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MULTIPLE DRUG RESISTANT *KLEBSIELLA PNEUMONIAE* RECOVERED FROM HUMAN AND ANIMAL SOURCES IN DIYALA PROVINCE, IRAQ

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Abstract

Klebsiella pneumoniae is a common opportunistic nosocomial pathogen, accounting for about one third of all Gram-negative infections. The emergence of multidrug-resistant *K. pneumoniae* has prompted the WHO to classify it on the global priority list of antibiotic-resistant bacteria. The objectives of the current study is exploration of the antimicrobial resistant pattern of *K. pneumoniae* isolates recovered from both human and animal sources and to determine the rate of multiple drug resistance of these isolates. This is cross-sectional laboratory based study conducted in Diyala province for the period from October 2019 to April 2020. A total of 182 different pathological specimens were collected from hospitalized and out patients of both sexes. Their age range was 1-65 years. The human specimens include; blood, urine, stool, sputum specimens, wound and burn swabs. Furthermore, 111 different specimens collected from different animal species including goats, sheep, chicken and cows. Animal specimens include stool, milk and urine. Standard bacteriological culture on different agar plates that incubated at 37°C for 24 hours. Macroscopical colony morphology, Gram's stain, biochemical tests plus ViTeK 2 techniques were followed to confirm *K. pneumoniae* diagnosis. All human and animal isolates were submitted to antimicrobial susceptibility testing using disc diffusion technique interpreted by the standards of Clinical and Laboratory Standards Institute to determine the antimicrobial susceptibility of *K. pneumoniae* isolates against 14 antimicrobial agents. The results shows that a total of 41 macroscopically, biochemically and Vitek 2 confirmed *K. pneumoniae* isolates were recovered; 37 from human and 4 from animal specimens. Human isolates were mostly from inpatients particularly children and elderly. Urine and stool were the most human specimens harboring K. *pneumoniae*. On the animal side, chicken showed highest isolation rate and stool and milk were the most animal specimens harboring K. *pneumoniae.* Regarding the antimicrobial susceptibility, all (100%) human isolates were resistant to Rifampicin, Ampiclox and Methicillin. Higher resistant rates were also recorded against Carbenicillin and Piperacillin. The MDR rate of human isolates was 70.3%. Whereas, all animal isolates were resistant to Rifampicin, Ampiclox and Methicillin. Higher resistance rate was also recorded against Carbenicillin. The MDR rate of human isolates was 75.0%. Multiple drug resistant *K. pneumoniae* are prevalent among human population in Diyala community particularity in children and elderly hospitalized patients. Domesticated animal may play a role as an additional source of the MDR bacterium in the community.

Key words: *K. pneumonia*, Diyala province, MDR Klebsiella.

Introduction

Klebsiella pneumoniae is classified as a Gram-negative, non-motile and capsulated bacillus which occurs naturally both in the environment and on human mucous membranes of the gastrointestinal tract and oropharynx microbiome. The bacteria can spread from these colonized areas to distinct tissues causing acute infections, including pneumonia, urinary tract infections, wound infections, bacteremia and liver abscesses (Efah *et al*., 2020). *Klebsiella pneumoniae* is a common opportunistic nosocomial pathogen, accounting for about one third of all Gram-negative infections overall (Navon-Venezia *et al*., 2017). These infections occur mainly in patients with an impaired immune system, although it has been revealed that hypervirulent strains can invade immunocompetent individuals as well (Cantalan-Najera *et al*., 2017; Zhao, *et al*., 2019). Furthermore, *K. pneumoniae* is also responsible for serious community-onset infections Along with its high prevalence and virulence, *K. pneumoniae* is a major source of antimicrobial resistance (Lagerba *et al*., 2016; Ferreira *et al*., 2019).

Table 1: *K. pneumonia* isolation rate of human and animal specimens.

