible remedies and treatments can be devised and administered to *klebsiella pneumoniae* patients. However, numerous virulence factors can be used to treat and curb the spread of *K. pneumoniae* infection and these include biofilm, iron acquisition, fimbriae, lipopolysaccharide (LPS) and capsular polysaccharide (CPS), (Ahmadi et al., 2022). As

a result, Zhu et al. (2021) highlight that *K. pneumoniae* can carry distinct virulence factors with unique clinical and pathogenic features.

Meanwhile, attempts to uncover the possible factors contributing to multi-drug resistant *K. pneumoniae* have seen factors like associated diseases, use of associated gaseous devices, long hospital stays, previous hospitalization in the Intensive Care Unit (ICU) and age being listed as the main contributing factors (Tugal et al., 2015; Ruiz et al., 2019). Besides, detecting multidrug-resistant *K. pneumoniae* is presumed to be a complex and difficult task and supporting evidence cites the presence of strains carrying several distinct multiple resistance determinants that cannot be easily treated (Datta et al., 2012; Ogalo et al., 2016). Moreover, *Klebsiella pneumoniae* infection is highly common in hospitalized patients because of the excessive use of empiric antibiotic treatment and ICU units are a very susceptible habitat for bacterial colonization (Baier et a., 2019; Pachori, Gothalwal & Gandhi, 2019), it remains inevitable that hospital-acquired resistant microorganisms will increase as *Klebsiella pneumonia* infections rates increase following increased colonization among prolonged hospitalized patients (Medina & Pieper, 2016; Sood et al., 2018). These problems can pose huge adverse effects, especially when considerations are made that certain isolates resistant to multidrug. This can be evidenced by empirical examinations which uncovered that 34% is against ceftazidime, imipenem 26%, gentamicin 67%, and amikacin (Girschick et al., 2008). Such resistance is foreseeable as studies concur that the high resistance of *Klebsiella pneumoniae* to antibiotics is significantly linked to the overuse of broad-spectrum antibiotics in hospitals (Pessoa-Silva et al., 2003; Silva et al., 2006; Ben-David et al., 2012). Therefore, the aims of this study are to:

- Detect the rate of *K. pneumonia* in sputum samples among patients with *K. pneumoniae*.
- Determine the highest *Klebsiella pneumonia* isolates among ICU patients.
- Determine the frequency of multidrug-resistant (MDR) *K. pneumonia* isolates.

MATERIALS AND METHODS

The study is a cross-sectional study that is part of a qualitative research design that applies descriptive methods in analyzing *K. pneumonia* in 150 sputum samples collected from Vin and Duhok emergency hospital patients with *K. pneumoniae*. Such a research design was applied to aid in ascertaining the highest *Klebsiella pneumonia* isolates and determining the frequency of multidrug-resistant (MDR) *K. pneumoniae* isolates among ICU patients. Hence, the study variables were examined using a combination of frequency tabulations and percentages. Chi-square tests were used to determine the significance of *K. pneumoniae* between men and women as well as among the adult male and female patients between the age of 18 years to 65 years in ICU were tested using the Chi-square test with the help of SPSS version 24.

Study population

The study involves the testing of 150 sputum samples that were collected from Vin and Duhok emergency hospitals from February to June 2022. The samples were collected from hospitalized adult male and female patients between the age of 18 years to 65 years in ICU to detect the isolates of *K. pneumoniae*. Thus, sputum samples from unconscious patients as well as patients below 18 years and above 65 years were excluded from the study. The MacConkey culture media together with the chocolate and blood agar were used to culture the sputum specimens, respectively, followed by gram staining.

Detection of K. pneumonia using VITEK2 system

A Vitek2 device with the AST–N326, AST-N327 BioMérieux-France and GN-6BioMérieux-France (Gram-negative bacteria detection kit) was used to detect the bacteria using 22 different types of antibiotics. Subsequently, confirmation of *Klebsiella pneumoniae* isolates was performed via PCR utilizing these primers Pf: 5-ATT TGA AGA GGT TGC AAA CGA T-3 and Pr1: 5-TTC ACT CTG AAG TTT TCT TGT GTT C-3 for the amplification of 16S rRNA gene the primers were obtained from (macrogen/korea) following the standard protocol for DNA extraction were used in confirming all the isolates of *Klebsiella* pneumonia (Ranjbar et al., 2016). For this, 19 Polymerase chain reaction (PCR) (25 μL) was prepared by mixing 12.5 μL master mix with 1 μL of each forward and reverse primers (10 pcm / ml), 7.5 μL nuclease-free PCR water and 3 ml a DNA sample (50 ng/ml). The mixture was processed at 94°C denaturing for 4 minutes. 94°C for 30 seconds, 55°C for 40 seconds, 72°C for 60 seconds, and 30 cycles.

