CHAPTER 5: DISCUSSION

Majority of the infections caused by *Klebsiella* species are seen in healthcare settings, especially in aged people and immunosuppressed individuals. Infections may be associated with significant morbidity and mortality and therapeutic options are becoming increasingly limited. The continued emergence and spread of infections by antibacterial drug resistant strains, i.e., ESBL producing *Klebsiella* with plasmid-mediated quinolone resistance (PMQR) genes in particular is a therapeutic challenge. Presence of multiple virulence factors in *Klebsiella* isolates make them strong pathogens, so that these bacteria are responsible for chronic infections in wounds, on surgical implants, in the respiratory tract, and immunocompromised patients. However, the present study has showed that the production of biofilm, and haemagglutination is probably the most important virulence factors, co-existing with PMQR mechanisms in *Klebsiella*.

5.1. Body site distribution, antibiotic resistance and ESBL production in clinical isolates of *Klebsiella* species

In the present study out of 201 ESBL producing *Klebsiella* isolates, 35.3% were isolated from pus, followed by 19.9% were from urine, 17.9% were from sputum, 12.9% were from blood culture (Table 4.1). Out of the 201 ESBL producing *Klebsiella* isolates, maximum number of isolates were obtained from medicine ward, i.e., 77 (38.1%) followed by surgery 49 (24.4%), and orthopedics 17 (8.5%) (Table 4.2). Among the 201 ESBL positive isolates, 137 (68.2%) were from male patients and 64 (31.8%) were from female patients, and maximum numbers of ESBL positive isolates were obtained from people aged 40-70 years (Table 4.3). Out of 201 isolates, 196 (97.5%) isolates were *K. pneumoniae* and five (2.5%) isolates were *K. oxytoca*. The antibiotic susceptibility pattern of the ESBL isolates revealed 91.5% (184/201) isolates were resistant to amoxyclav (AMC), piperacillin (PI), and 76.1% (153/201) were resistant to all fluoroquinolone antibiotics tested, i.e., nalidixic acid (NA), ciprofloxacin (CIP), and levofloxacin (LEV), sparfloxacin (SPX), Moxifloxacin (MO). It is notable that, 142 isolates were resistant to all fluoroquinolone drugs with maximum MIC value, such as nalidixic acid >256µg/mL, and ciprofloxacin, levofloxacin, sparfloxacin, moxifloxacin >32µg/mL each. Among 201 ESBL producing *Klebsiella* isolates, 79.6% (160/201) isolates were multi drug resistant (MDR). Among the MDR isolates, 95.6% (153/160) were NA, CIP, LEV, SPX and MO resistant. The
overall prevalence of fluoroquinolone (FQ) resistance was 76.1% (153/201) (Table 4.4). All MDR isolates were susceptible to colistin (CL) 100% (160/160) and 89.3% (143/160) were tigecycline (TGC) susceptible (Table 4.5).