Bacterial isolates	Human		Animal	
	No.	%	No.	%
K. pneumonia	37	20.3	4	3.6
Other G- bacteria	52	28.6	66	59.4
G + bacteria	37	20.3	2	1.8
No growth	56	30.8	39	35.1
Total	183	100	111	100

The most common resistance mechanisms observed in *K. pneumoniae* are beta-lactamases, including extended-spectrum enzymes (ESBL) and carbapenemases. ESBLs hydrolyze penicillin, first, second and third generation cephalosporins and monobactams. Cephamycins and carbapenems remain active against ESBL-producing strains. Although the majority of ESBLs belong to class A which is divided into the following families: TEM, SHV, CTX-M, PER, VEB, GES, BES, OXA, TLA, SFO, IBC, BEL and PME (Martha *et al.,* 2017; Ferreira *et al*., 2019; Chen *et al.,* 2020). Multiple drug resistant *K. pneumonia* isolates were defined as those isolate which were resistant to at least one antimicrobial drug in three or more antimicrobial categories, while the extensively drug-resistant (XDR) is the non-susceptibility of the bacterium to all antimicrobial agents except in two or less antimicrobial categories, lastly the pandrug-resistant (PDR) is the non-susceptibility of the bacterium to all antimicrobial agents in all antimicrobial categories (Magiorakos *et al*., 2011).

The horizontal transfer of highly mobile plasmids containing beta-lactamases-encoding genes determines the rapid spread of multidrug resistance among bacteria (Mrowiec *et al*., 2019). Moreover, genes conferring resistance to other antimicrobials (aminoglycosides, trimethoprim, sulphonamides, tetracyclines and chloramphenicol) could be localized on these mobile genetic elements (Lagerba *et al*., 2016; Arhoune *et al*., 2017). The multidrug-resistant (MDR) strains, due to their high epidemic potential, have been responsible for many outbreaks of hospital-acquired infections (Li *et al*., 2019; Jernigan *et al*., 2020). *Klebsiella pneumoniae* is one of the most worrisome and threatening MDR pathogens. The principal vehicle for the introduction and spread of MDR Gram-negative bacilli in humans is fecal carriage (Hawkey, 2015; Gorrie *et al*., 2017). Therefore, to prevent dissemination of MDR organisms, every patient admitted to a hospital should be screened for rectal carriage of MDR K. pneumoniae (Sekowska *et al*., 2014).

Materials and methods

This is cross-sectional laboratory based study conducted in Diyala province for the period from October 2019 to April 2020. A total of 182 different human pathological specimens were included. These were including; blood aspirate, urine, stool and sputum samples plus wound and burn swabs. 43 patients were from Al-Batool Teaching Hospital for Maternity and Children, 61 patients from Baquba Teaching Hospital and the remaining 78 were outpatients from different Health Care Centers. 77 were males and 105 were females. Their age range was 1-65 years. Furthermore, 111 different specimens collected from different animal species including goats, sheep, chicken and cows. Animal specimens include stool, milk and urine samples.

Standard bacteriological culture on blood, MacConky and EMB agar plates were incubated at 37°C for 24 hours. Post-culture identification of *K. pneumoniae* isolates was based on the followings: Macroscopical colony morphology on different agar plates plus starching test. Gram's stain smears. Biochemical tests including, Urease test. Finally, ViTeK2 system were followed to confirm *K. pneumonia* diagnosis.

All human and animal isolates were submitted to antimicrobial susceptibility testing using disc diffusion technique interpreted by the standards of Clinical and Laboratory Standards Institute (CLSI, 2019; CLSI, 2018) to determine the antimicrobial susceptibility of *K. pneumonia* isolates against 14 antimicrobial agents. MDR *K. pneumonia* isolates were defined as those isolate which were resistant to at least one antimicrobial drug in three or more antimicrobial categories (Magiorakos *et al*., 2011).

Table 2: Culture positivity rate according to specimen collection site.

