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

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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 (Sonel 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, cephemycins, 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, 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 hydrolyze and 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 0.5-0.6 McFarland standard with Densi-check. 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, Ceftriaxone, Ceftazime, 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 Enzyme 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/Cla valanic acid (43.8%), Ampicillin/Sublactam (48.2%), Pipercillin/Tazobactam (40.8%), Cefazolin (62.9%), Cefuroxime (37.1%), Cefoxitin (34.6%), Cefotaxime (58.5%), Ceftazidime (57.7%), 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)

<table>
<thead>
<tr>
<th>β-lactamases</th>
<th>Frequency</th>
<th>percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure ESBL</td>
<td>81</td>
<td>29.8</td>
</tr>
<tr>
<td>Pure carbapenemases</td>
<td>19</td>
<td>7.0</td>
</tr>
<tr>
<td>ESBL+carbenemases</td>
<td>14</td>
<td>5.1</td>
</tr>
<tr>
<td>AmpC+carbenemases</td>
<td>5</td>
<td>1.8</td>
</tr>
<tr>
<td>ESBL+carbenemase+AmpC</td>
<td>7</td>
<td>2.6</td>
</tr>
<tr>
<td>ESBL+AmpC</td>
<td>2</td>
<td>0.7</td>
</tr>
</tbody>
</table>

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. None of the isolates showed AmpC production alone (Table 1).

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 carbapenemase producer were detected in 24.4% and 62.2% respectively. *Bla VIM* and *bla IMP* genes were not detected in this study.

![Figure 1](image-url) 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

<table>
<thead>
<tr>
<th>Genes</th>
<th>Nucleotide sequence</th>
<th>Annealing temperature</th>
<th>Fragment length(bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bla TEM</td>
<td>F- 5’TCGGGAAATGTGCGCG-3’ R-5’TGCTTAATCAGTGAGGCACC-3’</td>
<td>54</td>
<td>972</td>
<td>Sima et al., 2016</td>
</tr>
<tr>
<td>Bla SHV</td>
<td>F 5’-GGGTTATCTTATTTTGTGC-3’ R 5’-TTAGCGTTGGCCAGTGCTC-3’</td>
<td>56</td>
<td>928</td>
<td>Sima et al., 2016</td>
</tr>
<tr>
<td>Bla CTX-M</td>
<td>F-5’-CGCTTTGCGATGTGCAG-3’ R-5’ACCGCGATATCGTTGGT-3’</td>
<td>54</td>
<td>557</td>
<td>Moussé et al., 2016</td>
</tr>
<tr>
<td>Bla KPC</td>
<td>F-5’-GATGGTTGGTTGTCGATA-3’ R-5’CGAAATCGGCAGCACCCAG-3’</td>
<td>52</td>
<td>538</td>
<td>Martha et al., 2014</td>
</tr>
<tr>
<td>Bla VIM</td>
<td>F-5’-GGAATAGAGTGCTCATTCTTC-3’ R-5’CCAACACCCTAGCTAT-3’</td>
<td>55</td>
<td>189</td>
<td>Elligton et al., 2007</td>
</tr>
<tr>
<td>Bla OXA-48</td>
<td>F-5’-TTGTTGCGCATCATTGTCG-3’ R-5’GAGACCTCCTTTTGGATGC-3’</td>
<td>55</td>
<td>741</td>
<td>Moini et al., 2015</td>
</tr>
</tbody>
</table>

Primers sequences used in this study.

Table 3. Antimicrobial susceptibility patterns for K. pneumoniae isolates

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

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%), Cefotaxin (34.6%), Ceftriaxone (58.5%), Cefazidimine (75.7%), 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%).
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