In a study done by Bora et al., (2014), total of 219 K. pneumonia were isolated from different body sites, of which 55.2% were obtained from urine, 26.9% were from sputum, 10.5% were from pus, 11.9% were from blood were studied. Of these isolates, 87.6% were MDR which is slightly higher compared to the present study. The present study showed that the majority of ESBL producing Klebsiella species is also resistant to all fluoroquinolones used, i.e., up to 76.1%, which is higher than a study done by Nandihal et al., (2015), which reported only 38.5% resistance to quinolones. The rising trend of MDR has emerged over the successive years. A study done by Basavaraj et al., (2011), showed that Klebsiella infections are more common in 41-60 years age group, which is similar to the present study. However, the prevalence was more among the females than male patients, which is discordant with the present study in which the infections were more prevalent in male patients. In the study done by Basavaraj et al., (2011), ESBL producers were more from the medicine ward (10.9%), followed by surgery (8.9%), which is similar to the present study in which ESBL producers were also more from medicine (38.1%) ward followed by surgery (24.4%). Carbapenems are often considered to be the last line of effective treatment available for infections caused by MDR Enterobacteriaceae. In the present study, 52.2% and 51.3% isolates were sensitive to imipenem and meropenem, respectively, whereas another study done by Sarojamma et al., (2011), found that 84% of their isolates were sensitive to imipenem. Most of these ESBL producers were MDR with a maximum level of resistance to more than three groups of antibiotics. A study done by Kaur et al., (2013), has reported that 60.3% were ESBL producers, of which 10.1% were CIP resistant. Whereas, a study done in Iran showed, that the Klebsiella isolates were resistance to NA, CIP, and LEV, i.e., 61.9%, 65.2% and 52.1%, respectively. Another study done by Sharma et al., (2016), showed that 67% were MDR Klebsiella and 72.7% were fluoroquinolone resistant. As per a study done in Assam, (2015), it is reported that quinolone resistance of K. pneumoniae, i.e., NA resistance in 71.4%, followed by LEV in 78.6%, CIP in 71.4%, and SPX in 71.4%, which is similar to the present study, i.e., 76.1% resistance to quinolones antibiotics. However, several other studies show higher resistance to ciprofloxacin as compared to the present study, which was 2.3%, 100%, and 84.5% respectively. It is very much evident that the prevalence of
ESBL producing *Klebsiella* species varies across different parts of the world.\(^\text{183-184}\) This could be partly due to the irrational use of various β-lactam antibiotics and partly due to the lack of accurate laboratory data, as many laboratory methods in routine practice fail to detect ESBL production.

### 5.2. Prevalence of virulence potential among drug resistant clinical isolates of ESBL *Klebsiella* species

The role of biofilm formation and development by bacteria is suggested to be an important stage in the pathogenesis of numerous infections by several bacterial species. Biofilm formed on the device surfaces and host tissue is believed to inhibit the antimicrobial effect, help evade the host immune mechanism and improve bacterial communication, helping them to express more virulence factors.\(^\text{191}\)

In the present study, bacterial resistance profile with ESBL production and MDR *Klebsiella* isolates was 76.9%, which might add to the virulence properties and other nosocomial factors to make them strong pathogens. In the present study, 88.6% of the isolates showed the ability to produce biofilms. Strong biofilm was seen in 7% of the isolates, moderate biofilm forming isolates were 30.4%, 51.2% were weak formers, and 11.4% of the isolates were non biofilm producers (Table 4.8). Among the MDR *Klebsiella* isolates; 143/160 (89.3%) isolates were biofilm producers (Table 4.11). *K. pneumoniae* has the ability to adhere, multiply and persist on non-living surfaces in the hospital environment and this has been reported as a significant cause of severe hospital acquired infections. Biofilm formation by *Klebsiella* is one of the most important virulence factors in the pathogenesis of this organism.\(^\text{192}\) Biofilm formation in the present study was quite high, such isolates were obtained from the specimens, i.e., 31.8%, 18.4%, 15.4%, 11.9%, and 4.5% of isolates from pus, urine, sputum, blood culture, and ventilator aspirate, respectively (Table 4.8), amounting to an overall percentage of 88.6 of isolates showing biofilm formation. Whereas, a study done in Nepal, (2017), also observed a high rate of biofilm producers, i.e., 83.3%, 71.7%, and 66.6% of isolates from pus, urine, and sputum, respectively.\(^\text{193}\) According to Seifi *et al.*, (2016), who reported that 93.6% were biofilm producing *K. pneumoniae*, isolated from urine, sputum, blood, and wound swabs, i.e., 96.5%, 100%, 50%, and 100%, respectively.\(^\text{192}\) The results of the present study is similar to the finding of Mishra *et al.*,
(2015), who reported that 75% of *K. pneumoniae* isolates from various clinical specimens were biofilm producers. According to Prasad *et al.*, (2017), it was reported that 73.3% of the *K. pneumoniae* strains from their study were biofilm producers, in which CIP was 15.9% resistant, which was very less compared to the present study. A study conducted in Pondicherry, (2012), reported that, 63% *Klebsiella* isolates from urine samples were biofilm producers and 37% were non biofilm producers, in which 93.3%, 73.3%, and 80% resistant to NA, CTX, and COT respectively.

