View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by International Institute for Science, Technology and Education (IISTE): E-Journals

Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.8, No.22, 2018
www.iiste.org

Phenotypic and Genotypic Characterization of Antibiotics Resistance Klebsiella pneumoniae Isolated from Clinical Samples at The Nairobi Hospital, Kenya.

Celestin NIBOGORA [1*] Andrew K.NYERERE[1] Caroline W.Ngugi [1] Paul MAKAU[2]
1.College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology
2.Pathology Department, Nairobi Hospital, Kenya

This study was financially supported by the research grant from Government of BURUNDI.

Abstract
Antimicrobial resistance is a growing threat worldwide. The present study aimed to determine the molecular basis of antibiotics resistant characteristics of *Klebsiella pneumoniae* isolates. Identification of Clinical isolates and detection of Beta lactamase enzymes with their antibiotic susceptibility pattern was done by automated VITEK 2 system. The distribution of genes was assessed by PCR method. The resistance rate of *Klebsiella pneumoniae* isolates to antibiotics was 100% to Ampicillin. Among 272 isolates, ESBLs were seen in 81(29.8%) isolates, carbapenemases were seen in 19(7.0%) isolates, ESBL and carbapenemases co-existed in 14(5.1%) isolates, AmpC and carbapenemases co-occurred in a 5 isolates (1.8%), 7 (2.6%) isolates produced ESBL + AmpC+carbapenemases,2 (0.7%)showed ESBL +AmpC producers. None of the isolates showed AmpC production alone. The occurrence of TEM, SHV and CTX-M was 5.77%, 1.92% and 2.88% respectively. The combination of TEM/CTX-M, SHV/CTX-M, TEM/SHV, TEM/SHV/CTX-M, TEM/SHV/CTX-M/OXA48 genes was carried by 4.81%, 8.65%, 13.46%, 52.88% and 9.62% respectively. The distribution of KPC and OXA-48 genes was 24.4% and 62.2% respectively. VIM and IMP were not detected. The presence of beta lactamases complicates treatment option; hence their prompt detection is critical to patient's wellbeing.

Keywords: *Klebsiella pneumoniae*, Resistance, Antibiotics, Beta lactamases, Co-existence, ESBLs genes, Carbapenemases genes.

1. Introduction

Klebsiella pneumoniae (K. pneumoniae) is a common opportunistic pathogen of nosocomial as well as community acquired infections (Du *et al.*,2014;Shemar *et al.,*2016).Antimicrobial resistance is a growing threat worldwide. Resistance mechanisms have been found for every class of antibiotic agents. Accurate and timely detection of these resistant mechanisms is very important in deciding the appropriate treatment schedule. Detection of the resistant mechanisms is always a serious challenge to the clinical laboratories (Sonal *et al.*,2016).Beta-lactamases are enzymes that are major cause of bacterial resistance to the beta-lactam family of antibiotics such as penicillins, cephalosporins, cephamycins, and carbapenems. These enzymes catalyze the hydrolysis of the amide bond of four-membered beta-lactam ring and render the antibiotic inactive against its original cellular target, the cell wall transpeptidase. On the basis of their primary structure, beta-lactamases are grouped into four classes A, B, C, and D enzymes. Enzymes of classes A, C, and D have serine at the active site, whereas the class B enzymes are zinc-metallo-enzymes (Iraz *et al.*, 2015; Bajpai *et al.,* 2017)

Resistance of *K. pneumoniae* to β-lactams is a clinical problem of the highest concern world-wide and most often is associated with a variety of acquired β lactamases, particularly ESBLs, AmpC-type cephalosporinases, carbapenemases (Iraz *et al.*, 2015;Shemar *et al.,*2016).The genes of these enzymes are carried on plasmids, facilitating spread between micro-organisms and often co-expressed in the same isolate(Vijaya & Achut,2017). Resistance to the carbapenemases in *K. pneumoniae* involves multiple mechanisms, including the production of carbapenemases (e.g. KPC, IMP, VIM, OXA-48-like), as well as alterations in outer membrane permeability mediated by the loss of porins, and the up regulation of efflux systems(Srinivasan *et al.*,2015;Meletis,2016).

Extended-spectrum β lactamases (ESBLs) are a group of enzymes with the ability to hydrolyzeand cause resistance to the oxyimino-cephalosporins (i.e.cefotaxime, ceftazidime, ceftriaxone, cefuroxime and cefepime) monobactams (i.e. aztreonam). There are several ESBLs genotypes such as SHV, TEM, and CTX-M (Rimal *et al.*, 2017)

The specific objectives of this study were to determine antimicrobial susceptibility patterns, to determine the co-existence of ESBLs, AmpC β-lactamase and carbapenemases, to identify ESBL genes and carbapenemases genes in *K.pneumoniae* isolates obtained from clinical samples referred to The Nairobi Hospital Laboratory (Microbiology and Parasitology unit).

2. Material and methods

2.1 Study design

A cross-sectional study was conducted from May 2017 to April 2018 at The Nairobi Hospital Laboratory

(Microbiology and parasitology unit), Kenya.

2.2 Sample size and sampling technique

A total of 272 of *K. pneumoniae* isolates were obtained from various clinical samples (urine, pus swab, sputum, blood, tracheal aspirate, catheter tip, body fluid and bile) except stool samples. All clinical specimens were received in laboratory for culture and processed according to standard procedures.

2.3 Isolation, identification and antibiotic susceptibility test

Urine sample was cultured on CLED agar and blood agar and other clinical samples (pus swab, sputum, blood, tracheal aspirate, catheter tip, body fluid and bile) were cultured on MacConkey agar and blood agar. All *K. pneumoniae* clinical isolates were examined morphologically for colony characteristics on agar media. *K. pneumoniae* are lactose fermenter and the colonies appeared pink and mucoid. Identification and AST was performed by using VITEK-2 system. Colonies from an overnight agar plate culture of each isolate were suspended in 3 mL of 0.45% saline and adjusted to a turbidity of 0.5-0.63McFarland standard with Densicheck. Identification of the *K.pneumoniae* isolates was performed by VITEK-2 system. The card "VITEK 2 GN" was used in this study as it is specifically made for identification for *K.pneumoniae* by biochemical tests. E.coli ATCC 35218 was used as quality control. The card "VITEK- 2 AST-GN83" was used in this study as it is specifically made for resistance determination for Gram negative rods.The card test susceptibility to the following antibiotics: Ampicillin, Amoxicillin/Clavulanic Acid, Ampicillin/subactam, Piperacillin/Tazobactam, Cefazolin, Cefuroxime, Cefoxitin, Cefotaxime, Ceftazidime, Ceftriaxone, Cefepime, Aztreonam, Meropenem,Amikacin, Gentamycin, Ciprofloxacin, Nitrofurantoin and Trimethoprim/ Sulfamethazole. MIC values are calculated and interpreted as R (resistant), I (intermediate), or S (sensitive) depending on the MIC value. The classification of MIC values for each drug/bug combination was determined according to Clinical Laboratory Standards Institute (CLSI, 2017).

2.4 Enzymes screening

The detection of ESBLs, AmpC and carbapenemases production was performed by automated VITEK 2 system.

2.5 DNA extraction

DNA was extracted from pure colonies of an overnight growth of *K.pneumoniae* isolates onto Nutrient agar by using Quick-DNA ™ Miniprep Plus Kit according to the manufacturer's procedure.

