Indian Journal of Experimental Biology
Vol. 62, August 2024, pp. 670-677
DOI: 10.56042/ijeb.v62i08.5306

Incidence of potential β-lactam resistance genes and related mobile genetic elements in uropathogenic *Escherichia coli* from pregnant women from Kolkata

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Received 01 September 2023; *Revised 17 January 2024*

Uropathogenic *Escherichia coli* (UPEC) infection is very common in pregnancy. Antimicrobial resistance (AMR) in UPEC especially against β-lactams, limits treatment options. In this study prevalence of β-lactam resistance (BLR) and associated genetic determinants was investigated in UPEC collected from pregnant women to delineate the underlying cause of AMR and thus design efficacious and safe therapeutics during pregnancy. All the UPEC isolates exhibited the highest resistance against ampicillin (100%). Phenotypically confirmed ESBL, BLIR and carbapenemase producing isolates were 63.64, 36.33 and 33.33%, respectively. Molecular studies showed co-occurrence of β-lactamase genes; $bla_{\text{OXA-I}}$, $bla_{\text{OXA-II}}$, $bla_{\text{OXA-III}}$, bla_{TEM}, bla_{CTXM}, bla_{NDM}, $bla_{\text{OXA-48}}$ in different combinations with significant (P <0.05) occurrence of bla_{TEM} and bla_{TEM}, bla_{CTXM} combination in multiple-replicon plasmids with predominance of IncFrepB and IncF1B, followed by IncX. Heatmaps showed that the UPECs belonged to two discrete clusters with respect to the presence and absence of bla_{TEM}. UPEC isolates with bla_{TEM} exhibited the highest occurrence of different combinations of integrons (int*I1*, int*I2*) and insertion elements (IS*5*, IS*Ecp1*, IS*26*), although their presence was statistically significant (P <0.05) in bla_{TEM} negative isolates. Therefore, this is the first report from India, that demonstrated co-occurrence of potential β-lactamase genes and associated mobile genetic elements in UPEC from pregnant females and demands a necessity of comprehensive surveillance to formulate appropriate therapeutics to protect both maternal and fetal health.

Keywords: Antimicrobial resistance, Insertion elements, Integrons, Multidrug resistance, Plasmid replicon typing, Urinary tract infections (UTIs)

Urinary tract infections (UTIs) caused by *Escherichia coli* remain one of the most frequent medical complications in pregnancy[1,2]. Therapeutic guidelines for UTI during pregnancy are available globally that assist clinicians in prescribing suitable antibiotics to each patient accounting for maternal and fetal safety. Empiric treatment in UTI was reported as a common prevalence in non-pregnant population which caused the alarming emergence of antimicrobial resistance (AMR) amongst uropathogens, especially uropathogenic *E. coli* (UPEC). The unavailability of suitable drugs to eradicate these resistant and multidrug-resistant (MDR) microbes complicated the overall clinical management[3]. Carbapenems were considered the last resource antibiotic to treat MDR infections, but their use was restricted in UTI treatment during pregnancy. However, pregnant women are at risk of being infected with these circulating MDR-UPEC strains which might generate further complications in UTI treatment. Acquisition of several resistance genes conferred AMR in UPEC, most frequently variants of extended-spectrum β-lactamases (ESBLs) such as bla_{TEM}, bla_{OXA}, and $bla_{\text{CTX-M}}$ which imparted resistance to broad-spectrum penicillin, cephalosporins, and monobactams[4,5]. Earlier studies classified β-lactam resistant UPECs as ESBL or carbapenemase producers according to the enzyme-substrate[6]. NDM and OXA-48 were the most frequent carbapenemases detected in this pathogen[7]. The mobile genetic elements (MGEs), especially those encompassed plasmids of different incompatibility (*Inc*) groups, that encoded antibiotic-resistant genes (ARGs) including β-lactam resistance, together with the insertion elements, transposons and integrons play a pivotal role in the accretion and transmission of the resistant determinants along with ARGs in varied pathogens including UPEC[8,9].

There are several studies on the occurrence and transmission of ESBL and carbapenemase in UPEC isolated from non-pregnant populations across the globe[7,10]. However, there is a scarcity of reports on the molecular determinants of β-lactam resistance and their dissemination in UPEC isolated from pregnant

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populations, both worldwide and nationwide. Therefore, in this study, we have made an attempt to detect β-lactam resistance, identify the related genes and the associated MGEs in UPEC isolated from pregnant women from Kolkata, the eastern region of India.

Materials and Methods

Bacterial isolates

A total of 126 urine samples were collected from pregnant women admitted to a tertiary care hospital in Kolkata, India. Culture-positive isolates were tested biochemically for the presence of *E. coli* and maintained in Luria Bertani (LB) broth for further investigation. Ethical approval was obtained from the Institutional Clinical Research Ethics Committee (CREC-STM/2020-AG-13) dated 19.12.2020.

