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Research Article

ASSOCIATION OF *TETA* AND *TETB* GENES AND DRUG RESISTANCE PATTERN OF *K.PNEMONIAE* ISOLATED FROM PATIENTS VISITING GONDAR UNIVERSITY TEACHING HOSPITAL

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ABSTRACT

***Klebsiella pneumoniae* found as a common flora in intestinal tract, mouth and skin of humans do not cause disease. However, when the bacterium founds other parts of the body, it is associated with many different types of human disease. *K. pneumoniae* is one of the pathogen that contains a variety of resistance genes. The objective of this study was to detect *tetA* and *tetB* resistance genes in *K. pneumoniae*. Experimental study was conducted on a totality of 50 *K. pneumoniae* isolates from different specimens of patients at Gondar University hospital from November, 2014 to April, 2016. The *Klebsiella pneumoniae* genomic DNA was isolated by phenol chloroform method. PCR was performed to detect *tetA* and *tetB* genes by specific pair of primers. *K. pneumoniae* showed resistance against Amoxicillin 50(100%), followed by Streptomycin 23 (60%). From total, forty (80%) of *K. pneumonia* isolates were multidrug resistance which showed a resistance to two or more antibiotics. The occurrence of the genes *tetA* and *tetB* were 2 (4%) and 1(2%) for *tetA tetB* genes, respectively. The presence of multidrug resistance *K. pneumoniae* strains that harbor resistance genes (*tetA and tetB*), might be serving as indicators for the role of these genes in antibiotic resistance.**

Key words*: Klebsiella pneumoniae,* resistance, tetA, tetB, tetracycline.

INTRODUCTION

Klebsiella pneumoniae is a gram negative, capsulated, facultative anaerobe, rod shaped bacterium which belongs to the family *Entrobacteriaceae*. *K. pneumoniae* found as a common flora in intestinal tract, mouth and skin of people does not cause any disease. However, when the bacterium finds other parts of body, it is associated with a range of different types of human disease which includes urinary tract infections, respiratory tract infection like pneumonia, septicemia, diarrhea, liver abscess, and wound infections in community and healthcare settings (Onuoha and Fatokun, 2014; Kim *et al.*, 2011; Chien *et al.*, 2002; Fung *et al.*, 2002).

K. pneumoniae is a typical nosocomial pathogen which mainly associated with healthcare associated infections that transmitted within the hospital or healthcare settings. Immuno-compromised people who have feeble immune systems, sick or injured people who undergone procedures for various health issues in healthcare settings are at high risk for *Klebsiella pneumoniae* infection. However, the bacterium doesn't cause an infection in healthy people and they are not at risk of getting *K. pneumoniae* infections, according to the Centers for Disease Control and Prevention (CDC, 2013).

K. pneumoniae infection multiply by direct people-to- people contact, like whenever someone with contaminated hands touches a wound. The disease can also be spread by the use of dirty medical equipment. For example; people on ventilators can contract *K. pneumoniae* if breathing tubes are contaminated with the bacteria. Similarly, the use of contaminated intravenous catheters can lead to bloodstream infections. Extensive usage of antibiotics over a long course of time can also increase a person's risk of getting a Klebsiella infection (Arnold *et al.*, 2011).

Epidemic and endemic nosocomial infections caused by Klebsiella species are leading causes of morbidity and mortality (Manikandan and Amsath, 2013). The study in Nigeria reveals that urinary tract infection (UTI) is a main health problem among pregnant women and *Klebsiella pneumonia* is the predominant uropathogen that causes urinary tract infection (Onuoha and Fatokun, 2014). Similarly, the study from Taiwan, have also shown that this pathogen has become the predominant cause of liver abscess (Fung *et al.*, 2002).