2.6 Polymerase Chain Reaction (PCR)

A. Primers

PCR amplification was performed using published primer pairs which are shown in **Table 2.**

B. Preparation of primers

For 100 pmol/ml from each primer we dissolved them in sterile distilled water as instructed by manufacture, then for 10 pmol/ml we dissolved 10 µl of each primer in 90 µl sterile distilled water.

C. Preparation of reaction mixture

The following reagents were used for each gene in the following volumes (total reaction volume was 25 µl). Working solution was diluted from the stock solution with sterile, nuclease free water (10µM) by using Taq DNA polymerase Master Mix with standard buffer 12.5µl, template DNA 2.5 µl, 10 µM forward primer 0.5 µl, 10 µM reverse primer 0.5 µl and nuclease-free water to 25µl.

D.DNA amplification and gel electrophoresis

Amplification of the template DNA was performed on ProFlex PCR System using the specific program for each gene. In order to visualize the PCR amplicons, 5µl of sample was mixed with 3 µl of Thermo Scientific loading dye and loaded into the wells of a 1.2% agarose gel in 1× Tris–acetate–EDTA (TAE) buffer. The gel was run at 110 V for 35 min. DNA fragments were stained by EZ- vision in gel and visualized by using ultraviolet light and image captured using a gel imaging system (UVITEC Cambridge). The gel had one well containing a DNA ladder (100 bp; Thermo Scientific) in order to be able to estimate the size of the DNA amplicons.

2.7 Statistical analysis

The software Microsoft Office Excel 2010 was used to process the data. The software SPSS (version 21) was used to perform the chi-square test. The test was considered statistically significant if p values < 0.05.

2.8 Ethics statement

This study was approved by the Bioethics and Research committee of The Nairobi Hospital (Ref: TNH /ADMIN/CEO/08/12/17).

3. Results

This cross-sectional study was performed on 272 isolates of *K.pneumoniae* from outpatients (50.7%) and inpatients (49.3%). There were (54%) females and 46% (males).Males and females had a big number of *K.pneumoniae* on age group greater than 50 which was 57(46.5%) for males and 65 (53.5%) for females. They were isolated from urine (n=171; 62.9%), pus swab (n=41; 15.1%), sputum (n=17; 6.3%), blood (n=17; 6.3 %), tracheal aspirate (n=11; 4.0%), Catheter tip (n=9; 3.3%), body fluid (n=3; 1.1%) and bile (n=3; 1.1%).

The resistance rates of *K.pneumoniae* isolates to antibiotics revealed that all *K.pneumoniae* were 100% resistant to Ampicillin. The resistance pattern of isolates to other antibiotics were: Amoxicillin/Clavulanic acid (43.8%), Ampicillin/Sulbactam (48.2%), Piperacillin/Tazobactam (40.8%), Cefazolin (62.9%),Cefuroxime (37.1%), Cefoxitin (34.6%), Cefotaxime (58.5%), Ceftazidime (57.7%), Ceftriaxone (53.7%),Cefepime (55.1%), Aztreonam (42.3%), Meropenem(21.7%), Amikacin (18.0%), Gentamycin (21.0%), Ciprofloxacin (25.4%), Nitrofurantoin (21.0%), Trimethoprim/Sulfamethoxazole (56.6%).The susceptibility pattern of *K.pneumoniae* isolates are shown in **Table 3**.

High resistance to antibiotics was observed among inpatients than outpatients and was found to be statistically significant ($p<0.05$) except Amoxicillin/clavulanic acid, Meropenem, Gentamycin, Nitrofurantoin and Trimethoprim /Sulfamethoxazole as shown in **Table 4**.

Of a total of 272 clinical isolates, 81(29.8%) screened positive for pure ESBLs, 19 (7.0%) pure carbapenemases,14 (5.1%)ESBL and carbapenemases co-producers, 5(1.8%) AmpC and carbapenemases co-producers, 7(2.6%) ESBLs and carbapenemases co-producers, 2(0.7%) ESBLS and AmpC co-producers. None of the isolates showed AmpC production alone **(Table 1)**

Table 1. Distribution of total ESBL (in combination with other enzymes), AMPc (in combination with other enzymes) & carbapenemase (in combination with other enzymes)

β-lactamases	Frequency	percentage
Pure ESBL	81	29.8
Pure carbapenemases	19	7.0
ESBL+carbapenemases	14	5.1
AmpC+carbapenemases	5	1.8
ESBL+carbapenemase+AmpC	7	2.6
ESBL+AmpC	2	0.7

A total of 272 clinical isolates, 81(29.8%) were screening positive for pure ESBLs, 19 (7.0%) pure carbapenemases, 14(5.1%) ESBL and carbapenemases co-producers, 5(1.8%) AmpC and carbapenemases co-producers, 7(2.6%) ESBLs and carbapenemases co-producers, 2(0.7%) ESBLS and AmpC co-producers

The single and the mixed enzymes of *K.pneumoniae* isolates were analyzed using Chi-square.The difference in resistance levels between ESBL producers and non-ESBLs producers, Carbapenemases producers and non carbapenemases producers; co-production enzymes and non co-production isolates for the antibiotics were statistically significant, $p<0.05$ as shown in **Table 5**.

A total of 104 out of 272 (38.2%) *K.pneumoniae* isolates were ESBLs producers. With regards to the PCR results, the occurrence of *bla TEM* gene, *bla SHV and bla CTX-M* genes in *K.pneumoniae* was 5.77%, 1.92% and 2.88% respectively in this study.

The combination of TEM/CTX-M, SHV/CTX-M, TEM/SHV, TEM/SHV/CTX-M, TEM/SHV/CTX-M/OXA-48 genes was carried by 4.81%, 8.65%, 13.46%, 52.88% and 9.62% respectively. *Bla KPC* and *bla OXA-48* genes of *K.pneumoniae* isolates carbapenamase producer were detected in 24.4% and 62.2% respectively. *Bla VIM* and *bla IMP* genes were not detected in this study.

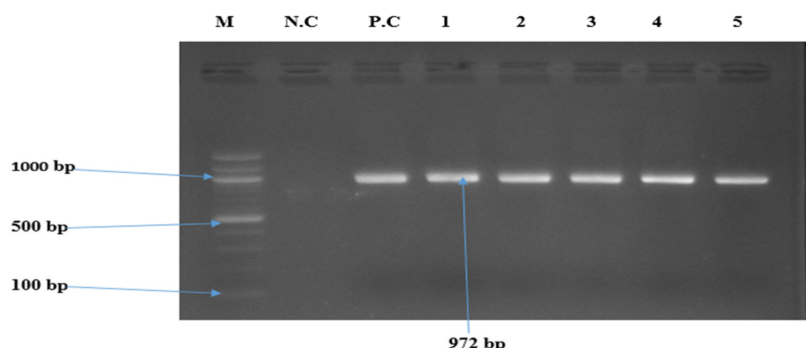

Figure 1. Detection of the presence of *bla TEM* genes producing *K.pneumoniae* by PCR.
Lane M: Ladder, N.C: Negative control, P.C: Positive control, Lanes 1-5: The 972 bp by PCR product of *bla TEM*.

