Indian J Med Res 149, February 2019, pp 222-231
DOI: 10.4103/ijmr.IJMR_2098_17

Quick Response Code:

Plasmid profiles among some ESKAPE pathogens in a tertiary care centre in south India

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Received December 31, 2017

***Background & objectives*: Plasmid has led to increase in resistant bacterial pathogens through the exchange of antimicrobial resistance (AMR) genetic determinants through horizontal gene transfer. Baseline data on the occurrence of plasmids carrying AMR genes are lacking in India. This study was aimed to identify the plasmids associated with AMR genetic determinants in ESKAPE pathogens.**

***Methods*: A total of 112 ESKAPE isolates including *Escherichia coli* (n=37), *Klebsiella pneumoniae* (n=48, including 7 pan-drug susceptible isolates), *Acinetobacter baumannii* (n=8), *Pseudomonas aeruginosa* (n=1) and *Staphylococcus aureus* (n=18) were analyzed in the study. Isolates were screened for antimicrobial susceptibility and whole genome sequencing of isolates was performed using Ion Torrent (PGM) sequencer. Downstream data analysis was done using PATRIC, ResFinder, PlasmidFinder and MLSTFinder databases. All 88 whole genome sequences (WGS) were deposited at GenBank.**

***Results*: Most of the study isolates showed resistant phenotypes. As analyzed from WGS, the isolates included both known and unknown sequence types. The plasmid analysis revealed the presence of single or multiple plasmids in the isolates. Plasmid types such as IncHI1B(pNDM-MAR), IncFII(pRSB107), IncFIB(Mar), IncFIB(pQil), IncFIA, IncFII(K), IncR, ColKP3 and ColpVC were present in *K. pneumoniae*. In *E. coli*, IncFIA, IncFII, IncFIB, Col(BS512), IncL1, IncX3 and IncH were present along with other types. *S. aureus* harboured seven different plasmid groups pMW2 (*rep*5), pSAS1 (*rep*7), pDLK1 (*rep*10), pUB110 (*rep*US12), Saa6159 (*rep*16), pKH12 (*rep*21) and pSA1308 (*rep*21). The overall incidence of IncF type plasmids was 56.5 per cent followed by Col type plasmids 18.3 per cent and IncX 5.3 per cent. Other plasmid types identified were <5 per cent.**

***Interpretation & conclusions*: Results from the study may serve as a baseline data for the occurrence of AMR genes and plasmids in India. Information on the association between phenotypic and genotypic expression of AMR was deciphered from the data. Further studies on the mechanism of antibiotic resistance dissemination are essential for enhancing clinical lifetime of antibiotics.**

Key words Antimicrobial resistance - β-lactamase - col-horizontal gene transfer - IncF - plasmids

Nosocomial infections are life-threatening and are a significant cause of morbidity and mortality rates. ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species) is a group of Gram-negative and Gram-positive bacterial pathogens causing severe nosocomial infections. Infections caused by these pathogens are difficult and challenge to treat due to the high rates of antimicrobial resistance (AMR) seen[1].

Most of these pathogens carry AMR encoding genes on mobile genetic elements favouring the rapid dissemination of intra- and inter-species spread. The maintenance of AMR genes under the selection pressure determines the presence of plasmids in the host and has led to the evolution of plasmids over time[2]. Plasmids are typed based on the replicon/incompatibility types (Inc) in Gram-negative organisms[3,4] and *rep* gene-based typing in Gram-positive organisms[5]. Some of the most common Inc plasmid types observed among Gram-negative bacterial pathogens are IncF, IncH, IncN, IncA/C, Incl, IncX, IncR, IncQ and Col plasmids[6]. Such plasmids are known to carry multiple AMR genes through accumulation by vertical and horizontal transfer, and this rapid dissemination phenomenon increases the multidrug-resistant (MDR), extremely drug resistant (XDR) and pan drug-resistant pathogen rates especially in ESKAPE pathogens.

AMR gene classes that are carried on plasmids include β-lactamases [extended-spectrum β-lactamases (ESBLs), AmpC and carbapenemases], aminoglycoside modifying enzymes, 16S rRNA methyltransferases (16S RMTases) and the recently reported mobile colistin resistance gene (*mcr*) are of concern. ESBLs (bla_{TEM}, bla_{SHV}, bla_{CTX-M}), carbapenemase (bla_{SPM}, bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{KPC} and bla_{OXA48}) and plasmid-mediated quinolone resistance genes [*qnr*A, *qnr*B, *qnr*S, *aac(6')lb-cr* and *qep*A] were most commonly reported worldwide in IncF type plasmids[6]. This includes the subtypes IncFIA, IncFIB and IncFII plasmids reported in *Escherichia coli*[7,8]. IncI type plasmids were reported predominantly in Europe with genes coding for resistance to aminoglycosides, tetracyclines and quinolones[9]. Another common plasmid among the Gram-negatives includes IncH type. These were regarded for their association with multidrug resistance as these carry ESBLs and other genes encoding for resistance to sulphonamides, aminoglycosides, tetracyclines and streptomycin, most commonly in *Salmonella* Typhi[10]. IncH plasmid includes its association with *mcr-1* and *mcr-3* plasmid-mediated colistin resistance genes in *E. coli*[11,12]. The most worrisome is the co-existence of multiple classes of AMR genes on the same plasmid compromising the use of two broad antimicrobials for therapy. This includes the co-existence of ESBLs and carbapenemases, carbapenemases and 16S RMTases, ESBLs and quinolone resistance[13].