Antimicrobials such as Tetracycline, Carbapenem, Cephalosporin, and ciprofloxacin have been used for the treatment of bacterial diseases. However, the emergence of antibiotic resistances compromises the treatment, prevention and control of infectious diseases. Like other bacteria Klebsiella has also become resistant to some common antibiotic. *K. pneumoniae* is generally resistant to a broad range of antibiotics, and almost always naturally resistance to Ampicillin and Amoxicillin. Drug resistant *K. pneumoniae* infections are challenging to treat compared to non-resistant bacteria (Manikandan and Amsath, 2013).

K. pneumoniae recently has gained attention for the appearance of multiple drug-resistant strains in healthcare settings that produce extended spectrum beta-lactamase. This form of resistance is due to

the production of unique beta-lactamase enzymes, referred to as extended spectrum beta-lactamases (ESBLs) (Nordmann *et al*, 2009; Sarathababu *et al*, 2012 and Kumar, 2013). *K. pneumonia* produce an enzyme known as carbapenemase also referred to as, '*K. pneumoniae* carbapenem producing organisms,' the class of antibiotics known as carbapenems will not work to kill the bacteria or treat the infection (CDC, 2013).

The carbapenem antibiotics was the last line of defense against gram-negative infections. However, resistance to carbapenems, resulting from carbapenemase production, reduces the possibility of treating infections of multidrug-resistant strains (Barguigua, *et al.*, 2012). According to CDC (2013), Carbapenem-resistant Klebsiella is the most common type of Carbapenem-resistant *Enterobacteriaceae* (CRE), and is responsible for about 7,900 infections and 520 deaths.

Bacterial drug resistance could occur by various mechanisms such as reduced permeability or uptake, enhanced efflux, enzymatic inactivation, alteration or over expression of the drugs target and loss of enzymes involved in drug activation, this mechanism is relatively new. Tetracycline resistance commonly can be conferred by efflux proteins and ribosomal protection proteins. The genes encoding efflux proteins belong to the major facilitator super family (MFS). All the tet efflux genes code for membrane-associated proteins which export tetracycline from the cell. Export of tetracycline reduces the intracellular drug concentration and thus protects the ribosomes within the cell (ARDB) (Bo *et al*., 2008) recently over 40 *tet* genes have been identified (Roberts, 2005). The objective of this study was to determine the presence of tetracycline resistance genes (*tetA* and *tetB*) in *K. pneumoniae* infected patients at Gondar University hospital.

MATERIALS AND METHODS

Study area and period

This study was carried out from November, 2014 to April, 2016 at Gondar University hospital, Gondar, Ethiopia. Gondar University hospital is found in North Gondar zone, Northwest Ethiopia. The hospital is a tertiary-level teaching hospital that serves around 4 million people from the surrounding zones and nearby regions. Gondar is located in Amhara regional state in the Northwest of Ethiopia bordering Sudan Republic and lies between 9° to 13° 45'N latitude and 36° to 40° 30'Elongitude. It covers approximately 16, 828.4 km^2 in area. The region is composed of 11 administrative zones. It has variable altitudes that range from 550 m (in western lowland) to 4,620 m (Semen Mountain in North) above sea level and it is the homeland of Walia ibex and Ethiopian wolf. The annual average rainfall varying from 510 mm to 2,000 mm that is characterized by bimodal type of distribution. The annual average temperature ranges from 10^0C (in the highland) to 44.5^0C (in the lowland) (Central Statistical Agency, 2008).

Data collection

A total of 50 culture confirmed of *K. pneumoniae* isolates from different clinical samples were collected from patients who visited Gondar University hospital from November 2014 to April 2016. Socio-demographic and other relevant data such as

age, gender, inpatient, outpatient and source of specimen (urine, blood, wound discharges, *etc*) were collected from each study participants by following standard operational procedures.