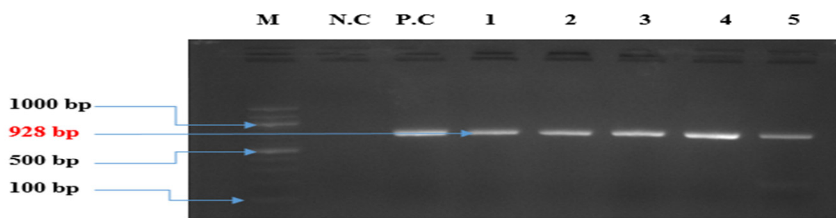

Figure 2. Detection of the presence of *SHV* genes producing *K.pneumoniae* by PCR.
Lane M: Ladder, N.C: Negative control, P.C: Positive control, Lanes 1-5: The 928 bp by PCR product of *bla SHV*.

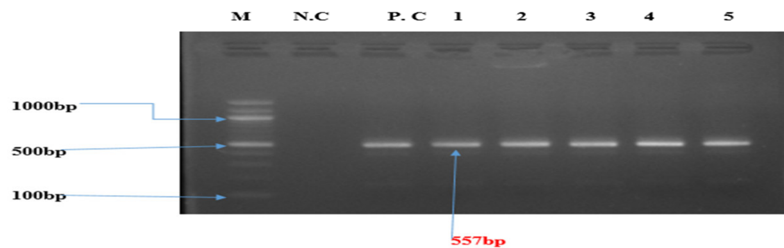

Figure 3. Detection of the presence of *bla CTX-M* genes producing *K.pneumoniae* by PCR.
Lane M: Ladder, N.C: Negative control, P.C: Positive control, Lanes 1-5: The 557 bp by PCR product of *bla CTX-M.*

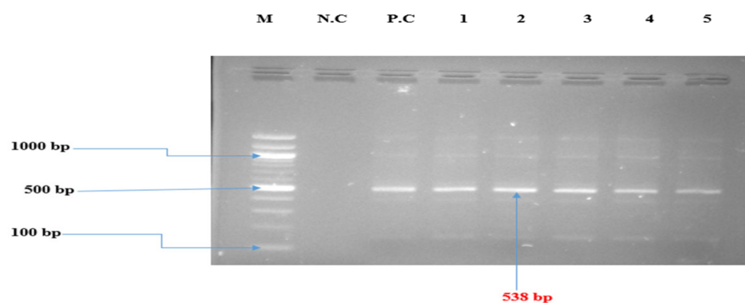

Figure 4.Detection of the presence of *bla KPC* genes producing *K.pneumoniae* by PCR.
Lane M: Ladder, N.C: Negative control, P.C: Positive control, Lanes 1-5: The 538 bp by PCR product of *bla KPC*

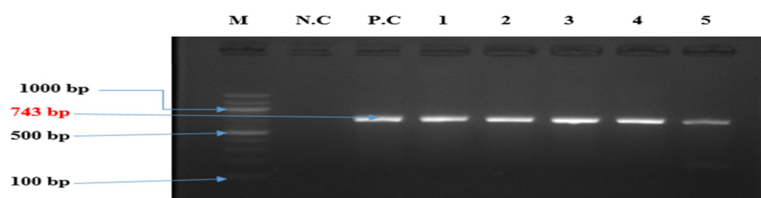

Figure 5. Detection of the presence of *bla OXA-48* genes producing *K.pneumoniae* by PCR.
Lane M: Ladder, N.C: Negative control, P.C: Positive control, Lanes 1-5: The 743 bp by PCR product of *bla OXA-48*

Table 2.Oligonucleotides primers were used for the detection of genes

Genes	Nucleotide sequence	Annealing temperature	Fragment length(bp)	Refence
Bla TEM	F- 5'TCGGGGAAATGTGCGCG-3' R-5'TGCTTAATCAGTGAGGCACC-3'	54	972	Sima *et al.*,2016
Bla SHV	F 5'-GGGTTATTCTTATTTGTCGC-3' R 5'-TTAGCGTTGCCAGTGCTC-3'	56	928	Sima *et al.*,2016
Bla CTX-M	F-5'-CGCTTTGCGATGTGCAG-3' R-5'ACCGCGATATCGTTGGT-3'	54	557	Moussé *et al.*,2016
Bla KPC	F-5' CATTCAAGGGCTTTCTTGCTGC-3' R-5'ACGACGGCATAGTCATTTGC-3'	52	538	Martha *et al.*,2014
Bla VIM	F-5' GATGGTGTTTGGTCGCATA-3' R-5'CGAATGCGCAGCACCAG-3'	52	390	Elligton *et al.*,2007
Bla IMP	F-5' GGAATAGAGTGGCTTAATTCTC-3' R-5'CCAAACCACTACGTTATC-3'	55	189	Li *et al.*,2012
Bla OXA-48	F-5' TTGGTGGCATCGATTATCGG-3' R-5'GAGCACTTCTTTTGTGATGGC-3'	55	741	Moini *et al.*,2015

Primers sequences used in this study.

Table 3.Antimicrobial susceptibility patterns for *K.pneumoniae* isolates

Antibiotics	Sensitive	%	Intermediate	%	Resistant	%
Ampicillin(10µg)	0	0	0	0	272	100
Amoxicillin/Clavulanic acid(20/10µg)	128	47.1	25	9.2	119	43.8
Ampicillin/Sulbactam(10/10)µg	116	42.6	25	9.2	131	48.2
Piperacillin/Tazobactam (100/10µg)	110	40.4	51	18.8	111	40.8
Cefazolin(30µg)	92	33.8	9	3.3	171	62.9
Cefuroxime(30µg)	104	38.2	7	2.6	101	37.1
Cefoxitin(30µg)	164	60.3	7	2.6	94	34.6
Cefotaxime(30µg)	108	39.7	5	1.8	159	58.5
Ceftazidime(30µg)	107	39.3	8	7.0	157	57.7
Ceftriaxone(30µg)	107	39.3	19	7.0	146	53.7
Cefepime (30µg)	112	41.2	10	3.7	150	55.1
Aztreonam(30µg)	139	51.1	18	6.6	115	42.3
Meropenem(10µg)	210	77.2	3	1.1	59	21.7
Amikacin(30µg)	208	76.5	15	5.5	49	18.0
Gentamycin(10µg)	205	75.3	10	3.7	57	21.0
Ciprofloxacin(5µg)	192	70.6	11	4.0	69	25.4
Nitrofurantoin(300µg)	124	45.5	91	33.5	57	21.0
Trimethoprim/Sulfamethoxazole(1.25/23.75µg)	108	39.7	10	3.7	154	56.6

The rate of resistance to antibiotics was follow: Ampicillin (100%),Amoxicillin/clavulanic acid (43.8%), Ampicillin/Sulbactam(48.2%),Piperacillin/Tazobactam(40.8%),Cefazolin(62.9%),Cefuroxime(37.1%),Cefoxitin (34.6%),Cefotaxime(58.5%),Ceftazidime(57.7%),Ceftriaxone(53.7%),Cefepime(55.1%),Aztreonam(42.3%),

Meropenem (21.7%), Amikacin (18.0%), Gentamycin (21.0%), Ciprofloxacin (25.4%), Nitrofurantoin (21.0%), Trimethoprim/Sulfamethoxazole (56.6%).