The information of plasmid profiles among ESKAPE pathogen is lacking, especially from India. Hence, we undertook this study to identify the common plasmid types among ESKAPE pathogens causing nosocomial infections in a tertiary care centre in south India using whole-genome sequencing. The objectives of this study were as follows: (*i*) identification of AMR genes associated with plasmids, (*ii*) identification and comparison of sequence types (STs) with AMR genes and plasmid types, and (*iii*) association of phenotypic expression and AMR genes profile.

Material & Methods

A total of 105 non-repetitive isolates including *E. coli* (n=30), *K. pneumoniae* (n=48, including 7 pan-drug susceptible isolates), *Acinetobacter baumannii* (n=8), *Pseudomonas aeruginosa* (n=1) and *Staphylococcus aureus* (n=18) from blood cultures were included in this study. The isolates were received from November 2015 to October 2017 at the department of Clinical Microbiology, Christian Medical College, Vellore, India. Identification of pathogens up to species level was done by using standard microbiological methods[14].

Antimicrobial susceptibility testing (AST): Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI)[15], using cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), piperacillin/tazobactam (100/10 µg), cefoperazone/sulbactam (75/30 µg), gentamicin (10 µg), amikacin (30 µg), netilmicin (30 µg), ciprofloxacin (5 µg), imipenem (10 µg), meropenem (10 µg), minocycline (30 µg) and tigecycline (15 µg) for *E. coli* and *K. pneumoniae*. In addition, aztreonam (30 µg), levofloxacin (5 µg) and tobramycin (10 µg) were tested for *P. aeruginosa* and *A. baumannii*. For *S. aureus*, the following antibiotics were tested: Cefoxitin (30 µg), gentamicin (10 µg), erythromycin (15 µg), clindamycin (2 µg), netilmicin (30 µg), trimethoprim-sulphamethoxazole (1.25/23.75 µg), rifampicin (5 µg) and linezolid

(30 µg). Susceptibility to colistin and vancomycin was determined by using the broth microdilution method (BMD), according to the CLSI guidelines[15]. *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 29213 were used as the quality control strains.

Whole genome sequencing: Genomic DNA of the isolates was extracted with QIAamp DNA mini kit (Qiagen, Hilden, Germany). Whole genome sequencing was performed using Ion Torrent (PGM) sequencer with 400 bp read chemistry (Life Technologies, CA, USA) according to manufacturer's instructions. Assembly of the data was performed *de novo* using AssemblerSPAdes v5.0.0.0 embedded in Torrent suite server version 5.0.3 (Life Technologies). The sequence annotation was done using PATRIC, the bacterial bioinformatics database, and analysis resource[16] and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (*http://www.ncbi.nlm.nih.gov/genomes/static/Pipeline.html*). ST was analyzed by MLST 2.0 tool (*https://cge.cbs.dtu.dk//services/MLST/*). Plasmid replicon identification was performed using PlasmidFinder 1.3 (*https://cge.cbs.dtu.dk//services/PlasmidFinder/*) and plasmid typing was done in pMLST 1.4 (*https://cge.cbs.dtu.dk/services/pMLST/*). Further downstream analysis was performed using Center for Genomic Epidemiology (CGE) server (*http://www.cbs.dtu.dk/services*), Rapid Annotation and Subsystem Technology (RAST) server v2.0 (*http://rast.theseed.org/FIG/rast.cgi*) and Pathosystems Resource Integration Center (PATRIC) v3.5.36 (*https://www.patricbrc.org/*). Resistance genes profile was analyzed using ResFinder 3.1 from the CGE server (*https://cge.cbs.dtu.dk//services/ResFinder/*). The genome and plasmid sequences were also screened for AMR genes in Antibiotic Resistance Genes Database (ARDB) and Comprehensive Antibiotic Resistance Database (CARD) through PATRIC. Plasmid groups were identified based on *inc* typing in Gram-negative pathogen and replicon (*rep*) typing in *S. aureus*. These whole genome sequences were deposited at GenBank.

Results

Among *E. coli*, two of the 30 isolates were identified as multidrug-resistant (MDR). Majority of the tested isolates were extreme drug resistant (XDR), which includes *E. coli* (n=30), *K. pneumoniae* (n=41), *P. aeruginosa* (n=1) and *A. baumannii* (n=8). In addition, seven *K. pneumoniae* were pan drug-susceptible, which were used as an internal control for plasmid analysis. Overall, nineteen *K. pneumonaie* and all *A. baumannii* isolates were resistant to colistin by BMD. All *S. aureus* (n=18) were identified as methicillin resistant *S. aureus* (MRSA), and all were susceptible to vancomycin and linezolid.