Bacterial species	Al-Batool Tech. Hospital		Baquba Tech. Hospital		Others HC centers	
	No.	%	No.	%	No.	%
K. pneumonia	23	53.5	10	16.4	4	5.1
Other G- bacteria	13	30.2	23	37.7	16	20.5
G+ bacteria	2	4.6	16	26.2	19	24.4
No growth	5	11.6	12	19.7	39	50.0
P value	0.001*					
*Significantly higher difference at P < 0.05						

Results

The results of bacteriological culture of human specimens yield 37 (20.3%) *K. pneumoniae* isolates (19 were pure growth of *K. pneumoniae*, 12 were mixed growth with E. *coli* and 6 were mixed with other lactose fermenter). 52 (28.6%) were G- bacteria (15 were pure *E. coli* and 37 were mixed lactose

Table 3: Bacteria species according to age groups.

Bacterial species	Age groups (Ys)					
	<18		18-49		=>50	
	No.	%	No.	%	No.	%
K. pneumonia	23	38.3	12	12.6	2	7.4
Other G- bacteria	18	30.0	25	26.3	9	33.3
G+ bacteria	4	6.7	23	24.2	10	37.0
No growth	15	25	35	36.8	6	22.2
P value	0.001*					
*Significantly higher difference at P < 0.05						

fermenter and non-lactose fermenter). Gram's positive bacteria was yielded in 37 (20.3%) and finally, 56 (30.8%) cultures have no growth. For the animal specimens, only 4 isolates of *K. pneumoniae* were recovered (2 were in pure growth and the other 2 were mixed with *E. coli*). 66 (59.4%) were other Gram's negative lactose fermenters and lactose non-fermenters. 2 isolates were Gram's positive bacteria and 39 (35.1%) were yield no growth, table 1.

Data presented in table 2 showed that the *K. pneumoniae* isolation rate was 23 (53.5%) from Al-Batool Teaching Hospital, 10 (16.4%) from Baquba Teaching Hospital and 4 (5.1%) from other health care centers. Therefore, the isolation rate is significantly higher (P= 0.001) from Al-Batool Teaching Hospital.

The isolation rate of *K. pneumoniae* was significantly higher 23 (38.3%) among those less than 18 years old compared to other age groups (p= 0.001), table 3.

According to the type of specimen, data presented in table 4 showed that the *K. pneumoniae* isolation rate from stool samples is significantly higher (59.1%) compared to other clinical specimens. (P= 0.001).

Table 4: Bacterial species according to type of specimen.

Bacterial species	Specimens					
	Blood No. (%)	Urine No. (%)	Wound No. (%)	Stool No. (%)	Sputum No. (%)	Burn No. (%)
K. pneumonia	2 (28.6)	14 (12.5)	3 (27.3)	13 (59.1)	3 (13.6)	2 (25.0)
Other G- bacteria	3 (42.8)	28 (25.0)	6 (54.5)	7 (31.8)	3 (13.6)	5 (62.5)
G+ bacteria	2 (28.6)	17 (15.2)	0 (0)	2 (9.1)	16 (72.7)	0 (0)
No growth	0 (0)	53(47.3)	2(18.2)	0(0)	0 (0)	1(12.5)
P value	0.001*					
*significant higher at P < 0.05						

Table 5: Bacterial species according to animal types.

Bacterial species	Animal types			
	Chicken	Goat	Cow	Sheep
	No. (%)	No. (%)	No. (%)	No. (%)
K .pneumoniae	2 (5.7)	1 (4.3)	1 (5.3)	0 (0)
Other G negative bacteria	33 (94.3)	5 (21.7)	11 (57.9)	16 (47.0)
Gram positive bacteria	0 (0)	0 (0)	1 (5.3)	1(2.9)
No growth of bacteria	0 (0)	17 (73.9)	6 (31.6)	17 (50.0)

According to animal types, 2 (5.7%) of *K. pneumoniae* isolates were recovered from chicken specimens, 1(4.3%) and 1(5.3%) isolates were recovered from goat and cow specimens respectively, table 5.

Culture of stool specimens yield 2 (3.6%) isolates of *K. pneumoniae*. Another 2 (8.7%) isolates were recovered from milk specimens, more details in table 6.