Table 4.Antimicrobial resistance pattern of the isolates among outpatients and inpatients

Antibiotics	Number of resistance isolates among inpatients (%)	Number of resistance isolates among Outpatients (%)	p-value
Amoxicillin/clavulanic acid	68(50.7%)	51(37.0%)	0.063
Ampicillin/sulbactam	79(59.0%)	52(37.7%)	0.002
Piperacillin/Tazobactam	72(53.7%)	39(28.3%)	0.001
Cefazolin	94(70.1%)	77(55.8%)	0.048
Cefuroxime	91(67.9%)	70(50.9%)	0.013
Cefoxitin	63(47.0%)	38(27.5%)	0.004
Cefotaxime	88(65.7%)	71(51.4%)	0.040
Ceftazidime	88(65.7%)	69(50.0%)	0.028
Ceftriaxone	82(61.2%)	64(46.4%)	0.014
Cefepime	84(62.7%)	66(47.8%)	0.022
Aztreonam	68(50.7%)	47(34.1%)	0.010
Meropenem	36(26.9%)	23(16.7%)	0.096
Amikacin	35(26.1%)	14(10.1%)	0.003
Gentamycin	34(25.4%)	23(16.7%)	0.066
Ciprofloxacin	42(31.3%)	27(19.6%)	0.001
Nitrofurantoin	31(23.1%)	26(18.8%)	0.327
Trimethoprim/Sulfamethoxazole	83(61.9%)	71(51.4%)	0.149

High resistance to antibiotics was observed among inpatients than outpatients and was found to be statistically significant ($p<0.05$) except Amoxicillin/clavulanic acid, Meropenem, Gentamycin, Nitrofurantoin and Trimethoprim /Sulfamethoxazole

Table 5. Sensitivity Pattern of *K.pneumoniae* Isolates producing single & mixed Enzymes

Antibiotics	ESBLS producers	Non ESBLs producers	p	carbapenemases producers	Non carbapenemases producers	p	Co-production enzymes	Non co-production enzymes	p
A/C	40(49.4%)	79(41.4%)	0.001	13(68.4%)	106(41.9%)	0.018	17(60.7%)	102(41.8%	0.070
A/S	48(59.3%)	83(43.5%)	0.003	13(68.4%)	118(46.6%)	0.046	17(60.7%)	114(46.7%)	0.279
PT	48(59.3%)	63(33.0%)	0.001	14(73.7%)	97(46.6%)	0.010	14(50.0%)	97(39.8%)	0.563
Cfz	77(95.1%)	94(49.2%)	0.001	17(89.5%)	154(60.9%)	0.024	16(57.1%)	155(63.5%)	0.068
Cfx	75(92.6%)	86(45.0%)	0.001	17(89.5%)	144(56.9%)	0.020	15(53.6%)	146(59.8%)	0.794
Cx	33(40.7%)	68(35.6%)	0.027	9(47.4%)	92(36.4%)	0.424	14(50.0%)	87(35.7%)	0.254
Ctx	79(97.5%)	80(41.9%)	0.001	18(94.7%)	141(55.7%)	0.004	15(53.6%)	144(59.0%)	0.590
Caz	79(97.5%)	78(40.8%)	0.001	18(94.7%)	139(54.9%)	0.003	15(53.6%)	142(58.2%)	0.494
Ctr	78(96.3%)	68(35.6%)	0.001	17(89.5%)	129(51.0%)	0.004	14(50.0%)	132(54.1%)	0.277
Cpe	76(93.8%)	74(38.7%)	0.001	17(89.5%)	133(52.6%)	0.005	13(46.4%)	137(56.1%)	0.600
Azm	42(51.9%)	73(38.2%)	0.044	13(68.4%)	102(40.3%)	0.025	12(42.9%)	103(42.2%)	0.629
MP	7(8.6%)	26(13.6%)	0.512	17(89.5%)	42(16.6%)	0.001	4(14.3%)	57(23.4%)	0.249
Sxt	49(60.5%)	105(55.0)	0.042	15(78.9%)	139(54.9%)	0.087	15(53.6%)	139(57.0%)	0.460
F	18(22.2%)	39(20.4%	0.941	7(36.8%)	50(19.8%)	0.211	8(28.6%)	49(20.1%)	0.069
CN	15(18.5%)	42(22.0%)	0.812	7(36.8%)	50(19.8%)	0.039	24(85.7%)	33(13.5%)	0.001
Amk	10(12.3%)	39(20.4%)	0.166	4(21.1%)	45(17.8%)	0.106	22(78.6%)	27(11.1%)	0.001
Cip	18(22.2%)	51(26.7%)	0.711	8(42.1%)	61(24.1%)	0.196	24(85.7%)	45(18.4%)	0.001

The table shows the antibiotic resistance patterns of single and mixed enzymes producers in *K.pneumoniae* isolates.

4. Discussion

In this study, females had a higher incidence of *K.pneumoniae* (54%) as compared to males (46%) considering gender classification. This study agreed with the study carried out by Deshmukh *et al*., (2016) but disagreed with the study conducted by Jesmin *et al.* (2014) in which reported that males had a higher prevalence of *K.pneumoniae* as compared to females (Jesmin *et al.,* 2014; Deshmukh *et al.,* 2016;). Females may be especially prone to *K.pneumoniae* infection because they have shorter urethras and being close to anus, which allow *K.pneumonia* quick access to the bladder. High frequency in *K.pneumoniae* was observed in age groups 50 + in the present study which agreed with others studies (Lina *et al*., 2007; Meatherall *et al*., 2009; Jesmin *et al*., 2014). This data suggested that age is a risk factor for *K.pneumoniae* infection.The highest percentage of *K.pneumoniae* isolates was isolated from urine(62.9%).In a comparable study, others studies showed the similar results with the high rate of *K.pneumoniae* isolates in urine samples (Moini *et al.,* 2015; Jesmin *et al.,* 2014; Mate *et al.,* 2014).The data of this study showed that the *K.pneumoniae* is most notorious organism to cause UTIs and can hit other parts of the body.

K.pneumoniae showed 100 % resistance to ampicillin in the present study.Previous studies have shown

similar resistance pattern with this drug(Ali *et al.*,2014;Moini *et al.*,2015;Ntirenganya *et al.*,2015;Deshmukh *et al.,*2016;Ghanem *et al.,*2017). Intrinsically resistance of *K.pneumoniae* to ampicillin could be the reason of this resistance to ampicillin. The high resistance to cephalosporins in this study agreed that *K.pneumoniae* were resistant to cephalosporins as reported by others studies.

In the present study,*K.pneumoniae* strains were found low resistant to (amikacin(18.0%),gentamycin(21.0%),ciprofloxacin (25.4%) and meropenem (21.7%) which was simular with the previous studies (Archana&Harch,2011;Manikandan &Amsath,2013; Mansury *et al*.,2016;Tulara 2018).Nitrofurantoin was resistant with 21% in the present study but Manikandan *et al.(2013);* Khamesipour &Tajbakhsh (2016) reported that nitrofurantoin was resistant with 50% and 41.1% respectively (Manikandan &Amsath,2013;Khamesipour &Tajbakhsh,2016). Manikandan & Amsath(2013)reported cotrimoxazole resistance(70.8%) which was high than what has observed in the present sudy (56.6%) (Manikandan &Amsath,2013). Based on the results of this study in comparison with others studies regarding antibiotics resistance,the difference of the resistance rate could be to the sample size,sampling method, the types of antimicrobial agents commonly used in certain areas and the rate at which antibiotics are prescribed for treatment of various infectious diseases causing gene mutation leading to β lactamases production.