In the present study, fimbriae detection was observed from isolates, i.e., 32.3%, 16.9%, 15.9%, 10.9%, 4.5%, and 6.5%, from pus, urine, sputum, blood culture, ventilator aspirates, and other samples (Body fluid, HVS, ETA, and BAL), respectively. Among the fimbriae, this study found that in *Klebsiella* isolates, type 3 fimbriae (MRHA) 139/201 (69%) was more frequent as compared to the type 1 fimbriae (MSHA) 36/201 (18%) and 26/201 (13%) isolates were non hemagglutinated (Table 4.9). Among MDR *Klebsiella* isolates; 144/160 (90%) isolates were hemagglutination positive (Table 4.11). A study done by Tarkkanen *et al.*, (1992), has reported that the total 39 strains studied (100%) were positive for type 3 fimbriae, which may be due to the low sample size used in their study. Whereas another study done by Chaudhary *et al.*, (2013), and reported that type 1 fimbriae were seen in 57.4% and type 3 fimbriae in 14.28%, which is discordant with the present study. However, a study done by Podschun *et al.*, (2000), reported type 1 fimbriae in 86% and type 3 fimbriae in 70% from the clinical isolates of their study. According to Schroll *et al.*, (2010), reported that type 3 fimbriae are more essential compared to type 1 fimbriae for biofilm production. Moreover, according to the present study, and several other studies, expression of type 3 fimbriae was more in *K. pneumoniae* which is essential for the typical biofilm formation in these strains.

5.3. Prevalence of hypermucoviscosity genes among *Klebsiella* isolates

Hypermucoviscosity is an emerging virulence factor which is the main cause of endophthalmitis and pyogenic liver abscess described in several other studies. In the present study 19/201 (9.5%) ESBL *Klebsiella* isolates were hypermucoviscosity positive; which were obtained from pus (3.5%), urine (2%), sputum (2%) and blood culture (1%), and ventilator aspirates and bronchoalveolar lavage (0.5%) each. Among 19/201 hypermucoviscosity positive samples; 15/19...
isolates were MDR (Table 4.11). Out of 19 isolates, 63.2% (12/19) were positive for $rmpA/magA$ genes; in which 15.8% (3/19) isolates were positive only for $rmpA$ genes and 47.4% (9/19) isolates were both $rmpA$ and $magA$ genes (Table 4.14). To check the hypermucoviscosity, 18 phenotypically HV negative isolates were collected randomly and checked for detection of genes. Out of which 55.6% (10/18) were positive genotypically, in which 8/18 (44.4%) isolates were positive only for $rmpA$ gene and 11.2% (2/18) were both $rmpA$ and $magA$ genes positive (Table 4.15). A study from Taiwan, (2010), showed among 91 isolates of K. pneumoniae; the most common sample from which the isolates were obtained was blood 58.2% (53/91), followed by urine were 19.8% (18/91), pus 7.7% (7/91), and sputum 5.5% (5/91). Of these isolates phenotypically HV positives were identified in 38.5% (35/91) isolates, and genotypically 46.2% (42/91) isolates were positive for $rmpA$ gene, and 8.8% (8/91) isolates were positive for $magA$ gene. Another study from Taiwan, (2006), showed that prevalence of $rmpA$ and $magA$ were 48% and 17%, respectively. However, a study done by Yu et al., (2015), ESBL-KP was identified in 57 isolates from various specimens, including sputum (24), urine (18), blood (10), ascites (2), bile (1), pleural fluid (1), and broncho-alveolar lavage fluid (1). Yu et al., (2015), have also shown that among the 57 ESBL-KP isolates, 21.1% (12/57) were positive for $rmpA$ gene, which is a little higher than the present study in which $rmpA$ was 15.8% (3/19). According to Amraie et al., (2014), of 173 isolates in their study; 73 (42.19%) were positive for HV test and 100 (57.80%) were negative; 2.3% (4/173) were positive and 97.7% (169/173) were negative for $magA$ gene. Another study done by Zamani et al., (2013), 60.95% (64/105) isolates of K. pneumoniae were HV positive phenotypically, in which 3.8% (4/105) isolates were positive for $magA$ gene, 2 of them were HV positive and 2 were HV negative phenotypically. Of these 4 isolates, 3 (75%) were obtained from blood samples and 1 (25%) was from an abscess sample. A study done by Abduljabbar et al., (2016), reported that of the 32 Klebsiella isolates, 13 were isolated from urine, 12 were from burns, 4 were from sputum, and 3 were from blood samples; 62.5% (20/32) were positive for $rmpA$ gene, which were identified from 5 urine samples, followed by 11 were from burns, 1 was from sputum, and 3 were from blood samples. According to a study from China, (2015), among 45 K. pneumoniae strains, 100% (45/45) were $rmpA$ gene positive, and 68.9% (31/45) were $magA$ gene positive. A study done by Cubero et al., (2015), has shown 5.4% (53/878) K. pneumoniae isolates were hypermucoviscous positive (by string test). Of these, 30.2% (16/53) were positive for $magA$ and $rmpA$ genes, in
which 22.6% (12/53) isolates were only positive for *rmpA* gene, and the remaining 47.2% (25/53) isolates were negative for *magA/rmpA* genes. However, several other studies have reported that the rate of hypermucoviscosity strains were 62.5% (20/32), 24.62% (16/65), and 33% (29/88), respectively, which is higher as compared to the present study. Therefore, according to the present study hypermucoviscous phenotype, as detected by string test, is not enough to recognize these clones, additional molecular studies are needed.