Genome analysis: Assembly of the raw reads of the isolates showed 70 average contigs (≥500 bp), with an average of 50X coverage. Each pathogen was identified with the unique STs, except in *E. coli* (n=3). ResFinder analysis showed different combination of AMR genes in each pathogen. All these isolates carrying AMR genes specifically coding for different classes of antibiotic were confirmed from ARDB and CARD. All the genome data were submitted in GenBank database.

Plasmid analysis: Plasmids in Gram-negative (*E. coli* and *K. pneumoniae*) pathogens and *S. aureus* were identified by using *inc* and *rep*, respectively. The analysis of sequenced genome for plasmid showed the presence of single or combination of plasmids. The distribution of plasmid was highly heterogeneous and ST-specific plasmids were not observed. *E. coli* and *K. pneumoniae* isolates had 133 and 113 plasmid types respectively, where both had only 15 STs. In *S. aureus*, seven plasmid types were seen with 10 STs.

Majority of the *E. coli* isolates were found with IncFIA, IncFII, IncFIB, Col(BS512), IncL1, IncX3 and IncH plasmids (Fig. 1). While in *K. pneumoniae*, IncHI1B(pNDM-MAR), IncFII(pRSB107), IncFIB(Mar), IncFIB(pQil), IncFIA, IncFII(K), IncR, ColKP3, ColpVC were the most common plasmids. Overall, IncF (56.5%) was the predominant plasmid followed 18.3 per cent of Col and 5.3 per cent of IncX type plasmids. In addition, the frequency of other plasmid groups was found as <5 per cent among the tested isolates (Fig. 2). However, database for the screening of Inc plasmids in *P. aeruginosa* and *A. baumannii* using whole genome sequence was not available.

In most of the tested isolates, the presence of AMR genes correlated with phenotypic resistance pattern of tested antimicrobials. However, the level of agreement between the phenotypic and genotypic AMR positivity differed from species to species (Figs 3 and 4). The distribution and frequency of plasmids carrying different AMR genes in *E. coli* and *K. pneumoniae* are given in Figs. 5 and 6, respectively. All colistin resistant *K. pneumoniae* (n=19) isolates were negative for plasmid-mediated *mcr* gene coding for colistin

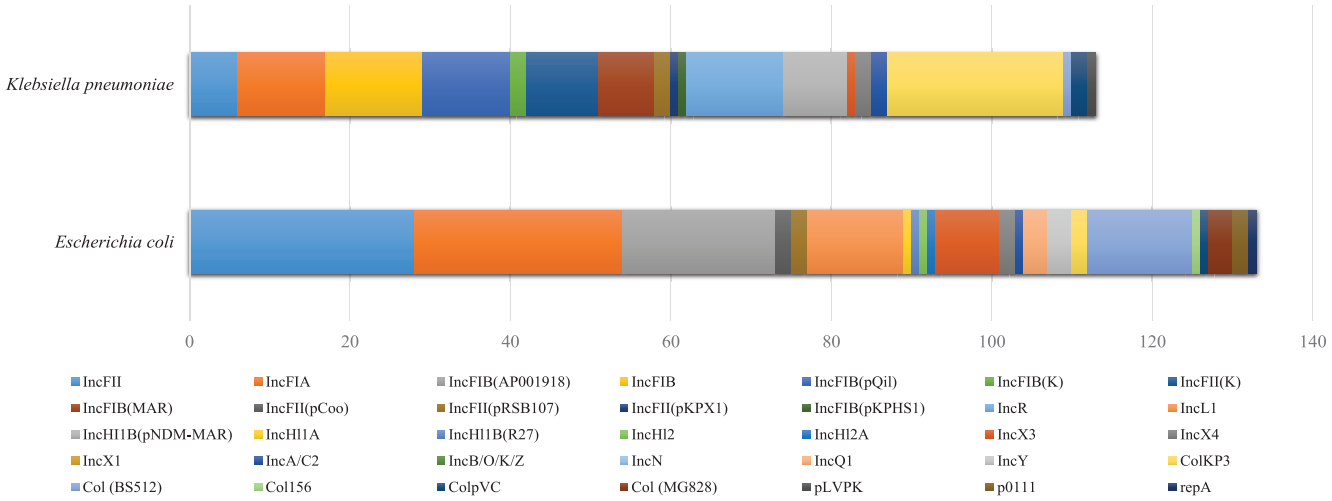

Fig. 1. Species-wise distribution of plasmids among *Escherichia coli* and *Klebsiella pneumoniae.*