The final extension was performed at 72°C for 10 minutes and at a final temperature of 4°C. The positive samples were obtained from the Duhok research center, College of Veterinary Medicine, University of Duhok and negative controls were used molecular to grade free nuclease distilled water. The PCR product was run on 2% agarose with 1x edta (tae) buffer and labelled with red a safe DNA staining solution (geNetBio/Korea).

RESULTS

Of the 39 individuals who were infected by *K. pneumoniae* 24 male and 14 female individuals tested positive for Klebsiella pneumonia. This reveals significant differences in *K. pneumoniae* infections between male and female individuals as supported by the computed chi-square test results that are significantly different at 5% (X = 3.27: P = 0.001). Further insights into the established results shown in Table 1 revealed that *K. pneumoniae* infections were significantly different among the examined 18-65 years age group at 5% (X = 5.09: P= 0.001).

Table (1): Demographic frequencies of *K. pneumoniae*

Variables	Description	Frequency (Percentage)	Chi-square	Probability
Gender	Male	25 (64.10%)		
	Female	14 (35.90%)	3.27	0.001
	Total	**39 (100%)**		
Age	18-25 years	2 (5.13%)		
	26-33 years	5 (12.82%)		
	34-41 years	8 (20.51%)		
	42-49 years	9 (23.08%)	5.09	0.001
	50 years and above	15 (38.46 %)		
	Total	**39 (100%)**		

As such, *K. pneumoniae* infections were higher among individuals between 42-49 years (9; 23.08%) and 50 years and above (15;38.46%) compared to young individuals between 18-25 years (2; 5.13%) and 26-33 years (5; 12.82%). The findings are significantly different at 5% (X = 5.09: Prob. = 0.00). Furthermore, result from the Vitek2 system uncovered that the most frequent isolates are Acinetobacter baumannii (34.67%), Klebsiella pneumonia (26%), Escherichia coli (10.67%), Pseudomonas aeruginosa (8%), Staphylococcus aureus (4.67%), Burkholderia cepaceia (3.33%), Staphylococcus epidermidis (2.67%), Stenotrophomonas maltophilia (1.33%), Staphylococcus hominis (1.33%) and Staph heaemolyticus (1.33%) as shown in Table 2. Table 2 further shows that 0.67% isolates existed in the form of Enterobacter aerogenes, Enterococcus faecium, Enterobacter cloacae spp dissolvens, Morganella morganii ssp morganii, Staph warneri, Streptococcus salivarius ssp salivarius, Streptococcus pneomonea, Streptococcus mitis, and Ochrobactrum anthropic.

Table (2): Frequency and percentage of microorganisms isolated from MDR K. pneumonia patients.

Microorganism	Number of isolations	Percentage
Acinetobacter baumannii	52	34.67
Klebsiella pneumonia	39	26.0
Pseudomonas aeruginosa	12	8.00
Staphylococcus aureus	7	4.67
Burkholderia cepaceia	5	3.33
Escherichia coli	16	10.67
Staphylococcus epidermidis	4	2.67
Stenotrophomonas maltophilia	2	1.33
Staphylococcus hominis	2	1.33
Enterobacter aerogenes	1	0.67
Enterococcus faecium	1	0.67
Enterobacter cloacae spp dissolvens	1	0.67

Staph heaemolyticus	2	1.33
Morganella morganii ssp morganii	1	0.67
Staph warneri	1	0.67
Streptococcus salivarius ssp salivarius	1	0.67
Streptococcus pneomonea	1	0.67
Streptococcus mitis	1	0.67
Ochrobactrum anthropic	1	0.67
Total	**150**	**100**

The pictorial description of PCR products is presented in Figure 1. The Products run on Agarose gel 2 % and the size of products was 130 bp (Figure 1).