In this study, the high resistance was significantly to inpatients as compared to outpatients .This is similar to results from a related study in Cameroon and Nigeria (Piéboji *et al.,* 2004; Nwosu *et al.,* 2014).The high resistance in inpatients may be due to, during hospitalization, their intestinal microbiome could have changed and others factors such as the underlying disease, antibiotic treatment and acquisition of (multi-resistant) microorganisms from the environment during the stay. Long hospital stay and antibiotic pressure select resistant strains which were colonized in susceptible patients.

In this study, none of the isolates showed AmpC production alone. This study differed with the results of several studies ranging from 2% to 63% (Chatterjee *et al*.2010;Oberoi *et al.,*2013;Patwardhan *et al.,*2013;Rawat *et al.,*2013;Doddaiah &Dhanalakshmi,2014;Vijaya &Achut,2017).

The present study demonstrated that 29.8% of the isolates showed ESBLs production. Previous studies showed variable results (Chatterjee *et al*.2010;Oberoi *et al.,*2013;Rawat *et al.,*2013;Doddaiah &Dhanalakshmi,2014;Anusuya &Rajesh,2016; Shahandeh *et al.,*2016;Vijaya &Achut,2017).

In the present study, carbapenemase production was in 7.0 % isolates of *K.pneumoniae.* Numerous studies represented different percentage of diverse carbapenemases producers(Oberoi *et al.,*2013; Patwardhan *et al.,*2013;Rawat *et al.,*2013;Wadekar *et al.,*2013; Doddaiah &Dhanalakshmi,2014;Anusuya &Rajesh,2016).

ESBL and AmpC co-existence was seen in 0.7% of our isolates in this study. The prevalence of the co-existence of ESBL/AmpC varies from as low as 2.3% to as high as 71.3% in previous studies(Oberoi *et al.,*2013;Rawat *et al.,*2013;Doddaiah &Dhanalakshmi,2014;Yusuf *et al.,*2014; Mohammed *et al.,*2015;Vijaya & Achut,2017).

The present study found 5.1% of our isolates showed co-expression of ESBL and carbapenemases. Previous studies showed different results (Rawat *et al.,*2013;Wadekar *et al.,*2013;Doddaiah &Dhanalakshmi,2014;Yusuf *et al.,*2014;Anusuya &Rajesh,2016).

AmpC and carbapenamase coexistence was reported in 1.8% of *K.pneumoniae* isolates in the present study. The co-existence of AmpC/carbapenemases has been reported by several investigators (Oberoi *et al.,*2013;Patwardhan *et al.,*2013;Rawat *et al.,*2013;Yusuf *et al.,*2014;Archana *et al.,*2016).

ESBL, AmpC and carbapenemases co-occurred in a 2.6% of isolate in the present study. Several studies showed different results ranging from 0.7% to 29.4% (Chatterjee *et al.,* 2010; Doddaiah &Dhanalakshmi, 2014; Yusuf *et al.,* 2014; Archana *et al.,* 2016) .Different phenotypic methods in various studies could be the reason of the difference regarding beta lactamases detection.

ESBLs producing *K.pneumoniae* isolates in present study showed 97.5%, 97.5%, 96.3% resistance rate to Ceftazidime, Cefotaxime and Ceftriaxone respectively, whereas in a study by Archana *et al*., (2016) showed a high rate resistance to Cefepime (94.29%), to Cefuroxime (92.39%) and to Ceftriaxone (88.58%)(Archana *et al.,*2016).Carbapenemases producing *K.pneumoniae* isolates in present study reported high resistance to cefotaxime(94.7%) and to Ceftazidime (94.7%) whereas Archana *et al*.,(2016) reported 87.50 % to Cefepime,81.25% to Amoxicillin/clavulanic acid and 75% to Cefuroxime %)(Archana *et al.,*2016). Inappropriate and incorrect administration antibiotics ,lack of appropriate infection-control strategies in the community and the the selective pressure created for the use of antibiotics should be responsible of the high resistance rate in ESBLs, carbapenemases and co-existence of beta lactamases producing *K.pneumoniae* in this study.

In the present study, the resistance to gentamycin, amikacin and ciprofloxacin showed the high resistance in *K.pneumoniae* producing all three beta lactamases (85.7%, 78.6% and 85.7% respectively). This was similar to results from a related study conducted by Chatterjee *et al*.(2010). Archana *et al.,(*2016) reported the resistance rate with 75% to gentamycin , 0 % to amikacin,91.67% to ciprofloxacin.The high prevalence of these antibiotics could be to these antibiotics are important alternative antibiotics for treating beta lactamases producers/or used in combination therapy with beta-lactams antibiotics and the mutants may be selected by exposure to

K.pneumoniae β lactamases producers.The popularity of ciprofloxacin in treating a variety of infections may be another reason.

The resistance of meropenem was low in ESBLs producers(8.6%) and in co-existence enzymes(14.3%).This low resistance should be to the injectable forms for treating *K.pneumoniae* infections and its use is limited.This drug remains useful for treating serious infections.

The detected $bla_{CTX\text{-}M}$, bla_{TEM}, bla_{SHV} and $bla_{CTX\text{-}M}$ genes were present alone or in combination with each other in the present study. TEM was detected in 5.77%,SHV in 1.92% ,CTX-M in 2.88%, the combination of TEM/CTX-M, SHV/CTX-M, TEM/SHV, TEM/SHV/CTX-M genes was carried by 4.81%, 8.65%, 13.46% and 52.88% respectively. Numerous studies of different prevalence of ESBLs genes and their combination has been reported (Alsultan *et al.,*2013;Jaskulski *et al.,*2013;Bora *et al.,*2014;Alibi *et al.,*2015;Al-Suboi&Nihad,2015; Zongo *et al.,*2015;Diagbouga *et al.,*2016;Juma *et al.,*2016;Moghadampour *et al.,*2018).

In the present study , the combination of TEM/SHV/CTX-M/OXA48 genes was carried by 9.62%.The combination has been detected with others investigators (Dimou *et al.,* 2012;Iraz *et al.,*2015;Srinivasan *et al.,*2015;Loucif *et al.,*2016).

The present study reported 24.4% and 62.2% of *K.pneumoniae* isolates carbapenamase producer were detected respectively for the presence of the *bla KPC* and *bla OXA-48* genes. OXA-48 was the most dominant gene in *K.pneumoniae* carbapenemases producers in this study which agreed with the study conducted by Oteo *et al.,(*2013) but disagreed with others in which KPC genes was the most prevalent genes(Oteo *et al.,*2013;Flores *et al.,*2016; Abdelhakam & Al-Fadhil,2017). The prevalence of OXA-48 in the present study is high compared to others studies.(Martha *et al.,*2014;Flores *et al.,*2016;Abdelhakam & Al-Fadhil,2017;Moemen & Massalat,2017;Sakarikou *et al.,*2017). KPC genes represented 24.4% in the present study. Previous studies revealed this gene as less detected or completely absent (Martha *et al.,* 2014; Sahin *et al.,* 2015).Others studies showed the most prevalent gene among *K.pneumoniae* isolates was bla KPC which discordant with this study (Wang *et al.,* 2012; Moemen & Massalat, 2017). The prevalence of genes in this study could be to the genetic factors and genomic rearrangements involved in *K.pneumoniae* antibiotic resistance and antibiotic resistance determinants are borne on transferable plasmids or mobiles elements. All strains were negative for VIM and IMP genes in the present study .This result agreed with the study from Brazil done by Flores *et al.,*. (2016) and by Moghadampour *et al*.,(2018) in Iran but disagreed with others studies conducted in the world (Giakkoupi *et al.,*2003;Pitout,2008;Tato *et al.,*2010;Martha *et al.,*2014;Abdelhakam & Al-Fadhil,2017).The geographic distribution should be explain the absence in this study.