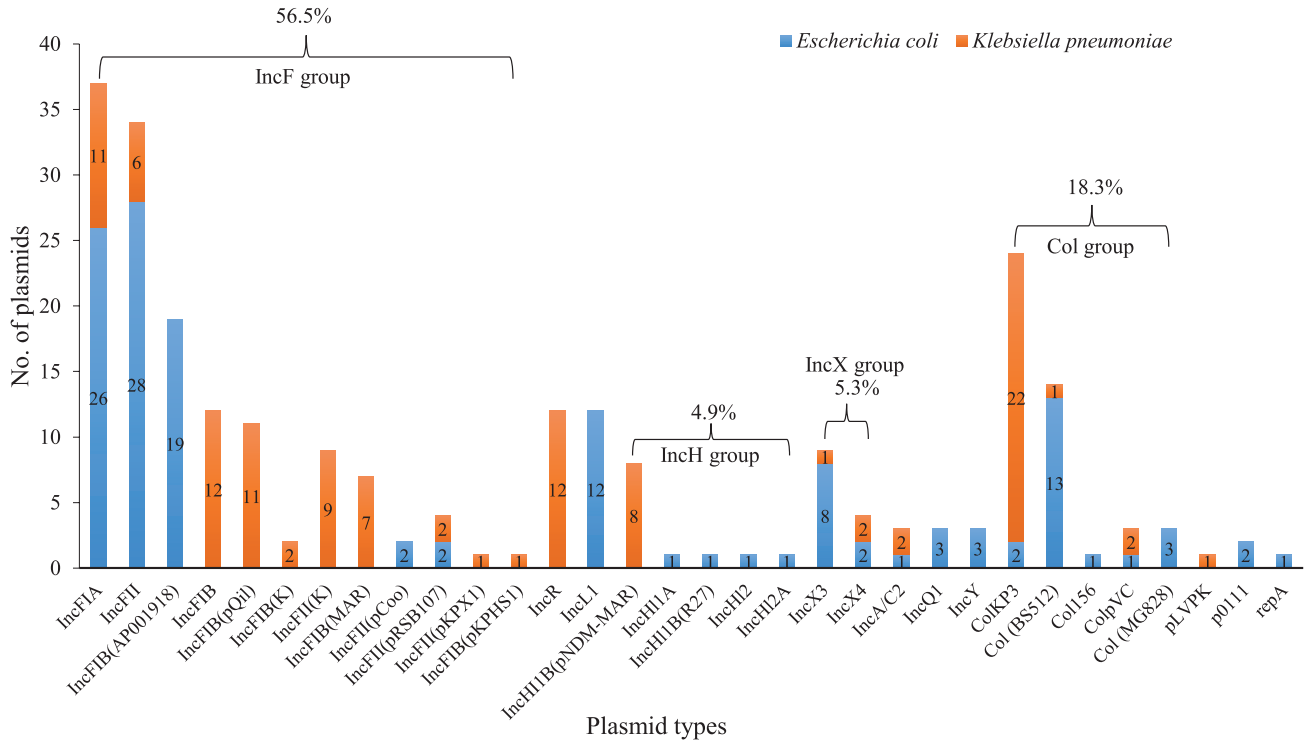

Fig. 2. Distribution of plasmid replicon types identified among *Escherichia coli* and *Klebsiella pneumoniae* (n=246).

resistance. Further, the genome of pan-drug susceptible *K. pneumoniae* (n=7) was found with IncHI1B(pNDM-MAR) plasmid.

In *E. coli*, β-lactamases encoding genes were predominant followed by aminoglycoside, trimethoprim, sulphonamide and macrolide resistance genes. Phenicol and tetracycline resistance genes were also seen. All these AMR genes were observed to carry on IncFII, IncFIA, IncFIB and Col(BS512) plasmids, while a similar observation was not seen with other plasmid types. Among β-lactamases, $bla_{CTX\text{-}M\text{-}15}$ was common followed by $bla_{NDM\text{-}5}$, $bla_{TEM\text{-}1B}$ and $bla_{OXA\text{-}1}$ in IncF plasmids. *sul*1 gene for sulphonamide resistance was common than *sul*2 gene; *tet*A was frequently seen

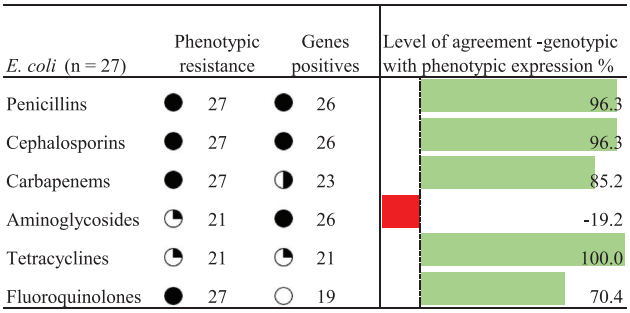

Fig. 3. Level of agreement between antimicrobial resistance genes and phenotypic resistance for *Escherichia coli*; genes present with no antimicrobial resistance correspond to non-expression of antimicrobial resistance genes. Full black circles represent total agreement between phenotype and genotype. Half-black circles represent lesser antimicrobial resistance genes than the phenotype. Empty circles represent even lesser antimicrobial resistance genes. Quarter black circle in aminoglycosides represents about 20 per cent non-expression of antimicrobial resistance genes, and quarter black circles in tetracyclines represent lesser genotype and phenotype compared to total numbers.