Antibiotic sensitivity and phenotype confirmatory test

The Kirby-Bauer disc diffusion method was used to determine the response of the isolates to various antibiotics, including ampicillin (AMP, 10 mcg), ceftazidime (CAZ, 30 mcg), cefotaxime (CTX, 30 mcg), imipenem (IPM, 30 mcg), meropenem (MRP, 10 mcg) and ertapenem (ETR, 10 mcg) (HiMedia, Mumbai, India) following Clinical Laboratory Standards Institute guidelines to determine the zone of inhibition. *E. coli* ATCC 25922 was used as a control. Isolates that were found to be resistant to either ceftazidime and/or cefotaxime, were subjected to an ESBL phenotype confirmatory test using a β-lactam-β-lactamase inhibitor combination[11]. Carbapenemase production was detected phenotypically using imipenem – EDTA combined disc synergy test, where carbapenemase positivity was defined as a 7 mm increase in the inhibition zone of the disc containing imipenem – EDTA relative to the disc with imipenem alone[12].

β-lactam resistant genes and mobile genetic elements identification

Plasmid DNA was prepared[13] from the UPEC isolates and used as template to identify the β-lactamase genes (oxacillinase genes; $bla_{\text{OXA-I}}$, $bla_{\text{OXA-II}}$, $bla_{\text{OXA-III}}$, ESBL genes; bla_{TEM}, bla_{CTXM}, carbapenemase genes; bla_{NDM} and $bla_{\text{OXA-48}}$), by PCR using the gene-specific primers[11,14]. The plasmid incompatibility types ($\text{IncF}_{\text{repB}}$, N_{rep}, X, W, FII_{S}, A/C, L/M, Y, I1, HI1, F1B) and associated integrons; class1 (int*I1*), class2 (int*I2*) and insertion elements; IS*5*, IS*Ecp1*, IS*26* were also detected by PCR using the respective gene-specific primers[8,15].

Statistical analysis

Cluster analysis was performed on Heatmaps generated using heatmap.2 function in the "gplots" library (version 3.1.3) in the R software package (Version 4.2.0) [R Core Team and the R Foundation for Statistical Computing]. Statistical significance was calculated using one sample t-test in the Prism software package (GraphPad Prism Version 8.0, La Jolla, California, USA)[15].

Result

Bacterial culture and β-lactam sensitivity

Culture positivity was observed in 50 out of 126 urine samples collected from pregnant women with symptoms of urinary tract infection. *E. coli* was detected in 36 out of the 50 (72%) culture-positive samples. All the *E. coli* isolates (100%) were resistant to ampicillin. Significant number of isolates exhibited resistance to the third generation of cephalosporins (61.11%, $P<0.05$) and carbapenems (66.67%, $P<0.05$) (Fig. 1). Extended-spectrum β-lactamase (ESBL) phenotype was detected in 14 (63.64%) and β-lactamase inhibitor-resistant (BLIR) phenotype in 8 (36.33%) out of the 22 *E. coli* isolates resistant to third generation of cephalosporins respectively. A phenotypic confirmatory test for carbapenemase production was detected in 8 (33.33%) of the 24 *E. coli* isolates resistant against at least one carbapenem antibiotic.

Prevalence and distribution of β-lactamase genes

Co-occurrence of β-lactamase genes; $bla_{\text{OXA-I}}$, $bla_{\text{OXA-II}}$, $bla_{\text{OXA-III}}$, bla_{TEM}, $bla_{\text{CTX-M}}$, bla_{NDM}, $bla_{\text{OXA-48}}$ in various combinations were observed amongst 17 out of the 36 *E. coli* isolates along with presence of $bla_{\text{OXA-I}}$, $bla_{\text{OXA-II}}$ and bla_{TEM} only in 2, 3 and 6 isolates, respectively. The remaining 8 isolates resistant to at least 1 β-lactam antibiotic revealed the absence of these respective β-lactamase encoding genes. Amongst all the β-lactamase genes, the prevalence of bla_{TEM} (22/36) was predominant. *E. coli* isolates those harbored bla_{TEM} either alone ($P<0.001$) or in combination with $bla_{\text{CTX-M}}$ ($P<0.001$) formed a discrete cluster (Cluster I) with respect to the distribution of the other β-lactamases (Cluster II). Additionally, two distinct clusters (A and B) with multiple sub-clusters were observed with respect to the absence of bla_{TEM} and its presence in combination with the other β-lactamases (Fig. 2).