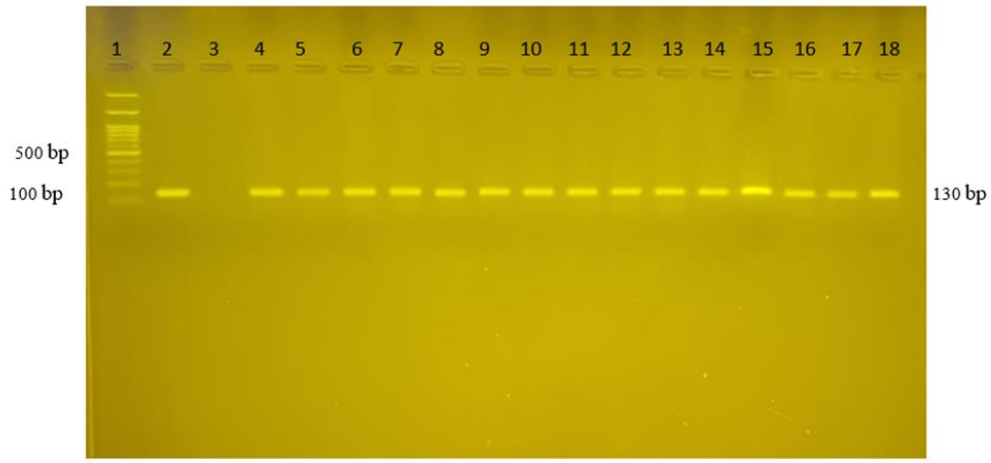

Fig. (1): PCR products on Agarose gel 2 %

Lane 1 is l100 bp ladder, Lane 2 is a positive control, Lane 3 is a negative control and Lanes 4-18 are positive samples.

Frequency tabulations were compiled to determine the most frequent microorganisms isolate from the MDR *K. pneumonia* patients. A description of the susceptibility test of *Klebsiella pneumoniae* isolated from MDR *K. pneumonia* patients is provided in Table 3. *K. pneumoniae* isolates were subjected to susceptibility tests involving 22 antibiotics and subsequent measurements of the bacterial growth inhibition zone around the antibiotic discs were taken. The findings presented in Table 3 denote different forms of sensitivity of the *K. Pneumoniae* isolates that were observed following exposure to the various forms of administered antibiotics. The *K. pneumoniae* isolates were completely resistant to Ampicillin and Piperacillin/Tazobactam and highly resistant to Cefixime (97.44%), Ceftazidime (97.44%), Ceftriaxone (97.44%), Cefuroxime (94.87%), followed by Amoxicillin/clavulanic acid and Cefepem with equal rates of resistance 92.31%. Nonetheless, cases of low resistance were predominantly linked to Tigecycline (41.02%) and Colistin (48.72%). Consequently, the findings portray that the *K. pneumoniae* isolates have distinct sensitivity and resistance potential that varies with the administered antibiotics. Hence, such findings caution medical practitioners into developing effective antibiotics and examine how two or more antibiotics can be administered together to deal with *K. pneumoniae* isolates.

Table (3): Susceptibility test of *Klebsiella pneumoniae* isolated from MDR K. pneumonia patients

	ANTIBIOTICS	Acronym	Isolate Number (%)		
			Sensitive No. (%)	Intermediate No. (%)	Resist No. (%)
1	Tigecycline	TIG	23 (58.98%)	0 (0%)	16 (41.02%)
2	Colistin	COL	20 (51.28%)	0 (0%)	19 (48.72%)
3	Fosfomycin	FO	14 (35.90%)	0 (0%)	25 (64.1%)
4	Co-Trimethoprim	SXT	11 (28.21%)	0 (0%)	28 (71.79%)
5	Gentamycin	GN	11 (28.21%)	0 (0%)	28 (71.79%)
6	Ciprofloxacin	CIP	10 (25.64%)	0 (0%)	29 (74.36%)
7	Amikacin	AK	9 (23.1%)	3 (7.69%)	27 (69.21%)
8	Nitrofurantoin	NFN	8 (20.51%)	7 (17.95%)	24 (61.54%)
9	Meropenem	MEP	6 (15.38%)	0 (0%)	33 (84.61%)
10	Pipracillin\Tazobactum	PRL/TAZ	5 (12.83%)	1 (2.56%)	33 (84.61%)
11	Imipenem	IMP	5 (12.82%)	3 7.69%	31 (79.49%)
12	Ertapenem	ERT	5 (12.82%)	0 (0%)	34 (87.2%)
13	Levofloxacin	LEV	4 (10.26%)	0 (0%)	35 (89.74%)
14	Cefuroxime	CFUR	2 (5.13%)	0 (0%)	37 (94.87%)
15	Amoxicillin/clavulonic acid	AMC	2 (5.13%)	1 (2.56%)	36 (92.31%)
16	Cefoxitin	CFN	2 (5.13%)	1 (2.56%)	36 (92.31%)
17	Cefixime	CFM	1 (2.56%)	0 (0%)	38 (97.44%)
18	Ceftazidime	CAZ	1 (2.56%)	0 (0%)	38 (97.44%)
19	Ceftriaxon	CRO	1 (2.56%)	0 (0%)	38 (97.44%)
20	Cefepem	CPM	1 (2.56%)	2 (5.13%)	36 (92.31%)
21	Piperacilin	PRL	0 (0%)	0 (0%)	39 (100%)
22	Ampicillin	AMP	0 (0%)	0 (0%)	39 (100%)