Inoculums preparation

K. pneumoniae isolates were isolated and collected separately from the samples by culturing overnight on Nutrient agar at 37 ^{0}C by using streak plate method. Then About 3-5 colonies of the test organism was transferred into a tube containing 1 ml of sterile normal saline (direct colony suspension) and mixed gently until the suspension becomes turbid then it was adjusted to 0.5 McFarland standard which is equivalent to a bacterial suspension of 1.5 x10^8 colony forming unit CFU/ml according to the guide line (CLSI, 2013). Using sterile cotton swab with applicator stick, the suspended bacteria from the broth was uniformly distributed on the surface of Muller Hinton agar plate.

Antibiotic susceptibility test

The susceptibility of all applied antibiotics was performed using disc diffusion method on Mueller-Hinton agar as recommended by Clinical and Laboratory Standards Institute (CLSI, 2013). The selected antibiotics were amoxacin (AX, 30μg) ceftriaxone (CT, 30μg), streptomycin (s, 15 μg), ciprofloxacin (CIP, 5 μg), and tetracycline (tet, 30 μg). Discs of the selected antibiotics were placed aseptically on the surface of inoculated media. The plates were dried for some minutes by putting upside down. Antibiotic discs were applied on the plate using sterile forceps ensuring complete contact with the agar surface and the plates were incubated at 37°C for 8-12 hrs. Finally, the diameters of the zone of inhibition around the discs were measured to the nearest millimeter using caliper, and the isolates were classified as susceptible, intermediate and resistant according to the interpretative standards of Clinical Laboratory Standards Institute (CLSI, 2013). Moreover, isolates showing resistance to two or more antimicrobial subclass were considered as multidrug resistant.

Genomic DNA isolation

Isolates which were resistant for two or more antimicrobial agent were cultured in nutrient broth at 37^{0}c for 24hr. Bacterial cultures of 2ml were transferred into a 2ml micro centrifuge tubes and centrifuged at 12,000 revolutions per minute (RPM). After harvesting enough bacterial cell, the DNA was isolated by HipurA^{TM} bacterial genomic DNA purification kit according to the manufacturer's instructions. The bacterial cells were resuspended in bead solution and transferred to a bead-beating tube containing microbeads and proteinase K. Subsequently, the cells were lysed through mechanical disruption using a standard vortex. The supernatant was loaded onto a Spin Filter mini column and washed twice with Wash buffer, and the genomic DNA was eluted with DNA-free Tris buffer. The quality of the isolated DNA was analyzed through spectrophotometric analysis.

Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) was implemented to detect the presence of (*tetA* and *tetB* gene**)** on the study organism. The following two sets of primers used to detect *tetA* and *tetB gene*. The primer for *tetA* 5'-

GTGAAACCCAACATACCCC-3' (F) and 5'- GAAGGCAAGCAGGATGTAG-3'(R) and primer for *tetB* 5'-CCTTATCATGCCAGTCTTGC-3' (F) and 5'-ACTGCCGTTTTTTCGCC-3'(R) were used. The PCR master mix was composed of 0.65µl 100pm of each primer, (3µl of 25mM Magnesium Chloride, 2.5µl of 10X PCR buffer, 1µl of 20mM dNTP mixture and 0.2µl 5U Taq DNA polymerase (Soils Bio dyne) with a molecular grade distilled water for 50µl reaction volume. The polymerase chain reaction conditions to amplify the two genes of interest were adjusted as follows: initial denaturation at 94^{o}C for 5 minutes, followed by; 94^{o}C as denaturation temperature for one minute, 59^{o}C as annealing temperature for one minute and 72^{o}c as an extension temperature for one minute; for 35 cycles of amplification. The final extension temperature was 72^{o}C for 10 minutes. After PCR amplification the PCR product was visualized and photographed by gel documentation system after running with 1.5% agarose gel electrophoresis. To visualize fragments of 888bp and 774bp PCR product for *tetA* and *tetB* gene respectively, 1.5% agarose gel electrophoresis was employed.

Data analysis and interpretation

The data from questionnaires and laboratory results was feed in to SPSS version 16 for analysis. Descriptive statistics and binary logistic regression analysis was employed. The results were analyzed by using descriptive statistics which includes of frequencies and mean. Difference of proportion was evaluated using Chi-square test. p–values < 0.05 were considered as statistically significant.