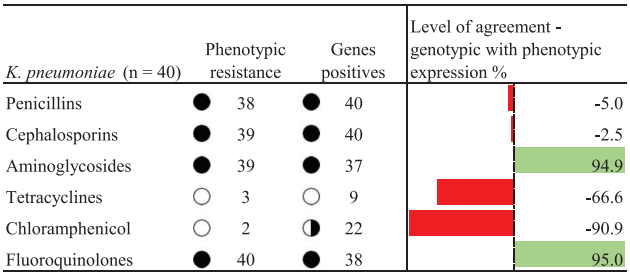

Fig. 4. Level of agreement of antimicrobial resistance genes and phenotypic resistance for *Klebsiella pneumoniae*; genes present with no antimicrobial resistance correspond to non-expression of antimicrobial resistance genes. Full circles in penicillins, cephalosporins, aminoglycosides and fluoroquinolones represent total agreement between phenotype and genotype. Half circle in chloramphenicol represents less number of gene positives among *K. pneumoniae*. Empty circles in tetracyclines and chloramphenicol represent less antimicrobial resistance genes and phenotypic resistance in *K. pneumoniae*.

with IncFII plasmids, whereas *tet*B was noticed with IncFIB plasmids.

ColKP3 plasmid type was prevalent among *K. pneumoniae* and harboured almost all classes of AMR genes. This was followed by IncHI1B and IncF group plasmids [IncFII(K), IncFIA, IncFIB, IncFIB(pQil)] and IncR. Among β-lactamases, $bla_{\text{CTX-M-15}}$ was most prevalent followed by $bla_{\text{OXA-232}}$ and $bla_{\text{TEM-1B}}$. Further, $bla_{\text{NDM-1}}$ was common than $bla_{\text{NDM-5}}$ and $bla_{\text{NDM-7}}$. Rare genes such as $bla_{\text{LEN-12}}$ (n=1), bla_{OKPB2} (n=1), $bla_{\text{TEM-124}}$ (n=1), *qep*A (n=1) and *qnr*B66 (n=4) were also seen in this study among *K. pneumoniae*.

Of the tested *S. aureus* (n=18), *rep* gene was not found in five isolates. Among the 14 *S. aureus* isolates, a total of six *rep* families (*rep5, rep7, rep10, rep16, rep*US12, *rep*21) were assigned. Based on the combination of *rep* genes identified, seven different plasmid groups were identified in *S. aureus*. Each plasmid had a unique combination of *rep* gene sequences as follows: pMW2 (*rep*5), pSAS1 (*rep*7), pDLK1 (*rep*10), pUB110 (*rep*US12) and Saa6159 (*rep*16). The plasmids pKH12 and pSA1308 were found with same *rep* gene (*rep*21). This shows a recombination between pKH12 and pSA1308 plasmids.

Discussion

IncF plasmids were the commonest plasmids seen among *E. coli* and *K. pneumoniae*. IncF plasmids were reported from several countries including 49 per cent in Tanzania, 71 per cent in Germany and 45 per cent in Switzerland[17]. In Japan, the IncFIB (18.5%) was most commonly reported in *E. coli* followed by IncF (17.6%) and IncFIA (7.6%)[18-20]. In this study, majority of the isolates were identified with IncF type followed by Col group. Other types seen included IncX, IncH, IncR, IncL, IncA/C, IncY and IncQ plasmids.

The IncF plasmids were reported to carry varied and increased number of AMR genes than other plasmid types[19]. This includes clinically relevant ESBLs, such as $bla_{\text{CTX-M-15}}$, plasmid-mediated AmpC genes (bla_{CMY} and bla_{DHA}), $bla_{\text{TEM-1}}$ and $bla_{\text{OXA-1}}$. In addition, IncF group plasmids were known to frequently harbour aminoglycoside and quinolone resistance genes *qep*A, *arm*A, *rmt*B, *aac(6')-Ib-cr* and *qnr*[17].

In this study, IncFII, IncFIA and IncFIB type plasmids seen among *E. coli* harboured high number of $bla_{\text{CTX-M-15}}$, $bla_{\text{NDM-5}}$, $bla_{\text{TEM-1}}$ and $bla_{\text{OXA-1}}$ β-lactamase genes followed by *aac(6')-Ib-cr*, *aad* and *rmtB* aminoglycoside resistance genes, *aac(6')-Ib-cr* and *qnr* for quinolones resistance, *tet*A and *tet*B for tetracycline resistance, *cat*B4 and *cat*A1 for chloramphenicol resistance, and *dfr*A and *sul* genes for trimethoprim and sulphamethoxazole resistance. In *K. pneumoniae*, IncFII, IncFIA and IncFIB plasmids harboured $bla_{\text{CTX-M-15}}$, $bla_{\text{NDM-5}}$, $bla_{\text{NDM-1}}$, $bla_{\text{TEM-1}}$, $bla_{\text{OXA-232}}$, $bla_{\text{OXA-181}}$ and $bla_{\text{OXA-1}}$ followed by *aac(6')-Ib-cr*, *aad, arm*A, *rmt*F, *rmtB, qnr*B1, *qnr*B66, *tet*A, *tet*B, *tet*D, *cat*A1, *cat*B3, *dfr*A *and sul genes.* The screening of $bla_{\text{NDM-5}}$ may be necessary in Indian settings, as this variant is found higher than $bla_{\text{NDM-1}}$ among *E. coli*. Among *K. pneumoniae*, $bla_{\text{NDM-5}}$ and $bla_{\text{NDM-7}}$ were seen in fewer isolates.