### 5.4. Prevalence of the *blaCTX-M* gene

ESBL producing bacteria have become a serious problem worldwide. The continued emergence of ESBL is probably because of improper anti-biogram schedule, long term antibiotic exposure and unnecessary prescribed drugs without checking sensitivity pattern. This poses the current diagnostic challenges to the clinical microbiology laboratories. Out of 201 ESBL producing *Klebsiella* isolates, 142 ESBL isolates were checked for *blaCTX-M* gene. Of which 85.9% (122/142) were *blaCTX-M* positive, therefore the remaining 14.1% (20/142) ESBL producing *Klebsiella* isolates were negative to *blaCTX-M* gene, which may be because of other genes, such as *blaTEM*, and *blaSHV*. According to two other studies ESBL production rate is gradually increasing in the last few decades in India, and varies from 17-86%. In the present study, the frequency of ESBL producing *K. pneumoniae* was higher in the age groups 40-50, 50-60 and 60-70 years, i.e., 17.9%, 20.9% and 19.9%, respectively, in which males were predominant, i.e., 68.2% as compared to females, i.e., 31.8%. A study conducted in Mangalore, (2010), which reported that 26.7% (16/60) *Klebsiella* isolates were ESBL producers, in which males were predominant, i.e., 62.5% as compared to the 37.5% seen in females. ESBL producing *Klebsiella* isolates were more in the age group 36-60 years, i.e., 81.3%, which is similar to the present study. A study from Bijapur, (2011), has reported that 38.9 % were ESBL producing *K. pneumoniae* among *Enterobacteriaceae*, which were mostly isolated from the age group 41-60 years, and however, in this study the isolates were mostly from the females. Prevalence of ESBL producing *K. pneumoniae* reported in the present study was mainly from the medicine ward (38.1%), followed by surgery (24.4%), orthopedics (8.5%), and medicine ICU (6%), which is in contrast to a study from Bijapur, (2011), in which the prevalence of ESBL producing *K. pneumoniae* was more from ICU (80%). In the present study, it was observed that ESBL positive isolates exhibited high levels of MDR and the prevalence of ESBL producing *K.
pneumoniae was found to be high, i.e., 97.5% and K. oxytoca was 2.5%, which is in accordance with the study done in Hyderabad, (2015), in which K. pneumoniae was 90.13%. The present study reports that 85.9% were blaCTX-M positive, of which pus sample was the major source, i.e., 31%, followed by urine 19%, sputum 12%, blood culture 10.6%, and body fluid 2.8%. However, a study from Jaipur, (2013), reported, urine was the major source among ESBL producing gram negative bacilli, i.e., 57.2%, followed by blood (31%), pus (48%), respiratory tract (63.8%), and body fluids (52.1%), in which 67% Klebsiella species were observed. Yet another study from Dehradun, (2015), also has similar results, in which out of 65 isolates of ESBL producing K. pneumoniae, urine was the major source, i.e., 41.5%, followed by pus, ET aspirate, and sputum, i.e., 20%, 13.8%, and 9.2%, respectively. A study from Taiwan, (2006), reveals predominant presence of blaCTX-M gene in ESBL-KP isolates. Among the 27 identified ESBL genes identified in their study, 21 (77.8%) were CTX-M gene, and 11 (40.7%) were SHV genes. Reports from Russia, (2003), have shown 34.9% isolates of K. pneumoniae were positive for blaCTX-M gene. A study done in Iran, (2015), reported that among 87 ESBL producing strains majority of isolates carried blaCTX-M gene, i.e., 80.5%, whereas, another study shows, out of 47.27% ESBL producers, 26.92% were blaCTX-M gene positive. Whereas, another study from Thailand, (2007), has reported, 50% Klebsiella isolates were blaCTX-M positive. According to the study from Malaysia, (2016), 87.5% (28/32) isolates of K. pneumoniae was positive for blaCTX-M gene, which is similar to the present study. A study from Kolkata, (2012), has reported blaTEM gene in 52% (38/73), blaSHV gene in 45% (33/73), and blaCTX-M gene in 37% (27/73) of the Klebsiella isolates. However, a study from Tumkur, (2013), and from Mangalore, (2014), have reported blaCTX-M gene was positive in 48.5% and 68% isolates, respectively. The present study, reports that blaCTX-M gene was present in 85.9%, quite high on ESBL producing K. pneumonia isolates, which is in accordance to studies done in Assam, (2014), and Jaipur, (2013), in which blaCTX-M was 77.58% and 85% respectively. Therefore, according to several studies, blaCTX-M gene is emerging rather than TEM and SHV genes among the ESBL producing isolates.
5.5. **Prevalence of plasmid mediated quinolone drug resistance (\textit{qnrA}, \textit{aac(6')Ib-cr}, \textit{qepA}, \textit{oqxA} and \textit{oqxB}) genes**