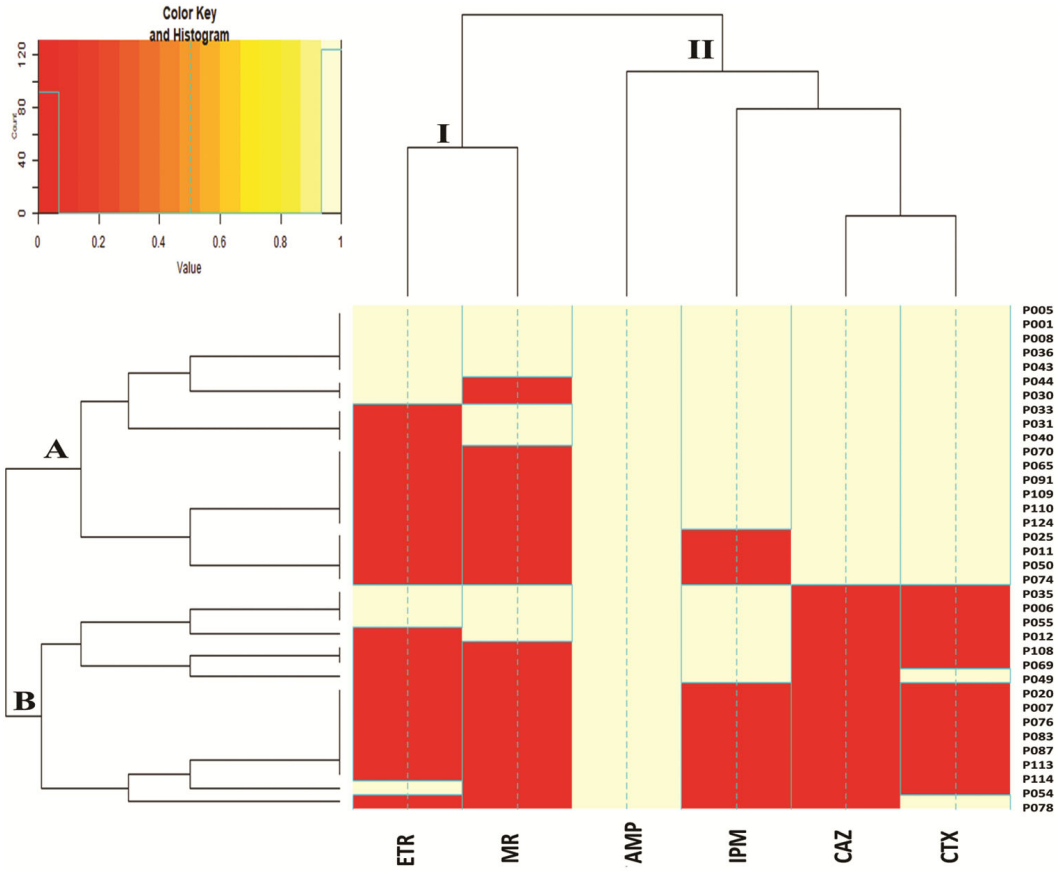

Fig. 1 — Heatmap representation of the distribution of β-lactam resistance in the UPEC isolates collected from pregnant women. The clusters analysis was based on resistance against ampicillin (AMP), third-generation cephalosporins; ceftazidime (CAZ), cefotaxime (CTX), and carbapenems; imipenem (IPM), meropenem (MRP) and ertapenem (ETR). The numbers on the right side indicate sample ID of each isolate. Colour key represents the variation in colours from red to white, illustrating the susceptibility towards a specific antibiotic to its resistance.

Distribution of plasmid replicon types and mobile genetic elements

Prevalence of multiple replicon plasmids were observed amongst the *E. coli* isolates. Plasmid replicon type IncF (FrepB, FIB) was most predominant followed by IncX type. Multiple replicon plasmids with varied combinations of 15 and 11 *Inc* types were observed in the bla_{TEM} positive and negative isolates respectively (Fig. 3). The distribution of multiple replicon plasmids in the two batches of isolates was statistically significant irrespective of the presence and absence of bla_{TEM} (P <0.01). Furthermore, the distribution of MGEs was also discrete in the two batches of *E. coli* isolates with and without the bla_{TEM} gene (Fig. 4). Nevertheless, the distribution of MGEs in the latter was only statistically significant (P <0.01).