5. Conclusion

The main contribution of this study was the disclosure of increasing data on the presence of *K. pneumoniae*, carrying genes responsible for ESBL and carbapenemase production. The data generated in this study indicate the importance of adopting measures of continuous prevention to control the spread of ESBL and carbapenemase-producing microorganisms in hospital settings and in the community. Measures such as active surveillance, rational use of antimicrobials, isolation precautions, hand hygiene, and education for health personnel are fundamental for the success of Health care Associated Infection prevention and control programs.

6. Conflict of interest

Author has not declared any conflict of interest.

7. Acknowledgments

I appreciate the cooperation of the staff of Nairobi Hospital Laboratory for their assistance in this study.

References

1. Abdelhakam, H.I.A., Al-Fadhil., A.O. (2017),"Molecular Characterization of Carbapenemase Producing *Klebsiella Pneumoniae* Dominance of OXA-48, KPC, VIM and NDM Producers in Khartoum, Sudan", *Journal of Clinical review & Case Reports*, **2**(3), 1-6.
2. Ali, A.R.K.A., Ali, M.A.M. (2014), **"**Prevalence of Extended Spectrum β-lactamase-Producing *Klebsiella pneumoniae* in Clinical Isolates," *Jundishapur Journal of Microbiology* **7**(11), 1-5.
3.Alibi,S.,Ferjani,A.,Boukadida,J.(2015),"Molecular Characterization of ESBLs produced by *K.pneumoniae* clinical strains from a Tunisian Hospital",*Med mal Infect*.,**45**(4),139-143.
4. Al-Subol,I.,Nihad ,Y.(2015)," Prevalence of CTX-M,TEM and SHV beta-lactamases in clinical isolates of *Escherichia Coli* and *Klebsiella Pneumoniae* Isolated From Aleppo University Hospitals, Aleppo, Syria", *Arch Clin Infect Dis*.,**10**(2),1-6.
5.Alsultan,A.A.,Aboulmagd,E.,Amin,T.T.(2013),"ESBL-producing *E.coli* and *K.pneumoniae* in Al-Ahsa,Saudi Arabia: antibiotic susceptibility and prevalence of bla SHV and bla TEM",*J Infect Dev Ctries* **7**(12),1016-9
6.AlTamimi,M.,AlSalamah,A.,AlKhulaifi,M.,AlAjlan,H.(2017), "Comparison of phenotypic and PCR methods

for detection of carbapenemases production by Enterobacteriaceae", *Saudi Journal of Biological Sciences* **24**(1),155-161.

7. Anusuya, D.D., Rajesh, R. (2016), "Occurrence of various β-lactamases enzymes producing Enterobacteriaceae in the hospital effluent: a wake-up call", *International journal of Medical Sciences and Public Health* **5** (6), 1204-1208.
8. Archana, B., Khatri, P.K., Aruna, S., Parihar, R.S., Chandora, A.K.(2016), "Incidence & Estimation of Beta – Lactamase Enzymes (ESBL Ampc, Carbapenemase Enzymes) singly and their coexistence in clinical isolates of Gram Negative bacteria by Vitek – 2 Compact System", *Indian J Microbiol Res* **3**(4),352-358.
9. Archana, S.S., Harsh, V.B. (2011), " Challenge to healthcare: Multidrug resistance in *Klebsiella pneumoniae*." *International Conference on Food Engineering and Biotechnology*, *IPCBEE vol.***9** *(2011),* IACSIT Press, Singapore, 130-134.
10. Bajpai, T., Pandey, M., Varma, M., Bhatambare, G.S. (2017), "Prevalence of TEM, SHV, and CTX-M Beta-Lactamase genes in the urinary isolates of a tertiary care hospital" *Avicenna Jounal of Medicine* **7**(1),12-16.
11. Bora,A.,Hazarika,N.K.,Shukla,S.K., Prasad,K.N. ,Sarma,J.B.,Ahmed,G.(2014),"Prevalence of bla TEM,bla SHV and bla CTX-M genes in clinical isolates of *E.coli* and *K.pneumoniae* from Northeast India", *Indian J Pathol Microbiol* **57**(2),249-254.
12. Chatterjee,S.S.,Karmacharya,R.,Madhup,S.K.,Gautam,V.,Das,A.,Ray,P.(2010),"High prevalence of co-expression of newer β lactamases (ESBLs,Amp-C-β-lactamases,and metallo-β-lactamases) in gram negative bacilli", I*ndian Journal of Medical Microbiology* **28**(3),267-268.
13. Deshmukh, P.M., Ukesh, C.S., Patil, S.D. (2016), "Antibiotic Resistance Pattern of Some Pathogens Isolated from Clinical Samples", *Bulletin of Environment, Pharmacology and Life Sciences* **5**(2), 66-72.
14. Diagbouga,S.,Salah,F.D.,Sadji,A.Y.,dabire,A.M.,Nadembega,C.,Kere,A.B.,Soubeiga,S.T.,Ouattara,A.K.,Zohoncon,T.,Belemgnegre,M.,Karou,S.,Simpore,J.(2016),"Detection of High prevalence of TEM/SHV/CTX-M genes in ESBL producing and Multidrug resistant *K.pneumoniae* and *K.oxytoca* ".*Journal of Clinical Diagnosis and Research* **4**(1),1-7
15. Dimou,V.,Dhanji,H.,Rachel,P.,David,M.L.,Neil,W.(2012), "Characterization of Enterobacteriaceae producing OXA-48-like carbapenemases in the UK",J*ournal of Antimicrobial Chemotherapy*,**67**(7),1660-1665.
16. Doddaiah,V.,Dhanalakshmi,A.(2014)," Prevalence of ESBL, AmpC and carbapenemase among gram negative bacilli isolated from clinical specimens", *American Journal of Life Sciences* **2**(2),76-81.
18. Du, J., Li, P., Liu, H., Lü , D., Liang, H., Dou, Y. (2014),"Phenotypic and molecular characterization of multidrug resistant *Klebsiella pneumoniae* isolated from a University Teaching Hospital, China,*PLoS One*,**9**(4).
19. Ellington, M.J., Kistler, J., Livermore, D.M., Woodford, N.(2007), "Multiplex PCR for rapid detection of genes encoding acquired metallo β lactamases", *Journal of Antimicrobial Chemotherapy*, **59(** 2),321-2.
21. Flores,C.,Romão,C.M.C.P.A.,Bianco,K.,Catia,C.D.M.,Angela,B.,Souza,A.P.S.,Rosana,M.R.S.,BiancamO.F.,Ivano,D.F.,Maysa,M.C.(2016),"Detection of antimicrobial resistance genes in beta-lactamase and carbapenemase producing *K.pneumoniae* by patient surveillance cultures at an intensive care unit in Rio de Janeiro,Brazil",*Jornalo Brasileiro de Patologia e Medicina Laboratorial* **52**(5),284-292.
22. Ghanem, S., Hatem, M. E. S., Tamer, E. K. A., Nikhat, M. (2017), "Antimicrobial resistance patterns of Klebsiella isolates from clinical samples in a Saudi hospital", *African Journal of Microbiology Research,* **11**(23), 965-71.
23. Giakkoupi,P.,Xanthaki,A.,Kanelopoulou,M., Vlahaki,A.,Miriagou,V.,Kontou,S.,Papafraggas,E., Malamou-Lada,H., Tzouvelekis,L.S., Legakis,N.J., Vatopoulos,A.C.(2003), "VIM-1 metallo-β-lactamase-producing *Klebsiella pneumoniae* strains in Greek hospitals", *Journal of Clinical Microbiology* **41**(8),3893-6.
24. Iraz , M., Özad Düzgün,A., Sandall,C., Doymaz, M.Z., Akkoyunlu, Y., Saral ,A., Peleg,A.Y., Özgümüş, O.B., Beriş, F.Ş., Karaoğlu, H., Çopur Çiçek, A.(2015)," Distribution of β-Lactamase Genes Among Carbapenem-Resistant *Klebsiella pneumoniae* Strains Isolated From Patients in Turkey" *,Annals of Laboratory Medicine*,**35**(6),595-601.
25. Jaskulski,M.R.,Medeiros,B.C.,Borges,J.V.,Zalewsky,R.,Fonseca,M.E.C.,Marinowic,D.R.,Rocha,M.P.,Nodari,P.,Machado,D.C.(2013),"Assessment of extended-spectrum β-lactamase, KPC carbapenemase and porin resistance mechanisms in clinical samples of *Klebsiella pneumoniae* and *Enterobacter* spp.",*Journal of Antimicrobial Agents*,**42**(1),76-79.
26. Jesmin, A., Masudul, A.C., Mohammad, A.F. (2014), "Study on Prevalence and Antibiotic Resistance Pattern of *Klebsiella* Isolated from Clinical Samples in South East Region of Bangladesh", *American Journal of Drug Discovery and Development* **4(**1), 73-79.
27. Juma,B.W.,Kariuki,S.,Waiyaki,P.G.,Mutugi,M.M.,Bulimo,W.D.(2016),"The prevalence of TEM and SHV genes among Extended-Spectrum Beta-Lactamase-producing *K.pneumoniae* and *E.coli*", *African Journal of Pharmacology and Therapeutics*,**5**(1),1-7.
28. Kapoor, G., Saigal, S., Elongavan, A. (2017), "Action and resistance mechanisms of antibiotics: A guide for