The resistance mechanisms against antimicrobial agents can take place in four major ways: by altering drug targets, protecting drug targets, enzymatic drug modification, and reducing drug accumulation.\textsuperscript{223} \textit{Klebsiella} resists quinolone antibiotics by all of these ways. While chromosomal mutations in genes coding for DNA gyrase, topoisomerase IV and genes coding for outer membrane, and efflux proteins are also responsible for the resistance.\textsuperscript{126} PMQR involves, the \textit{qnr} proteins protecting DNA gyrase and topoisomerase IV from quinolones and the \textit{aac(6')}Ib-cr enzyme, which is able to modify some fluoroquinolone antibiotics.\textsuperscript{141}

In the present study, the aim was to detect PMQR genes, i.e., \textit{qnrA},\textit{aac(6')Ib-cr}, \textit{qepA}, \textit{oqxA} and \textit{oqxB} among ESBL producing \textit{Klebsiella} species. Among 201 isolates, 153 were resistant to NA, CIP, LEV, SPR, and MOX by antimicrobial susceptibility testing. A total of 142 isolates were identified for the PMQR genes, of which maximum PMQR genes were \textit{aac(6')Ib-cr} gene, i.e., 96.5% (137/142), followed by 91.5% (130/142) \textit{oqxB}, 84.5% (120/142) \textit{oqxA} gene, 42.2% (60/142) \textit{qnrA}, and only 3.5% (5/142) were \textit{qepA} gene positive.