The common plasmid of IncHI1B type is pNDM-MAR. These are reported to carry the $bla_{\text{CTX-M-15}}$,

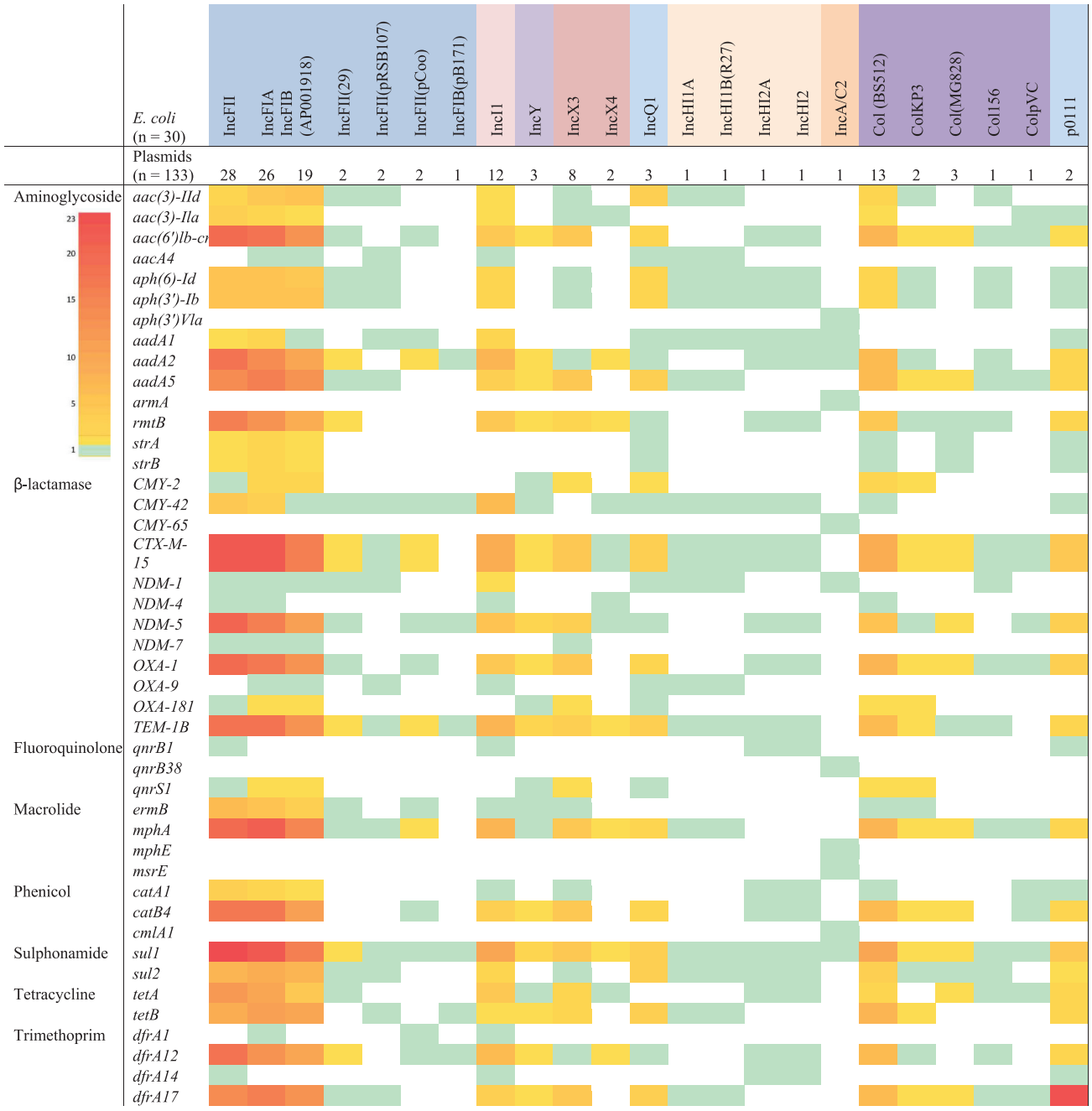

Fig. 5. Heatmap showing association frequency of antimicrobial resistance genes and plasmids in *Escherichia coli*. IncFII, IncFIA and IncFIB (AP001918) had highest association with the antimicrobial resistance genes in comparison to other plasmid types. The grading numbers in color strip depicts the number of genes associated with the particular plasmid type.