The results of antibiotic susceptibility of 37 confirmed isolates of *K. pneumoniae* from human source revealed that all isolates (100%) were resistant to Rifampicin, Ampiclox and Methicillin, while all (100%) isolates were sensitive to Imipenem. furthermore, 29 (78.4%) were resistant to Carbenicillin. Similarly, 23 (62.2%) were resistant to Piperacillin, More detailed were presented in table 7.

The rate of multiple drug resistant isolates of K. *pneumoniae* recovered from human specimen 70.3% while the other 29.7% isolated were non- MDR phenotype, table 8.

For the animal *K. pneumoniae* isolates, all (100%) isolates were resistant to Rifampicin, Ampiclox and Methicillin, while, all isolates (100%) were sensitive to Ciprofloxacin, Imipenem, Levofloxacin and polymyxin. Furthermore, 3(75%) of the isolates were resistant to Carbenicillin. More detailed presented in table 9.

The rate of multiple drug resistant isolates of *K. pneumoniae from animal specimens was* 75.0%, while only 25.0% was non- MDR phenotype, table 10.

Discussion

Antimicrobial resistance (AMR) is resistance of a microorganism to an antimicrobial drug that was originally effective for treatment of infections caused by it. The worldwide antimicrobial resistance crisis has a potentially devastating effect on human beings, livestock and the global economy. The global threat of deaths associated with AMR infections projected to exceed 10 million per year by the year 2050. The overuse and misuse of antibiotics is the primary driver of this resistance, with up to 50% of antibiotics prescribed in the hospital setting being either unnecessary or inappropriate (Editorial, 2014). Additionally, Health care–associated antimicrobial resistance share a substantial burden on nosocomial infections (Jernigan *et al*., 2020). In

Table 6: Bacterial species according to specimen types.

Bacterial species	Specimen types		
	Stool	Milk	Urine
	No. (%)	No. (%)	No. (%)
K. pneumoniae	2 (3.6)	2 (8.7)	0 (0)
Other G negative bacteria	49 (89.1)	4 (17.4)	12 (37.5)
Gram positive bacteria	0 (0)	0 (0)	2 (6.1)
No growth of bacteria	4 (7.3)	17 (73.9)	19 (57.6)

2017, WHO published new guidelines on the use of antibiotics in animals farmed for food. The document urged member states to reduce animal consumption of all classes of antibiotics vital for human health. Since it has been found that the restricting antibiotic consumption in the animal population resulted in a 10-15% decrease in resistance in animals. There was also a 24% decrease in resistance in humans, mainly among those in direct contact with animals (Editorial, 2014). Therefore, the present study is undoubtedly acquire it importance from the globalization and crucialness of this field.

The results of this study found that the isolation rate of *K. pneumoniae* from human specimens was higher than that of animal specimens (20.3% *Vs* 3.6%). Besides that, *K. pneumoniae* is a part of normal flora of the mouth, skin and intestines, exogenously it can enter the body by many routes as it is naturally occurs in the soil, sewage and feces (Gorrie *et al*., 2017; Jose and Joana, 2019). Furthermore, *K. pneumoniae* has many virulence factors, therefore the range of clinical diseases is wide includes pneumonia, thrombophlebitis, urinary tract infection, cholecystitis, diarrhea, upper respiratory tract infection, wound infection, osteomyelitis, meningitis, bacteremia and sepsis (Li *et al*., 2014; Clegg and Murphy, 2016). Accordingly, the more comprehensive collected human specimens may partly explain the high isolation rate compared to restricted animal specimens. Additionally, the life span of humans is longer than animals, thus, particularly during the elderlyhood may complain certain degree of immunocompromization probably as a result of chronic debilitating diseases that may enhance the endogenous *K. pneumoniae* infections (Liu *et al*., 2019).