RESULT

Demographics characteristics

A total of 50 *K. pneumoniae* isolates were collected from patients who visited Gondar University hospital during the study period. From all culture confirmed positive *K. pneumonia* infections, 28 (56%) were males and 22 (44%) were female patients. The minimum and maximum age of study participants was 1 and 85 years respectively with mean age 23.48 (±22.63) years. From total study participants, 38 (76%) were from inpatients and 12 (24%) were from outpatients. With respect to type of specimen, 16 (32%) and 16 (32%) of *Klebsiella pneumoniae* isolates were obtained from wound discharge and urine specimen, respectively while, 11 (22%) isolates were from blood, 6 (12%) from eye and ear discharge and 1 (2%) from Cerebrospinal fluid (CSF) as described in table no. 1 below

Table 1: Socio-demographic and clinical characteristics of *K. pneumoniae* infected patients in Gondar University hospital.

Variable	Category	Frequency (%)
Sex	Male	28 (56%)
	Female	22 (44%)
Age	≤ 15	22(44%)
	16-25	9 (18%)

	26-40	10 (20%)
	> 40	9(18%)
Specimen type	Eye and ear discharge	6(12%)
	Urine	16(32%)
	Wound discharge	16(32%)
	Blood	11(22%)
	CSF	1 (2%)
Patient site	Inpatient	38 (76%)
	Out patient	12 (24%)
Total		50 (100%)

CSF – Cerebrospinal fluid

Antimicrobial susceptibility pattern of *Klebsiella pneumonia* isolates.

Antimicrobial susceptibility testing of 50 cultures confirmed *Klebsiella pneumonia* isolates was done by disc diffusion method on Muller Hinton agar plates and zone of inhibition was interpreted according to CLSI guideline. On their resistance to antibiotics, isolates were categorized into three groups as susceptible (S), Intermediate (IR) and resistant (R) as presented in Table 2 below. Majority of the isolates were susceptible to ciprofloxacilin 40 (80%) and 29 (58%) for Tetracycline respectively. The highest resistance was observed against Amoxicillin 50(100%), followed by Streptomycin 23(60%) as described in Table 2.

Table 2. Antimicrobial susceptibility pattern of *Klebsiella pneumoniae* isolates among study subjects at Gondar University hospital.

Antimicrobial agents	Category	No/%
Tetracycline	S	29 (58)
	IR	1 (2)
	R	20 (40)
Amoxicillin	S	0 (0)
	IR	0 (0)
	R	50 (100)
Ciprofloxacin	S	40 (80)
	IR	6 (12)
	R	4 (8)
Ceftriaxone	S	18(36)
	IR	1(2)
	R	31(62)
Streptomycin	S	17 (34)
	IR	3(6)

	R	30(60)
S = Sensitive	IR=intermediate resistance	R = resistance

From total isolates, 40 (80%) of *Klebsiella pneumonia* isolates were multidrug resistance which showed a resistance to two or more antibiotics. Majority of multidrug resistance isolates were from inpatients 28 (70%) and from urine specimen, 14 (35%). Significantly high proportion of MDR *Klebsiella pneumonia* isolates was obtained from inpatients (p =0.047). In addition majority of MDR isolates were from age groups less than 15 years. The isolates which were resistant for two or more antimicrobial agents were subjected to PCR amplifying the efflux genes *tetA* (888bp) and *tetB* (774bp) in which only 2 (4%) isolates showed the presence of *tetA* gene and 1(2%) showed the presence of *tetB* gene as described in Table 3.