DISCUSSIONS

The established findings are congruent with other related studies regarding the effects of MDR *Klebsiella pneumonia* and reinforce the idea that ICU patients are at high risk of contracting pneumoniae infections. As such, an incidence of 52% was found to be highly prevalent among ICU patients (Joseph et al., 2010) and 57.1% acquired more than 5 forms of bacteria in ICU (Thuy et al., 2018). Of paramount importance is the highlighted observation showing that male individuals are at a high risk of contracting MDR *Klebsiella pneumonia* compared to women. This mirrors Nirwati et al.'s (2019) findings derived in Indonesia where numerous *K. pneumoniae* were isolated from male patients compared to female patients. This is possibly attributed to the fact that this study was done during the Covid-19 era during which Covid-19 infection rates in Iraq were higher among male individuals than female individuals (AL-Mosawe & Fayadh, 2021). Consequently, this increased their vulnerability or risk of contracting other infections like *Klebsiella pneumonia* compared to women. However, this is contrary to relatively similar study findings established in Zakho City of Kurdistan, which showed that more female patients tested positive for MDR *Klebsiella pneumonia* compared to male patients (Polse et al., 2020). On the same angle of analysis, Anosike et al.'s (2020) study findings conducted in Nigeria found a 5.6% prevalence in females which was higher than the 2.8% prevalence among male patients. This probably suggests the influence of economic, cultural and family-related factors influencing exposure, avoidance and immunity to MDR Klebsiella pneumonia. Another interesting feature that this study uncovered is that *Klebsiella pneumonia* infections were higher among elderly people that are at least 50 years old compared to young people. Thus, this study's findings suggest that this is attributed to a weakening immune system, a person faces as he or she ages. In a study conducted in Indonesia, Nirwati et al. (2019) found that *Klebsiella pneumonia* infections were higher among elderly people above the age group of 50 years. In a study conducted in Calgary Health Region, Meatherall et al. (2009) found that elderly patients and men were at the

highest risk for *K. pneumoniae* bacteremia. To further buttress this study's findings, Cristea et al.'s (2017) examinations of CKD patients admitted to the Nephrology Department of the County Emergency Clinical Hospital Craiova in Romania revealed that male patients had a higher risk to get Klebsiella infection than females. In addition, such *K. pneumoniae* resistance is attributed to poor over-the-counter (OTC) practices allowing individuals to get access to antibiotics without prescriptions and getting improper prescriptions from unqualified health providers in Iraq. This mirrors a common consensus among academic studies citing that poor OTC practices are one of the main reasons hindering the use of broad and narrow-spectrum antibiotics among host communities in countries such as Nepal (Pokharel & Adhikari, 2020) and India (Kotwani, Joshi & Lamkang, 2021).

Additional challenges linked to MDR *Klebsiella pneumoniae* are inevitable as it is famously known worldwide for prolonging the course of the disease (Medina & Pieper, 2016), and hindering antibacterial treatment (Sood et al., 2018). Besides, *Klebsiella pneumonia* infection has always been observed to be highly common in hospitalized patients because of the excessive use of empiric antibiotic treatment and ICU units are a very susceptible habitat for bacterial colonization (Baier et al., 2019; Pachori, Gothalwal & Gandhi, 2019). Hence, hospital-acquired resistant microorganisms will increase as *Klebsiella pneumonia*e infection rates increase following increased colonization among prolonged hospitalized patients (Medina & Pieper, 2016; Sood et al., 2018).

This study's findings exhibit relatively distinct establishments denoting that Acinetobacter baumannii (34.67%), Klebsiella pneumonia (26%), Escherichia coli (10.67%), Pseudomonas aeruginosa (8%) and Burkholderia cepaceia (3.33%) are the most five frequent isolates found in ICU patients in Dohuk as presented in Table 3. Other studies found that Pseudomonas aeruginosa, Acinetobacter baumannii and *Klebsiella pneumoniae* to be the most common resistant bacteria encountered in Greece's general public hospitals' ICUs and other wards (Feretzakis et al, 2019). Cai et al. (2017) found P. aeruginosa (60.3%), A. baumannii (22%) and E. coli. Or *K. pneumoniae* (17.7%) are the most prominent isolates found in CR patients. This denotes that the frequency of isolates found in *K. pneumoniae* varies significantly according to patients, medical, hospital geographical and other environmental conditions.