The human results also found that the *K. pneumoniae* was generally higher among hospitalized patients compared to outpatients (89.2% *Vs* 10.8%). Actually these facts were previously reported by many studies (Apondi *et al*., 2016; Martha *et al*., 2017; Jernigan *et al*., 2020). *K. pneumoniae*, an opportunistic pathogen, ranks among the most important causes of nosocomial infections in developing countries and among the eight most important causes in developed countries, especially in immunocompromised patients and those with indwelling medical devices on which the bacteria is able to grow in a biofilm (Murphy and Clegg, 2012; Cruz-Cordova *et al*., 2014; Alcantar-Curiel *et al*., 2018). The main reservoir for transmission of *K. pneumoniae* is the gastrointestinal tract of patients and the hands of medical staff (Calbo *et al*., 2011; Gorrie *et al*., 2017).

Table 7: Antibiotic susceptibility testing of human *K. pneumoniae* isolates.

Antibiotics	Resistant		Sensitive		Intermediate	
	No.	%	No.	%	No.	%
Rifampicin	37	100	-	-	-	-
Ceftriaxone	16	43.2	21	56.8	-	-
Ciprofloxacin	3	8.1	33	89.2	1	2.7
Carbenicillin	29	78.4	3	8.1	5	13.5
Imipenem	-	-	37	100	-	-
Streptomycin	15	40.5	17	45.9	5	13.5
Nalidixic acid	7	18.9	21	56.8	9	24.3
Trimethoprim-Sulfamethozal	16	43.2	18	48.6	3	8.1
Piperacillin	23	62.2	6	16.2	8	21.6
Azithromycin	5	13.5	31	83.8	1	2.7
Levofloxacin	4	10.8	30	81.1	3	8.1
Polymyxins	12	32.4	25	67.6	-	-
Ampiclox	37	100	-	-	-	-
Methicillin	37	100	-	-	-	-

Table 8: MDR *K. pneumonia* isolates from human samples.

Category	No. of resist isolates	%
Isolates resistant to $\leq$ 2 antimicrobial categories	11	29.7
Isolates resist to $\geq$ 3 antimicrobial categories	26	70.3
Total	37	100%

Furthermore, the current results found that the highest isolation rate of *K. pneumoniae* was from patients of childhood age. Similar results were also reported by others (Clegg and Murphy, 2016; Jose and Joana, 2019). The high isolation rate of *K. pneumoniae* from children in our community can be explained by the high rate of secondary bacterial infection following the paramyxoviruses and orthomyxoviruses respiratory infections which are more prevalent among children and young adults (Kentigern and Andrew, 2012; Christophe and François, 2019). The mechanisms underlying post-viral bacterial infections are complex and include multifactorial processes mediated by interactions between viruses, bacteria and the host immune system. As these viral infections of the

Table 9: Antibiotic susceptibility testing of animal *K. pneumoniae* isolates.

Antibiotics	Resistant		Sensitive		Intermediate	
	No.	%	No.	%	No.	%
Rifampicin	4	100	-	-	-	-
Ceftriaxone	1	25.0	3	75.0	-	-
Ciprofloxacin	-	-	4	100	-	-
Carbenicillin	3	75.0	-	-	1	25.0
Imipenem	-	-	4	100	-	-
Streptomycin	1	25.0	3	75.0	-	-
Nalidixic acid	1	25.0	3	75.0	-	-
Sulfamethozal	2	50.0	2	50.0	-	-
Piperacillin	1	25.0	1	25.0	2	50.0
Azithromycin	1	25.0	3	75.0	-	-
Levofloxacin	-	-	4	100	-	-
Polymyxin	-	-	4	100	-	0
Ampiclox	4	100	-	-	-	-
Methicillin	4	100	-	-	-	-

upper or lower respiratory tract suppress the innate local immunity of the host and considerable alterations in epithelial surfaces, thus enabling the existing non-pathogenic bacteria to flare-up and caused its disease (Amber and Jonathan, 2014; David *et al*., 2018). In this regard, Agnieszka *et al.,* (2015) stated that both innate and adaptive antibacterial host defenses are impaired in the context of preceding influenza virus infection, thus influenza-induced susceptibility to secondary bacterial pneumonia. Additionally, it has been reported that antiviral immune responses induced by acute respiratory infections such as influenza are associated with changes in microbial composition and function (dysbiosis) in the respiratory and gastrointestinal tract, which in turn may alter subsequent immune function against secondary bacterial infection or alter the dynamics of inter-microbial interactions, thereby enhancing the proliferation of potentially pathogenic bacterial species (Cawcutt and Kalil, 2017; Shigeo *et al*., 2018).