Table 3. Distribution of multidrug resistance *K. pneumoniae* isolates among study subjects in Gondar University hospital.

variable	Category	No	MDR *K. pneumoniae isolates* No (%)	χ^2 Value	*p-value*
Sex	Male	28	20 (50 %)	2.922	*0.087*
	Female	22	20 (50%)		
Age	≤ 15	22	16 (40%)	3.283	
	16-25	9	7 (17.5%)		*0.350*
	26-40	10	10 (25%)		
	> 40	9	7 (17.5%)		
Specimen type	Eye and ear discharge	6	5 (12.5%)	7.997	
	Urine	16	14 (35%)		
	Wound discharge	16	13 (32.5%)		*0.156*
	Blood	11	7 (15.5%)		
	CSF	1	1 (2.5%)		
Patient site	Inpatient	38	28 (70%)	3.947	
	Out patient	12	12(30%)		***0.047***
Total		50	40(100%)		

***note**: CSF =cerebrospinal fluid χ =chi-square test MDR = Multidrug resistance*

K. pneumoniae isolates which have resistance for two or more antibiotics were grouped as multidrug resistance. Bivariate analysis of independent variables with multidrug resistance *K. pneumoniae* isolates among patients attending Gondar University

hospital indicated that, all of the independent variables like age, sex, specimen type and patient site were not significantly associated with multi drug resistance pattern of isolates as described in Table 4.

Table 4. Bivariate analysis of independent variables with Multidrug resistance *K. pneumoniae* isolates among study subjects at Gondar University hospital.

Variables	Category	MDR No	Non-MDR No	COR (95% C.I)	*p-value*
Sex	Male	20	8	0.250 (0.047-1.327)	*0.103*
	Female	20	2	1*	
Age	≤ 15	16	6	1*	
	16-25	7	2	1.313 (0.210-8.184)	*0.771*
	26-40	10	0	6.058 (0.000)	*0.999*
	> 40	7	2	1.313 (0.210-8.184)	*0.771*
Specimen type	Eye and ear discharge	5	1	0.000 (0.000)	*1.000*
	Urine	14	1	0.000 (0.000)	*1.000*
	Wound discharge	13	3	0.000 (0.000)	*1.000*
	Blood	7	4	0.000 (0.000)	*1.000*
	CSF	1	0	1*	
Patient site	Inpatient	28	10	0.000 (0.000)	*0.999*
	Outpatient	12	0	1*	

*= Reference category, COR= crude odds ratio, 95% C.I = 95% confidence interval, MDR = Multidrug resistance

tetA and tetB resistance genes of *Klebsiella pneumonia*

In this study a total of 3 (3/40) Tetracycline resistance genes (both *tetA* and *tetB* genes) were detected from a total of 40 multidrug resistance *Klebsiella pneumonia* isolates. While in the rest multidrug resistance *Klebsiella pneumoniae* isolates drug resistance genes were not detected, 37/40 (92.5%). Majority of drug resistance genes were detected from male patients, 2/3 (66.7%) and age group ≤ 15years, 2/3 (66.7%). All of *tetA* and *tetB* resistance genes were detected from *K. pneumonia* isolates obtained from inpatients 3/3 (100%) as described Table 5.

Table 5. Distribution of *tetA* and *tetB* resistance genes of *K. pneumoniae* isolates among study subjects in Gondar University hospital.

variable	Category	No	MDR No	*TetA* and *tetB* genes		*X -value*	*p-value*
				yes	No		
Sex	Male	28	20	2 (66.7%)	26(55.3%)	0.147	*0.701*

	Female	22	20	1 (33.3%)	21(44.7%)		
Age	≤ 15	22	16	2 (66.7%)	20(42.6%)	2.002	*0.572*
	16-25	9	7	1(33.3%)	8(17.0%)		
	26-40	10	10	0(0.0%)	10(21.3%)		
	> 40	9	7	0(0.0%)	9(19.1%)		
Specimen type	Eye and ear discharge	6	5	0(0.0%)	6(12.8%)	2.349	*0.799*
	Urine	16	14	1(33.3%)	15(31.9%)		
	Wound discharge	16	13	2(66.7%)	16(29.8%)		
	Blood	11	7	0(0.0%)	11(23.4%)		
	CSF	1	1	0(0.0%)	1(2.1%)		
Patient site	Inpatient	38	28	3(100%)	35(74.5%)	1.008	*0.315*
	Out patient	12	12	0(0.0%)	12(25.5%)		
Total		50	40	3 (7.5%)	37(92.5%)		