Most importantly, the findings reveal that Tigecycline is the only antibiotic that is highly sensitive to K. pneumoniae isolates with a sensitivity of 58.98% and a lower resistance of 41.02%. This is contrary to Spanu et al.'s (2012) findings and suggests that the low tigecycline-resistant strains are due to the low number of normal-phenotype parent strains. Additionally, low tigecycline-resistant strains can be attributed to successful microbiological eradication as suggested in previous related examinations (Curcio, 2008; Elemam Rahimian & Mandell, 2009). Colistin was ranked second with a relatively moderate sensitivity of 51.28% and a resistance of 48.72% which is higher than that of Tigecycline. Similarly, Sah, Begum and Anbumani (2022) found high Colistin resistance by *K. pneumoniae* isolates of 99% compared to E. coli, Enterobacter spp., and C. freundit. This possibly suggests that the *K. pneumoniae* isolates would have been in the Dohuk for a long period and already spread to antibiotic resistance reservoirs such as the gut (Hu et al., 2019).

The *Klebsiella pneumoniae* isolates were lowly sensitive to Cefixime (2.56%), Ceftazidime (2.56%), Ceftiaxon (2.56%), and Cefepem (2.56%). No *Klebsiella pneumonia* isolates sensitivity was observed when Piperacillin (0%) and Ampicillin (0%) were involved and this is contrary to Piperacillin and Ampicillin susceptibility rates of 60.5% (Najim, Janan & Blind, 2012) and 5% (Patilaya, Husori & Marhafanny, 2019), respectively. However, with completely resistant to Ampicillin and Piperacilint and highly resistant to Cefixime (97.44%), Ceftazidime (97.44%), Ceftriaxon (97.44%), Cefuroxime (94.87%), followed by Amoxicillin/clavulanic acid and Cefepem with equal rates of resistance 92.31%. The study practically calls for improvements regarding the development and administration of Ampicillin, Piperacillin/Tazobactam, Cefixime, Ceftazidime, Ceftriaxon, Cefuroxime followed by Amoxicillin/clavulanic acid and Cefepem. Similar recommendations were made by Hu et al. (2019) and followed suggestions highlighting that high antibiotic resistance emanates from important risk factors like huge volumes of the susceptible patient population in ICU units, the frequent use of invasive devices and excessive consumption of antibiotics exerting selective pressure on bacteria (Ben-David et al., 2012). Most importantly, studies concur that the high

resistance of *Klebsiella pneumonia* to antibiotics is significantly linked to the overuse of broad-spectrum antibiotics in hospitals (Ben-David et al., 2012; Pessoa-Silva et al., 2003; Silva et al., 2006). Consequently, it is vital to determine various resist typing patterns of antibiotics so as to produce new and highly effective antibiotics.

Conclusions

MDR *Klebsiella pneumoniae* remains undesirable and demands immediate attention and treatments because it can prolong the course of the disease and hinder antibacterial treatment. Of paramount importance is the finding that male patients in ICU are at high risk of contracting MDR *Klebsiella pneumonia* due to excessive use of empiric antibiotic treatment and the fact that ICU units are a very susceptible habitat for bacterial colonization. Additionally, huge volumes of the susceptible patient population in ICU units, the frequent use of invasive devices and excessive consumption of antibiotics exerting selective pressure on bacteria are the most important risk factors linked to MDR *Klebsiella pneumonia*.

The *Klebsiella pneumoniae* isolates are lowly sensitive to Cefixime, Ceftazidime, Ceftiaxon, and Cefepem. Nonetheless, the study infers that *Klebsiella pneumonia* isolates are completely resistant to Ampicillin and Piperacillin/Tazobactam and highly resistant to Cefixime, Ceftazidime, Ceftriaxon, Cefuroxime followed by Amoxicillin/clavulanic acid and Cefepem with equal rates of resistance. The high resistance of *Klebsiella pneumoniae* to antibiotics is significantly linked to the overuse of broad-spectrum antibiotics in hospitals. Hence, it is vital to determine various resist typing patterns of antibiotics to produce new and highly effective antibiotics. Given that poor OTC practices and getting improper prescriptions from unqualified health providers are the behavioral causes of high *K. pneumoniae* resistance in Kurdistan. This study, therefore, underscores the importance of targeting consumers, prescribers and pharmacy dispensers to reduce OTC antibiotics dispensing. The study is not void of limitations. As such, the findings based on the examination of ICU patients in Dohuk and hence, they cannot be generalised to other patients, cities in Kurdistan and well as other countries. Additionally, the number of patients was limited to 150. Thus, apart from patients in ICU and Dohuk, it is vital to include a wide number of patients with various medical conditions that are drawn from other hospitals and cities for broader coverage.

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