A study done by Tripathi \textit{et al.}, (2012),\textsuperscript{167} from Kolkata, has reported that PMQR genes, i.e., \textit{qnrA} were detected in 37% (27/73), and \textit{qnrB} were 56% (41/73) of the \textit{Klebsiella} isolates, which is similar to the present study. Another study from Chennai, (2011), has reported among 23 ESBL producing \textit{K. pneumoniae} isolates, 70% (16/23) were \textit{qnrA} and 48% (11/23) \textit{qnrB} genes positive and 34.7% (8/23) isolates had both \textit{qnrA} and \textit{qnrB} genes, and \textit{aac(6')Ib-cr} was observed in 56.5% (13/23) isolates,\textsuperscript{168} which is higher than the present study in case of \textit{qnrA} gene, i.e., 42% were positive and less in case of \textit{aac(6')Ib-cr} gene, i.e., 96.5%. According to a study from Puducherry, (2016), the authors have reported highest prevalence of \textit{aac(6')Ib-cr} gene, i.e., 64.5% among \textit{Enterobacteriaceae} family, while, \textit{qnrB} and \textit{qnrS} genes were present in 15% (97) and 10% (64) respectively. None of the strains were positive for \textit{qnrA} and \textit{qnrD} genes.\textsuperscript{169} Whereas, the present study has shown, 42.2% of the isolates were \textit{qnrA} gene positive and also highest prevalence of \textit{aac(6')Ib-cr} gene, i.e., 96.5% in ESBL producing \textit{K. pneumoniae} isolates.

A study conducted in Assam, (2016), reported that 73.08% isolates of \textit{Klebsiella} species were positive for \textit{aac(6')Ib-cr} gene, in which 23.08% were \textit{K. pneumoniae} and 50% from \textit{K. oxytoca}. The prevalence of \textit{aac(6')Ib-cr} was highest among other PMQR determinants,\textsuperscript{170} which is
similar to the present study, i.e., 96.5% were $aac(6')Ib-cr$ positive. A study done by Al-Agamya et al., (2018), have reported that the $qnrS$ and $aac(6')Ib-cr$ genes are the foremost among PMQR genes in $E. coli$ and $K. pneumoniae$ isolates. Among those isolates, presence of $qnrS$ was 61.3% (19/31) followed by the $aac(6')Ib-cr$ gene 51.6% (16/31). Of the 21 $K. pneumoniae$ isolates, 61.9% (13/21) possessed $qnr$ genes, i.e., $qnrS$ were 57.1% (12/21), and $qnrB$ were 4.8% (1/21), and $aac(6')Ib-cr$ was detected in 47.6% (10/21) of the isolates.