***note:** CSF =cerebrospinal fluid* χ *=chi-square test* *MDR = Multidrug resistance*

Fig 1. Representative agarose gel electrophoresis picture of tetA and tetB genes. M.100 bp DNA marker. Lanes 1,3,8, 888bp tetA gene, lanes 2,4,6, 888 bp tetA and 747 bp tetB genes, lanes 9 and 1o indicate 747 bp tet B gene.

Bivariate analysis of independent variables with detection of both *tetA* and *tetB* genes among *K. pneumoniae* isolates indicated that all of the variables indicated were not significantly associated with resistance gene detection within the isolates (p-value >0.05) as described in Table 6.

Table 6. Bivariate analysis of independent variables with *tetA and tetB genes* of *K. pneumoniae* isolates among study subjects at Gondar University hospital.

Variable	Category	No	MDR No	*tetA* and *tetB* genes		COR (95% C.I)	*p-value*
				Yes	No		
Sex	Male	28	20	2	26	0.619(0.052-7.307)	*0.703*
	Female	22	20	1	21	1*	
Age	≤ 15	22	16	2	20	1*	
	16-25	9	7	1	8	0.80(0.063-10.11)	*0.863*
	26-40	10	10	0	10	1.615(0.00)	*0.999*
	> 40	9	7	0	9	1.615(0.00)	*0.999*
Specimen type	Eye and ear discharge	6	5	0	6	1.00(0.00)	*1.00*
	Urine	16	14	1	15	0.00(0.00)	*1.00*
	Wound discharge	16	13	2	16	0.00(0.00)	*1.00*
	Blood	11	7	0	11	1.00(0.00)	*1.00*
	CSF	1	1	0	1	1*	
Patient site	Inpatient	38	28	3	35	1.385(0.00)	*0.999*
	Out patient	12	12	0	12	1*	
Total		50	40	3	47		

*= Reference category, COR= crude odds ratio, 95% C.I = 95% confidence interval, MDR = Multidrug resistance

DISCUSSION

This study provides insights on detection of tetracycline resistance genes (*tetA* and *tetB*) and antibiotic resistance pattern of *K. pneumonae* isolated from different clinical specimens among patients at Gondar University teaching hospital. In the present study, *K. pneumonia* isolates showed different pattern of resistance to antibiotics, Amoxicillin (100%), Streptomycin (60%), Ceftriaxone (62%) and Tetracycline (40%) which is high. Similarly, a study conducted in Italy reported 100% resistance of *K. pneumonae* isolates for Amoxacin from different clinical specimens (Silva . *et al.*,2014), and in India, 100% resistance for Tetracycline, 88.8% for ciprofloxacilin, 60.2% for Amoxacin (Asati, 2013). Additionally, a study from Pakistan reported, 69% of *K. pneumoniae* isolates resistance for tetracycline and amoxicillin (Jamil *et al*., 2014), from Bangladesh it has been reported that 81% of *K. pneumoniae* isolates were resistant to Amoxicillin (Akter *et al*., 2013) and in Nigeria 96.1% of *K. pneumoniae* isolates from different specimen were resistant for Amoxicillin, for Tetracycline it was reported to be 77.8%

resistant strains from the same location (Olowe *et al.*,2013). A previous study at Gondar University hospital, Ethiopia reported that *Klebsiella* species were resistance for Tetracycline (82.9%) and Amoxicillin (97.7%) (Tiruneh *et al*., 2014). High antibiotic resistance of *K. pneumoniae* isolates can indicate that bacterial drug resistance is an ongoing public health threat within the health care settings and within the community at large which cause various infections. The spread of drug resistance likely due to the fact that inappropriate prescription of antibiotics and usage of commercially available antibiotics like Amoxicillin without prescription, incorrect dose and duration which mainly contributes to the occurrence and spread of resistant bacteria strain.