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>ESBLs producers</th>
<th>Non ESBLs producers</th>
<th>p</th>
<th>carbapenemases producers</th>
<th>Non carbapenemases producers</th>
<th>p</th>
<th>Co-production enzymes</th>
<th>Non co-production enzymes</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/C</td>
<td>40(49.4%)</td>
<td>79(41.4%)</td>
<td>0.001</td>
<td>13(68.4%)</td>
<td>106(41.9%)</td>
<td>0.018</td>
<td>17(60.7%)</td>
<td>102(41.8%)</td>
<td>0.070</td>
</tr>
<tr>
<td>A/S</td>
<td>48(59.3%)</td>
<td>83(43.5%)</td>
<td>0.003</td>
<td>14(73.7%)</td>
<td>97(46.6%)</td>
<td>0.010</td>
<td>14(50.0%)</td>
<td>97(39.8%)</td>
<td>0.563</td>
</tr>
<tr>
<td>CTx</td>
<td>77(95.1%)</td>
<td>94(49.2%)</td>
<td>0.001</td>
<td>17(89.5%)</td>
<td>154(60.9%)</td>
<td>0.024</td>
<td>18(57.1%)</td>
<td>155(63.3%)</td>
<td>0.068</td>
</tr>
<tr>
<td>Cfx</td>
<td>75(92.6%)</td>
<td>86(45.0%)</td>
<td>0.001</td>
<td>17(89.5%)</td>
<td>144(56.9%)</td>
<td>0.020</td>
<td>15(53.6%)</td>
<td>146(59.8%)</td>
<td>0.794</td>
</tr>
<tr>
<td>Cx</td>
<td>33(40.7%)</td>
<td>68(35.6%)</td>
<td>0.027</td>
<td>9(47.4%)</td>
<td>92(36.4%)</td>
<td>0.424</td>
<td>14(50.0%)</td>
<td>87(35.7%)</td>
<td>0.254</td>
</tr>
<tr>
<td>Cx</td>
<td>3(40.7%)</td>
<td>80(41.9%)</td>
<td>0.001</td>
<td>18(94.7%)</td>
<td>141(55.7%)</td>
<td>0.004</td>
<td>15(53.6%)</td>
<td>144(59.0%)</td>
<td>0.590</td>
</tr>
<tr>
<td>Ctx</td>
<td>79(97.5%)</td>
<td>78(40.8%)</td>
<td>0.001</td>
<td>18(94.7%)</td>
<td>139(54.9%)</td>
<td>0.003</td>
<td>15(53.6%)</td>
<td>142(58.2%)</td>
<td>0.494</td>
</tr>
<tr>
<td>Ctx</td>
<td>78(96.3%)</td>
<td>68(35.6%)</td>
<td>0.001</td>
<td>17(89.5%)</td>
<td>129(51.0%)</td>
<td>0.004</td>
<td>14(50.0%)</td>
<td>132(54.1%)</td>
<td>0.277</td>
</tr>
<tr>
<td>Cpe</td>
<td>76(93.8%)</td>
<td>74(38.7%)</td>
<td>0.001</td>
<td>17(89.5%)</td>
<td>133(52.6%)</td>
<td>0.005</td>
<td>13(46.4%)</td>
<td>137(56.1%)</td>
<td>0.600</td>
</tr>
<tr>
<td>Azm</td>
<td>42(51.9%)</td>
<td>73(38.2%)</td>
<td>0.001</td>
<td>17(89.5%)</td>
<td>102(40.3%)</td>
<td>0.025</td>
<td>12(42.9%)</td>
<td>103(42.2%)</td>
<td>0.629</td>
</tr>
<tr>
<td>MP</td>
<td>7(8.6%)</td>
<td>26(13.6%)</td>
<td>0.512</td>
<td>17(89.5%)</td>
<td>42(16.6%)</td>
<td>0.001</td>
<td>4(14.3%)</td>
<td>57(23.4%)</td>
<td>0.249</td>
</tr>
<tr>
<td>Sm</td>
<td>49(60.5%)</td>
<td>105(55.9)</td>
<td>0.042</td>
<td>15(79.9%)</td>
<td>139(54.9%)</td>
<td>0.087</td>
<td>15(53.6%)</td>
<td>139(57.0%)</td>
<td>0.460</td>
</tr>
<tr>
<td>CN</td>
<td>15(18.5%)</td>
<td>42(22.0%)</td>
<td>0.812</td>
<td>7(36.8%)</td>
<td>50(19.8%)</td>
<td>0.211</td>
<td>8(28.6%)</td>
<td>49(20.1%)</td>
<td>0.069</td>
</tr>
<tr>
<td>Amk</td>
<td>10(12.3%)</td>
<td>39(20.4%)</td>
<td>0.166</td>
<td>4(21.1%)</td>
<td>45(17.8%)</td>
<td>0.106</td>
<td>22(78.6%)</td>
<td>27(11.1%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Cip</td>
<td>18(22.2%)</td>
<td>51(26.7%)</td>
<td>0.711</td>
<td>8(42.1%)</td>
<td>61(24.1%)</td>
<td>0.196</td>
<td>24(85.7%)</td>
<td>45(18.4%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

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. (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*.pneumoniae 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; Ntireganya 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 similar with the previous studies (Archana & Harsh, 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 & Tajbaksh (2016) reported that nitrofurantoin was resistant with 50% and 41.1% respectively (Manikandan & Amsath, 2013; Khamesipour & Tajbaksh, 2016). Manikandan & Amsath (2013) reported cotrimoxazole resistance (70.8%) which was high than what has observed in the present study (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; Doddah & 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; Doddah & 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; Wadkar et al., 2013; Doddah & 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; Patwardhan et al., 2013; Rawat et al., 2013; Doddah & Dhanalakshmi, 2014; 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; Wadkar et al., 2013; Doddah & Dhanalakshmi, 2014; Yusuf et al., 2014; Mohammed et al., 2015; Vijaya & Achut, 2017).

AmpC and carbapenemase 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; Wadkar et al., 2013; Yusuf et al., 2014; Archana et al., 2016).

ESBL, AmpC and carbapenemases co-occurred in 2.6% of isolate in the present study. Several studies showed different results ranging from 0.7% to 29.4% (Chatterjee et al., 2010; Doddah & 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 Ceftriaxone (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 select 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 gentamicin, 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 gentamicin, 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-M, bla\_TEM, bla\_SHV and bla\_CTX-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 carbapenemase 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