In a study done by Xue et al., (2017), in China, prevalence of the PMQR genes was investigated, in which out of 243 isolates, 55 (22.63%) were positive for PMQR genes; the most frequently detected gene was $qnrS$ (13.2%), followed by $aac(6')Ib-cr$ (6.2%) and $qnrB$ (3.7%). The $qnrA$ and $qepA$ genes were not detected. A study from Argentina, (2017), has reported that 26% of isolates were non susceptible to at least one of the three quinolones antibiotics, i.e., NA, CIP, LEV. The overall prevalence of PMQR genes was 8.1%, 4.6% for $aac(6')Ib-cr$; 3.9% for $qnr$ genes, and 0.4% for $oqxA$ and $oqxB$, which is very less compared to the present study. However, a study from Egypt by Khalil et al., (2017), have reported that $qnr$ gene was detected in 39.4%, $aac(6')Ib-cr$ gene was detected in 72.7%. $qepA$ gene was detected in only one $K. pneumoniae$ isolate and $oqxA$ gene was detected in 48.5% of the isolates. A study from Nigeria, (2016), has reported 35.2% of the isolates were PMQR, of which 28.2% isolates carried various $qnr$ genes (of which 9.9% were $qnrA$, 5.6% were $qnrB$, 11.3% were $qnrS$, and 4% were $qnrD$), 25.4% isolates were positive for $aac(6')Ib-cr$, and 18.7% were $qepA$ gene. According to a study conducted in Hungary, (2016), the most commonly identified gene among 53 $Klebsiella$ isolates was $aac(6')Ib-cr$, i.e., 38 (70%), followed by $oqxA$ 26 (48%), $oqxB$ 22 (40%), and 5 (9%) were $qnrS$ gene positive. All isolates were negative for $qnrA$, $qnrB$, $qnrC$, $qnrD$, and $qepA$ genes. A study from Kuwait, (2015), the authors studied 173 ESBL producing $K. pneumoniae$, in which $qnr$ genes were identified in 27 (15.6 %) isolates, of which 1 (3.7%) was $qnrA2$, 21 (78%) were $qnrB1$, and 05 (18.5%) were $qnrS$ and $aac(6')Ib-cr$ gene were identified in 26 (96%) isolates. Another study was done in Sweden, (2010), which has reported a high prevalence of the PMQR genes, i.e., $qnr$ and $aac(6')Ib-cr$ among the ESBL producers (9.1% and 52.3%, respectively). This is in accordance with the present study in which PMQR was higher among ESBL producers.
A study done in Mexico, (2011), had reported that the prevalence of *qnr* gene in *K. pneumoniae* isolates was 13.7%, the *aac(6')Ib-cr* gene was 33.3%, and none of them were positive for *qepA* gene. In a study by Peymani *et al.*, (2015), in Iran, of the 200 *K. pneumoniae* isolates, 124 (62%) were quinolone resistant, of which *qnr* encoding genes were present in 49 (39.5%) isolates, among these *qnrB1* was positive in 30.6%, followed by *qnrB4* 9.7%, and *qnrS1* 1.6%, and no isolates were positive for *qnrA* gene. According to a study conducted in Taiwan, (2012), 66 (53.2%) isolates had at least one PMQR gene. Among these isolates, *qnrB2*, *qnrB4*, and *qnrS1* genes were positive in 26, 19, and 13 isolates, respectively, whereas all isolates were negative for *qnrA* gene. ESBL genes were transferable via conjugation with either *aac(6')Ib-cr* or *qnrB* in 63.6% of the isolates carrying PMQR genes. According to Stephenson *et al.*, (2010), in Jamaica, out of 83 fluoroquinolone resistant isolates 10/83 were *Klebsiella* species, of which, 20% isolates were positive for *qnrA* and 30% were positive for *qnrA* and *qnrS* genes, and Limoncu *et al.*, (2012), in Turkey studied the prevalence of PMQR genes in 27 *K. pneumoniae*, of which 2/27 isolates were positive for *qnrA* gene, 3/27 were *qnrB*, and 1/27 was positive for *qnrS* gene. Some isolates (16/27) were positive for *aac(6')Ib-cr* gene and none of the isolates were positive for *qepA* gene. A study done by Seo *et al.*, (2010), in Korea, reported that among 187 isolates of *K. pneumoniae*, 86 (46%) isolates had *qnr* gene, 12 (6%) had the *aac(6')Ib-cr* variant and 8 isolates (4%) were positive for both *qnr* and *aac(6')Ib-cr*. A study from China, (2008), has reported high prevalence of *qnr* and *aac(6')Ib-cr* genes in *Enterobacteriaceae* family. Among those isolates, *qnr* and *aac(6')Ib-cr* were 65.5% and 21.8%, respectively, in *K. pneumoniae* isolates. According to the Okadea *et al.*, (2014), in Japan, of the 24 *K. pneumoniae* isolates studied, *qnrB*, *qnrS*, and *aac(6')Ib-cr* genes were detected in 12 (50.0%), 4 (16.7%), and 1 (4.2%), respectively, while of the 126 *E. coli* isolates, *qnrA,aac(6')Ib-cr*, and *qepA* genes were detected in 1 (0.8%), 11 (8.7%), and 2 (1.6%), respectively. *qnr* genes were mainly found in *K. pneumoniae* (66.7%) and to a lesser extent in *E. coli* (0.8%). Whereas, according to the present study positivity of *aac(6')Ib-cr* gene was more followed by *oqxB* and *oqxA* genes in PMQR *K. pneumoniae* isolates, i.e., 96.5%, 91.5%, and 84.5% respectively, and 42.2% were positive for the *qnrA* gene. A study from Iran, (2014), had reported, 30.4% (7/23) isolates harbored *qnr* and *aac(6')Ib-cr* genes. None of the isolates were positive for *qnrA* gene. According to Nematzadeh *et al.*, (2015), the distribution of the antibiotic resistance genes in ESBL producing *K. pneumoniae, aac(6')Ib-cr* gene was 100%