In the present study the prevalence of multidrug resistance *K. pneumoniae* isolates was 40/50 (80%). This result is in line with a study conducted in India, which reported 80% MDR *K. pneumoniae* isolates from different specimens (Khadri *et al*., 2007),and similar scenario was observed in the study done in Iran33/41 (80.5%) of *K. pneumoniae* isolates were multidrug-resistant (MDR) strains (Masoume *et al.,* 2015).Similar study from Italy reported 40 multidrug resistances *K. pneumoniae* isolates from different clinical samples (Silva *et al.,* 2014). The finding of the present study is higher as compared to a study conducted in South Africa which is reported to be 23.1% multidrug resistance *K. pneumoniae* isolates (Habte *et al*., 2009*).* In Iran, 544.9% MDR *K. pneumoniae* isolates were reported from urine sample which is quite high number when compared to other studies at different locations (Latifpour *et al.,* 2016). Nevertheless, the finding of this study is low when compared to a study in Guinea that reported 91.7% of *K. pneumoniae* isolates with multidrug resistance pattern (Shatalov., 2015). This might be due to the difference in study population, the type of specimen in which *K. pneumoniae* was obtained and small sample size in the present study. Multidrug resistance *K. pneumonae* isolates from multiple clinical specimens indicated that antibiotic resistance is still a public health problem in the study area and bacterial antibiotic resistance is the most alarming situation in the treatment of infectious disease.

In molecular identification of *tet* genes in multidrug resistance *K. pneumoniae* isolates, showed that the existence of both *tetA and tetB* genes. In the present study a total of 3 *tet* (1 *tetA* and 2 *tetB*) genes were detected which gives 7.5%. The current result is by far lower compared to a study conducted in Iran, which reported *tetA* and *tetB* resistance genes from all 30/30 (100%) drug resistance *K. pneumoniae* isolates (Bokaeian *et al*., 2014), morever the current result is lower than the study done in Nigeria 43.8% of *K. pneumoniae* isolates from different specimens harbor *tetA* and 32.2% *tetB* genes, while both *tetA* and *tetB genes* were observed in 4.4% of *K. pneumonia* isolates (Olowe *et al.*, 2013). In comparison the study conducted on *E. coli* isolated from poultry drinking water, 8/25 (32%) isolates contained *tet*A gene whereas 17/25 (68%) isolates harbor both *tet*A and *tet*B genes

(Naseer *et al.,* 2014*)*. This difference might be due to difference in type of the specimen, small number of sample size, difference in antibiotic resistance pattern and also different frequency of harboring resistance genes. In addition, this difference might be due the small number of sample size in the present study which results in low number of drug resistance gene detection. However, multidrug resistance pattern with detected resistance genes in *K. pneumoniae* isolates indicated that the spread of drug resistance genes and the spread of multidrug resistance strains with in the hospital environment and surroundings.

In the present study significantly high proportion of *K .pneumoniae* isolates (76%) were obtained from inpatients ($P<0.05$). Among the specimens more *K. pneumoniae* isolates were recovered from urine and wound discharge (32%) each. Similar study conducted at Jimma specialized hospital indicated drug resistant isolates were significantly high among isolates from inpatients (46.4%) compared to outpatients (14.13%) (Shewki *et al*., 2015). This indicates that health care settings can be the source of infection. The spread of drug resistance *K. pneumoniae* isolates within the health care settings might be through inappropriate infection control and disinfection practices in hospital settings, contaminated intravenous catheters, hands of health care staffs and other related factors like irrational use of empirical therapy without antimicrobial susceptibility testing which contributes for the emergence of bacterial drug resistance.

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