The present results also revealed that among the domesticated animals included, the isolation rate of *K. pneumoniae* was highest in chickens and the stool the main specimens harboring the bacterium. These results are in agreement with other studies (Cheng *et al.,* 2018; Hayati *et al*., 2019). *K. pneumoniae* is widely existed in surface water, sewage, soil, plants and the mucosal surfaces of mammals (Siu *et al*., 2011). Due to its broad spectrum of virulence factors, the infection of *K. pneumoniae* usually plays an important role for the causing of pneumonia, bloodstream infection and pyogenic liver abscesses in mammals (Newire *et al*., 2013). The invasion of *K. pneumoniae* in domestic animals not only causes hazard in livestock production but also poses a potential threat to public health since these animals can act as the reservoir of multidrug-resistant *K. pneumoniae* strains (Efah *et al.,* 2020). Although antibiotic therapy is a widely tool for the treatment of infections caused by *K. pneumoniae*, however, antibiotic resistance in pathogenic bacteria from food-producing animals and environmental sources is recognized as a global problem for public health (Navon-Venezia *et al*., 2017).

Table 10: MDR *K. pneumonia isolates* from animal samples.

Category	No. resistant isolates	%
Isolates resistant to $\leq$ 2 antimicrobial categories	1	25.0
Isolates resist to $\geq$ 3 antimicrobial categories	3	75.0
Total	4	100%

The central dogma of the present study is the high resistance rates of *K. pneumoniae* isolates of both human and animals to wide range of antibiotics and antibacterial. Moreover, according to the definition of Magiorakos *et al*., (2011) actually more than two thirds of these isolates were MDR. In this regard, worldwide studies reporting an emergence of such MDR strains of *K. pneumoniae* have been extensively documented (Apondi *et al*., 2016; Ferreira *et al*., 2019; Heinz *et al*., 2019). Certainly these newly emerges stains were become more virulent (Cruz-Cordova *et al*., 2014; Zhao *et al*., 2019). Therefore, such strains are responsible for high mortalities particularly among elderly and those with comorbidities (Liu *et al*., 2019; Arij *et al*., 2019; Liu *et al*., 2019), Moreover, Extended drug resistant strains of *K. pneumoniae* also had been documented in both human and livestock (Newire *et al*., 2013; Hayati *et al*., 2019; Mrowiec *et al*., 2019).

The horizontal transfer of highly mobile plasmids containing beta-lactamases-encoding genes determines the rapid spread of multidrug resistance among bacteria (Mrowiec *et al*., 2019). The principal vehicle for the introduction and spread of MDR Gram-negative bacilli in humans is fecal carriage (Hawkey, 2015; Gorrie *et al*., 2017). The overuse and misuse of antibiotics is the primary driver of this resistance, with high rate of antibiotics prescribed in the hospital setting or outpatient clinics being either unnecessary or inappropriate (Editorial, 2014). Additionally, Health care-associated antimicrobial resistance share a substantial burden on nosocomial infections (Jernigan *et al*., 2020). In Diyala or probably whole Iraq, the principal reason behind this problem is the wide availability of antibiotics or antibacterials on counter without prescription for any types and any quantity. Additionally, what is aggravating the problem is the wide use

of antibiotics for food or meat a animals. Thus it is not surprising to find a XDR or even pandrug (PDR) resistant strains of *K. pneumoniae* or other Gram's positive or Gram's negative bacteria.

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