bla_{NDM} and quinolone-resistant determinant *qnr*B1[20]. In this study, *E. coli* harboured only one IncHI1B(R27) plasmid with bla_{NDM-1} and $bla_{CTX-M-15}$. While in *K. pneumoniae*, eight isolates (including 7 pan-drug susceptible) (<5%) harboured IncHI1B(pNDM-MAR) plasmids with only one $bla_{CTX-M-15}$. This highlights the risk of acquiring carbapenem-resistant gene (bla_{NDM}) among pan-drug susceptible *K. pneumoniae*. Non-pNDM-MAR IncHI1B plasmids harboured $bla_{CTX-M-15}$, bla_{NDM-1}, *qnr*B1 and *qnr*B66. Plasmids reported to carry *mcr-1* gene including IncFI and IncFIB were seen in this study. The presence of these plasmids in *E. coli* and *K. pneumoniae* may promote the acquisition of *mcr* gene and increase the threat of dissemination[21].

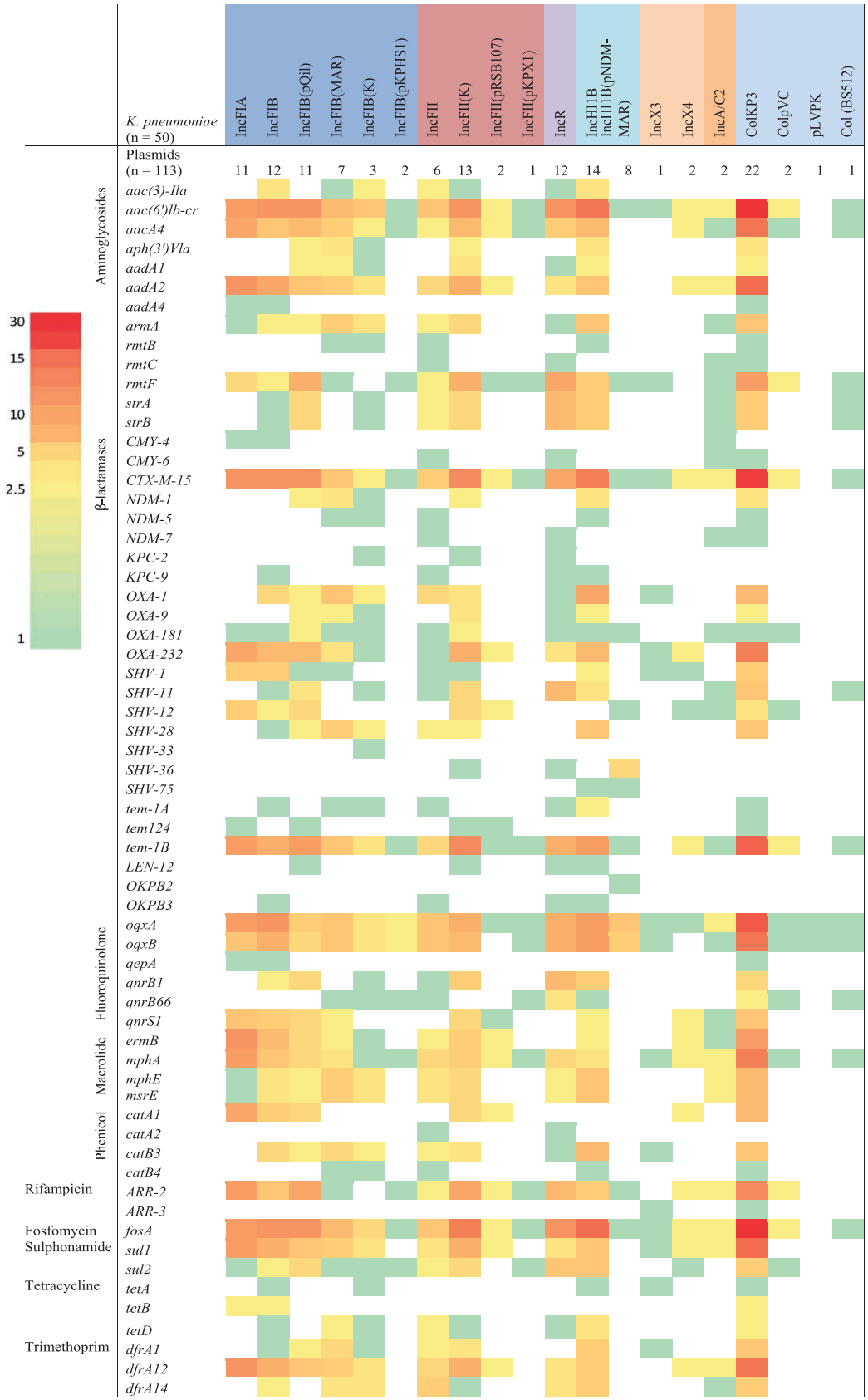

Fig. 6. Heatmap showing association frequency of antimicrobial resistance genes and plasmids in *Klebsiella pneumoniae*. ColKP3 followed by IncFII(K), IncHI1B and IncFIA had high association with antimicrobial resistance genes in comparison to other plasmid types. The grading numbers in color strip depicts the number of genes associated with the particular plasmid type.