(32/32), as the major determinant of PMQR it was detected in all CIP resistant isolates, and none of the isolates was positive for qnrA gene, whereas, a study from Syria, (2015), has reported, 62.5% (15/24) were aac(6')Ib-cr positive, followed by 50% (12/24) were qnrB, 37.5% (9/24) were qnrS positive, whereas, none of the isolates were positive for qnrA gene. According to a study done by Heidary et al., (2016), in Iran, out of 117 K. pneumoniae isolates, 4% (5/117) were qepA and 85% (100/117) were aac(6')Ib-cr genes positive, which is almost similar to the present study, in which 3.5%, and 96.5% were qepA, and aac(6')Ib-cr respectively. A study done by Meteab Alshammari et al., (2015), in Kufa, had reported the results of detection of the PMQR genes and revealed a wide distribution of aac(6')Ib-cr gene alone or combined with qnrS gene in 14 (41.18%) isolates, or with qepA in 2.94% isolates. About 8.82% of the bacterial isolates carried aac(6')Ib-cr, qepA, and qnrS genes, whereas only one isolate was positive for qnrB gene. As per the study from Tehran, (2015), of the 79 K. pneumoniae isolates, 47 (59.5%) carried the PMQR determinants. Among these, 42 (89.4%) isolates carried aac(6')Ib-cr gene, of which 21 (50%) also harbored qnrB, three isolates carried qnrB alone, two isolates (4.2%) harbored qnrS gene and none of the isolates had qnrA. In a study done by Shams et al., (2015), 77 ESBL producing K. pneumoniae isolates harbored PMQR genes, of which 70.1% were aac(6')Ib-cr, and 46% were qnrB, followed by 5.7% qnrS. However, all isolates were negative for qnrA and qepA. A study done in Korea, (2014), had reported that out of 22 K. pneumonia isolates, qnrA gene was not detected in any of the isolates; however, qnrB was detected in 50.0% (11/22) and qnrS was detected in 9.1% (2/22) of the K pneumoniae isolates. The aac(6')Ib-cr gene was detected in 90.9% (20/22), and the oqxA and oqxB genes was found in 11 (50%) positive isolates. PMQR genes are observed in many other studies where 58%, 84%, and 100% were positive for aac(6')Ib-cr gene. Gay et al., (2006), reported a high association between the PMQR genes (both the qnr and aac(6')Ib-cr genes) and genes encoding for ESBL, and this explains the prevalence of enterobacterial clinical isolates resistant to both fluoroquinolones and β-lactams antibiotics. A study from Iraq, (2016), has reported qnrB, qnrA, and qnrS were in 23%, 2.7%, and 1.4%, of the isolates respectively. However, a study from Morocco, (2017), has reported that qnrB, qnrS, and aac(6')Ib-cr genes were 11.86%, 5.93%, and 18.64%, respectively. A study done by El-Badawy et al., (2017), in Egypt has shown that aac(6')-Ib-cr (61%), oqxA (88%), oqxB (30%), qepA (12%), were positive and qnrA was negative. According to a study done in Iran, (2015), of the 247 ESBL producing K.
pneumoniae isolates, \textit{qnrA}, \textit{qnrB}, and \textit{qnrS} genes were present in 3.6%, 1.6%, and 1.2%, respectively. In this, the most prevalent of PMQR gene was \textit{aac(6')Ib-cr} (68.8%) followed by \textit{oqxA} (56.7%), \textit{oqxB} (54.6%), \textit{qnr} (6.4%), and \textit{qepA} (2%), therefore high frequency of \textit{aac(6')Ib-cr} gene among ESBL producers has been reported.\textsuperscript{130} Similarly, the present study reports that maximum isolates were positive for \textit{aac(6')Ib-cr} gene (96.5%) in ESBL producing \textit{K. pneumoniae} isolates, followed by \textit{oqxB} (91.5%), \textit{oqxA} (84.5%), \textit{qnrA} (42.2%), and \textit{qepA} (3.5%).

5.6. DNA sequencing

DNA sequencing was done in 10 randomly selected samples in order to find out if any novel mutation has occurred. The amplified products were sequenced to confirm the alleles. After comparing with the known alleles in NCBI’s GeneBank, it was observed that in the isolates studied in the present study that no novel mutations have been identified among all alleles, as all the nucleotide sequences showed 100% similarity with the other sequences. Similarly, a study done in Puducherry has also not shown any novel mutation among the PMQR study.\textsuperscript{169}