clinicians", *Journal of Anaesthesiology Clinical Pharmacology* **33**(3), 300-305.

29. Khamesipour, .F, Tajbakhsh, E. (2016)," Analyzed the genotypic and phenotypic antibiotic resistance patterns of *Klebsiella pneumoniae* isolated from clinical samples in Iran", *Biomedical Research,* **27**(4), 1017-1026.
30. Iraz , M., Özad Düzgün,A., Sandall,C., Doymaz, M.Z., Akkoyunlu, Y., Saral ,A., Peleg,A.Y., Özgümüş, O.B., Beriş, F.Ş., Karaoğlu, H., Çopur Çiçek, A.(2015)," Distribution of β-Lactamase Genes Among Carbapenem-Resistant *Klebsiella pneumoniae* Strains Isolated From Patients in Turkey" *,Annals of Laboratory Medicine*,**35**(6),595-601.
31. Lina, T.T., Sabita, R.R., Donald, J.G. (2007), "Multiple-Antibiotic Resistance Mediated by Plasmids and Integrons in Uropathogenic *Escherichia coli* and *Klebsiella pneumoniae", Bangladesh Journal of Microbiology* **24**(1), 19-23.
32. Loucif,L., Kassah-Laouar,A., saidi,M., Messala,A., Chelaghma,W., Rolain,J.M.(2016), " Outbreak of OXA-48-Producing *Klebsiella pneumoniae* Involving a Sequence Type 101 Clone in Batna University Hospital, Algeria", *Antimicrob Agents Chemother* **60**(12),7494-7497.
33. Manikandan, C., Amsath, A. (2013)," Antibiotic susceptibility pattern of *Klebsiella pneumoniae* isolated from urine samples" *International Journal of Current Microbiology and Applied Sciences* **2**(8), 330-337.
34. Mansury, D., Motamedifar, M., Sarvari, J., Shirazi, B., Khaledi, A. (2016), "Antibiotic susceptibility pattern and identification of extended spectrum β-lactamases (ESBLs) in clinical isolates of *Klebsiella pneumoniae* from Shiraz, Iran. *Iran", Journal of Microbiology* **8**(1), 55-61.
35. Martha, F.M., Stephen, E.M., Can, I., Freddie, B.(2014)," Carbapenemase Genes among Multidrug Resistant Gram Negative Clinical Isolates from a Tertiary Hospital in Mwanza, Tanzania", *BioMed Research International,*1-6.
36. Mate,P.H.,Devi,K.S.,Devi,K.M..,Damrolien,S.Devi,N.L.,Devi,P.(2014),"Prevalence of carbapenem resistance among gram negative bacteria in a Tertiary care Hospital in North –East India", *IOSR Journal of Dental and Medical Sciences* **13**(2),56-60.
37. Meatherall, B.L., Gregson, D., Ross, T., Pitout, J.D. (2009), Laupland, K.B., "Incidence, risk factors, and outcomes of *Klebsiella pneumoniae* bacteremia", *American journal of Medicine* **122**(9), 866-73.
38. Meletis, G.(2016), " Carbapenem resistance: overview of the problem and future perspectives",*Therapeutic Advances in Infectious Disease* **3**(1) ,15-21.
39. Moemen, D., Massalat, D.T. (2017),"Prevalence and Characterization of carbapenem-resistant K.pneumoniae isolated from intensive care units of Mansoura University hospitals", *Egyptian Journal of Basic and Applied Sciences* **4**, 37-41.
40. Moghadampour, M., Rezaei, A., Faghri,J.(2018), " The emergence of Bla OXA-48 and Bla NDM among ESBL-producing K.pneumoniae in clinical isolates of a tertiary hospital in Iran", *Acta Microbiol Immunol Hung*, **65**(3),335-344.
41. Mohammed,K.N.,Srivastava,P.,Chophi,V.,Nirwan, P. S.(2015), " Prevalence of ESBLs and AmpC β-Lactamase in gram negative bacilli in various clinical samples at Tertiary care Hospital ",*International Research Journal of Medical* Sciences **3**(8),1-6.
42. Moini, A.S., Babak, S., Abbas, T.A., Alireza, M., Mahzad, E., Mostafa, H.R., Mansoor, N. (2015)," Multidrug-Resistant *Escherichia coli* and *Klebsiella pneumoniae* Isolated From Patients in Kashan, Iran", *Jundishapur Journal of Microbiology* **8**(10), 1-8.
43. Moussé,W., Pacôme, A.N., Nicodème, W.C., Haziz, S.,Majoie, G.T., Théodora ,A .A., Lamine ,B.M.(2016), **"**Phenotypic and Genotypic Characterization of Extended Spectrum *β*-Lactamase *Klebsiella pneumoniae* and Fluorescent *Pseudomonas* spp. Strains from Market Garden Products and Their Watering Water in Benin (West Africa)" *Food and Nutrition Sciences* **7**(3),192-204.
44. Nwosu, L.,Amadi, E. S.,Nwanyanwu, C. E., Chikwendu, C. I. Madu, C. L.(2014)," The prevalence of extended spectrum β lactamases(ESBLs)among *E.coli* and *Klebsiell*a *species* urinary isolates from Abia State university Teaching Hospital (ABSUTH) aba,Abia State Nigeria", *International Journal of Microbiology and Mycology* **2**(3),20-28.
45. Ntirenganya, C., Manzi, O., Muvunyi, M.C., Ogbuagu, O. (2015)," High Prevalence of Antimicrobial Resistance Among Common Bacterial Isolates in a Tertiary Healthcare Facility in Rwanda", *American Journal of Tropical Medicine and Hygiene*, **92**(4), 865-70.
46. Oberoi,L.,Nachhatarjit,S.,Poonam,S.,Aruna,A.,"ESBL,MBL and Amp C β lactamases Producing Superbugs-Havoc in the Intensive Care Units of Punjab India", *Journal of Clinical and Diagnostic Research*,7,1.January 2013,pp.70-73.
47. Oteo.J.,Hernandez,J.M.,Espasa,M.,Fleites,A., Saez,D.,Perez-Vazquez,M.,Fernandez Garcia,M.D., Delgado-Iribarren,A.,Sanchez-Romero,I.,Garcia Picazo, L., Miguel,M.D., Solis,S., Aznar,E., Trujillo,G., Mediavilla,C., Fontanals,D., Rojo,S., Vindel,A., Campos,J.(2013), "Emergence of OXA-48 *K.pneumoniae* and the novel carbapenemases OXA-244 and OXA-245 in Spain",*J Antimicrobiol Chemother*. **68**(2), 317-