Replicon groups IncA/C and Incl1 were frequently seen with *Enterobacteriaceae* and harboured multiple resistance genes including resistance for extended-spectrum cephalosporins (bla_{CMY}) and carbapenems (bla_{NDM-1})[17]. In this study, Incl1 was identified nine per cent in *E. coli* with resistance genes to β-lactams, aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and sulphonamides, while in *K. pneumoniae* none were seen. ColKP3 was reported as the most common plasmid type seen among *K. pneumoniae* and known to harbour AMR genes. In the present study, 19.5 per cent ColKP3 was found, and almost all had AMR genes for all classes of antibiotics.

Plasmids observed among Gram-positives are mostly common between *S. aureus* and *Enterococcus* spp. In *S. aureus*, the β-lactamase gene (bla_Z) was encoded by pMW2 and pSAS1 plasmid, and both the plasmids belong to the pMW2 like plasmid group[22]. The present study showed pMW2 (*rep*5) plasmid carrying bla_Z among MRSA. The plasmid pUB110 was reported with *ant* (4')-1 which encodes for kanamycin and tobramycin resistance gene[23]. The pDLK1 plasmid had *erm*C gene encoding for erythromycin resistance[24], and the *qac*C gene seen in pSA1308 plasmid encodes for multi-drug efflux pump encoding[25]. However, in the present study, pUB110 and pSA1308 were seen in a few MRSA isolates.

In this study, the plasmid types identified among the specified pathogens and AMR genes corroborated well with the available literature worldwide[26]. However, the plasmid replicon types identified among the study isolates were diverse than the STs. This might be due to the presence of multiple replicon types in the same organism harbouring plasmid[27]. The number of STs for each organism is higher than the number of plasmid types available for that organism. However, for tracking the AMR genes and to identify a plasmid outbreak in a hospital situation, plasmid typing is essential in a local setting.

Although most of the isolates harbour AMR genes, expression percentage differs widely. The non-expression of a few genes might be due to various factors such as mutations in the protein coding region, insertion elements in the gene promoter region, and within-gene recombination[28]. In this study among *E. coli*, >70 per cent association was observed between AMR genes and phenotypic expression, except for aminoglycosides, where 19 per cent were non-expressive.

In *K. pneumoniae*, among penicillins and cephalosporins, very few isolates possessed non-expressive genes (5 and 2.5%). Aminoglycosides and fluoroquinolones did not possess any non-expressive genes, whereas, tetracyclines and chloramphenicol had high non-expressive genes (66.6 and 90%). Similar case for tetracycline was observed by Gow *et al*[28]. Aminoglycosides and fluoroquinolones showed agreement (95%) between phenotypic expression and genotypic AMR findings. Plasmid-mediated genes for colistin resistance were not seen in any of the study isolates. Presence or absence of a genotype does not specify the isolate as resistance or susceptible. The mechanism behind the AMR is numerous and complex[28]. Hence, a phenotypic test is necessary from a diagnostic point of view for confirmation of the genotype. Studying the plasmid prevalence among *Enterobacter, Enterococci, Pseudomonas* and *Acinetobacter* would give a complete understanding of plasmid prevalence and dynamics of AMR dissemination among ESKAPE pathogens. This acts as a limitation to the present study.

Results from this pilot study may act as a baseline data for plasmid among *E. coli* and *K. pneumoniae* in India. Only isolated cases of plasmids have been reported previously by our group from India[29-31]. These findings substantiate that occurrence of a few common plasmid types among the hospital pathogens may lead to a considerable amount of horizontal gene exchange.

In conclusion, plasmids analysis revealed that among β-lactamases, $bla_{CTX-M-15}$ was present both in *E. coli* and *K. pneumoniae*, followed by bla_{TEM-1B}. In addition, bla_{NDM-5} and bla_{OXA-1} were also seen in *E. coli*, whereas bla_{NDM-1} and $bla_{OXA-232}$ were common among *K. pneumoniae*. The study also showed the relation between phenotypic and genotypic expression of AMR for various classes. IncFII plasmid was observed in *E. coli*, while, ColKP3 followed by IncFII(K) was present in *K. pneumoniae*. Understanding on such vectors carrying the AMR genes could help in improving strategies on better control of AMR dissemination.

***Financial support & sponsorship*:** The authors acknowledge the Indian Council of Medical Research, New Delhi, for providing the grant for this research (Ref. No: AMR/TF/55/13ECDII dated 23/10/2013) and Fluid Research Grant of Christian Medical College, Vellore (IRB min. no. 9616 dated 01.09.2015).

***Conflicts of Interest*:** None.

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