21.
48. Patwardhan, N.S., Patwardhan, S.S., Deore, S. (2013)," Detection of ESBL, MBL, AmpC and Carbapenemases and their coexistence in clinical isolates of gram negative bacteria", *Journal of Medical Research and Practice* **2**(7), 179-190.
49. Piéboji,J.G., Koulla-Shiro,S., Ngassam,P., Adiogo,D.,Njine,T.,Ndumbe,P.(2004),"Antimicrobial resistance of Gram negative bacilli isolates from inpatients and outpatients at Yaounde Central Hospital, Cameroon" ,*International Journal of Infectious Diseases* **8**(3),147-54.
50. Pitout,J.D.(2008),"Multiresistant Enterobacteriaceae : new threat of an old problem", *Expert Rev Anti Infect Ther.***6** (5), 657-69.
51. Rawat, V.,Monil,S.,Pankaj,K.V.(2013),"Detection of different β-Lacatamases and their co-existence by using various Discs combinations methods in clinical isolates of Enterobactericeae and pseudomonas spp.",*Journal of Laboratory Physicians* **5**(1),21-25.
52. Rimal, U., Shovana, T., Roshani, M. (2017)," Prevalence of Extended Spectrum Beta-Lactamase Producing Escherichia coli and *Klebsiella species* from Urinary Specimens of Children attending Friendship International Children's Hospital", *Nepal Journal of Biotechnology* **5**(1),32-38.
53.Sahin,K.,Tekin,A.,Ozdas,S.,Akin,S.,Akin,D.,Yapislar,H.,Dilek,A.R.,Sonmez,E.(2015),"Evaluation of carbapenem resistance using phenotypic and genotypic techniques in Enterobacteriaceae isolates", *Annals of Clinical Microbiology and Antimicrobials* **14**(44),2-6.
54. Sakarikou, S., Ciotti, M., Dolfa, C., Angeletti, S., Favalli, C.(2017),"Rapid detection of carbapenemases producing *K.pneumoniae* strains derived from blood cultures by Matrix-Assisted Laser desorption Ionization-Time of Flight Mass Spectrometry(MALDI-TOF MS)",*BioMed Central Microbiology* **17**(54), 1-8.
55. Shahandeh, Z., Farahnaz, S., Khadije, B.R.(2016)," Phenotypic Detection of ESBL, MBL (IMP-1), and AmpC Enzymes, and Their Coexistence in *Enterobacter* and *Klebsiella* Species Isolated from Clinical Specimens", *International Journal of Enteric Pathogenes* **4**(2),1-7.
56. Sheemar, S., Chopra, S., Mahajan, G., Kaur, J., Chouhan Y.S. (2016)," Extended spectrum beta-lactamase and AmpC producing *Klebsiella pneumoniae*: A therapeutic challenge", *Tropical of Journal Medical Research* **19** (2), 114-7.
57. Sima,S.S.,Mehdi,.G.,Fattaneh,S.(2016),"Relation between bla TEM,bla SHV and bla CTX-M gnes and acute urinary tract infections",*Journal of Acute Disease* **5**(1),71-76.
58. Sonal, P.C., Sunanda, N.S., Ratna, R.P., Gajbhiye, S .R. (2016)," Detection of various β-lactamases amongst gram negative bacilli from clinical isolates with special reference to Metallo β-Lactamases", *International Journal of Advanced Research* **4**(5),22-29.
59. Srinivasan, R., Maanasa, B., Ellappan, K., Harish, B. N. (2015), "Prevalence and characterization of carbapenemase producing isolates of Enterobacteriaceae obtained from clinical and environmental samples: Efflux pump inhibitor study",*African Journal of Microbiology Research* **9**(17),1200-1204.
60. Tato, M., Morosini, M., Garcia, L., Alberti, M., Coque, T.,Canton,R.(2010), " Carbapenem Heteroresistance in VIM-1-Producing *Klebsiella pneumoniae* Isolates Belonging to the Same Clone: Consequences for Routine Susceptibility Testing", *Journal of Clinical Microbiology* **48**(11),4089-93.
61. Tulara, N. K. (2016), "Nitrofurantoin and Fosfomycin for Extended Spectrum Beta-lactamases Producing *Escherichia coli* and *Klebsiella pneumoniae*" *Journal of global infectious diseases,* **10**(1), 19-21.
62. Vijaya, S., Achut, R. (2017), "Detection of co-existence of β-lactamases in Gram negative bacteria using disc potentiation tests", *Indian J Microbiol Res* **4**(1),64-67.
63. Wadekar, M.D., Anuradha, K., Venkatesha,D.(2013),"Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae", *International Journal of Current Research and Academy Review* **5**(3),89-95.
64. Wang,L., Gu,H.,Lu,X.(2012),"A rapid low-cost real-time PCR for the detection of *Klebsiella pneumonia* carbapenemase genes", *Annals of Clinical Microbiology and Antimicrobials*, **11**(9).
65. Yusuf, I., Arzai, A.H., Haruna, M., Sharif, A.A., Getso M.I. (2014)," Detection of multi drug resistant bacteria in major hospitals in Kano, North-West, Nigeria", *Brazilian Journal of Microbiology* **45**(3), 791-798.
66. Zongo,K.J.,Metuor-Dabire,A., Compare,L.G., Sanou,I.,Sangare,L.,Simpore,J.,Zeba,B.(2015)," First detection of bla TEM, SHV and CTX-M among Gram negative bacilli exhibiting extended spectrum -lactamase phenotype isolated at University Hospital Center, Yalgado Ouedraogo, Burkina Faso", *African Journal of Biotechnology*, **14**(4) ,1174-1180.