Discussion

UTIs caused by *E. coli* pose a common complication in pregnancy. Our study reported a high prevalence of *E. coli* (72%) in the urine culture-positive samples collected from pregnant women, like other studies across the globe[3,16]. In recent times, the emergence of MDR *E. coli* in pregnant populations[17-19] was documented leaving limited treatment options for the MDR infection. Additionally, akin to our study, moderate to high ESBL *E. coli* occurrences were reported amongst pregnant women from different parts of the world[18,20,21]. The prevalence of carbapenemase-producing *Enterobacteriaceae* (CPE), predominantly *E. coli* was documented in mothers and newborn that primarily resulted from the direct transmission of the bacteria from the mother to her baby during childbirth. Therefore, mothers pose a major risk factor for the transmission of CPE to the neonates[3,22]. In our study co-occurrence of different β-lactamase (oxacillinase, ESBL carbapenemase) genes was observed with the highest prevalence of bla_{TEM} (61%) amongst *E. coli* isolated from pregnant

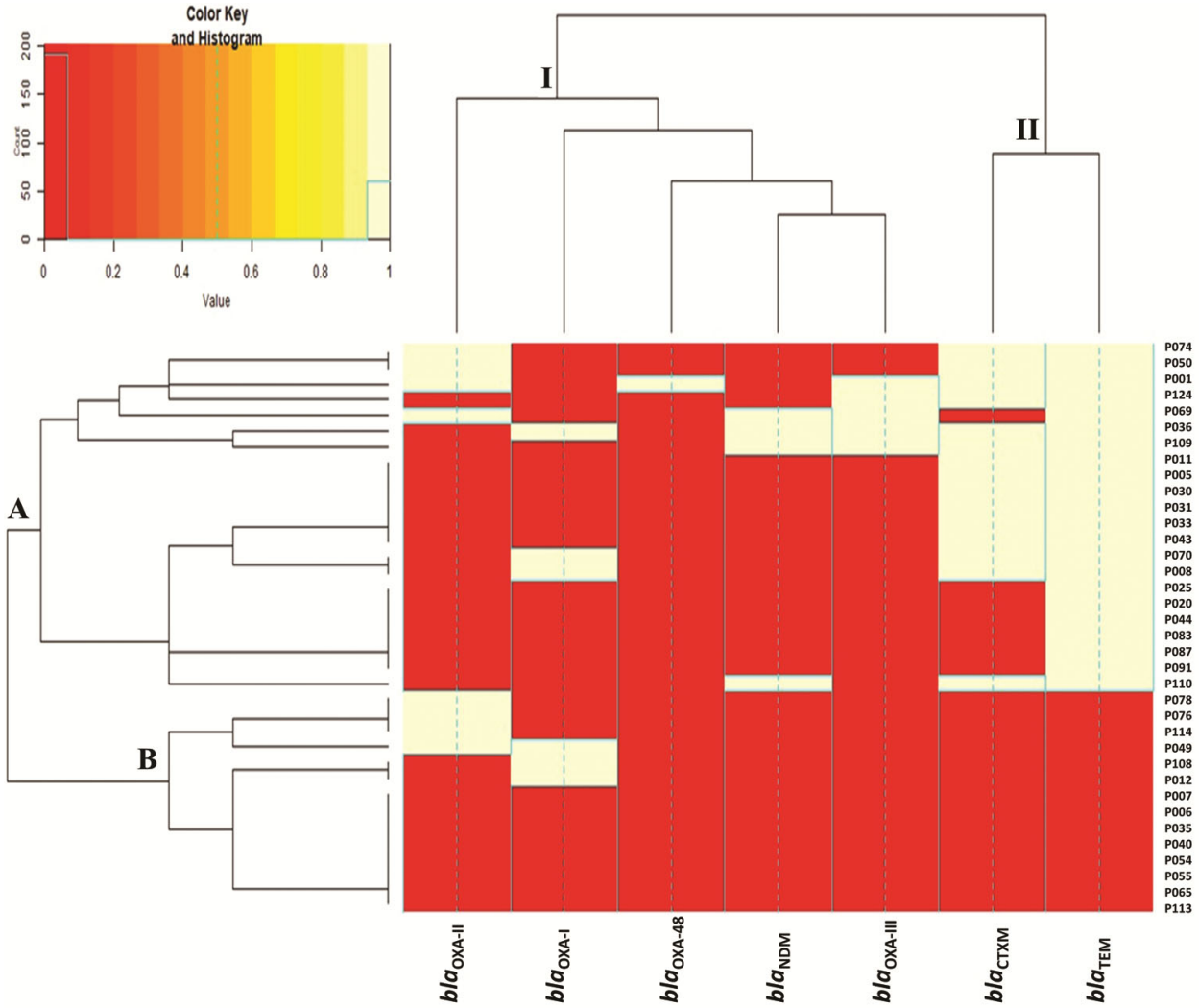

Fig. 2 — Heatmap representation of β-lactamase genes in the UPEC isolates collected from pregnant women. The cluster analysis was based on the presence and absence of bla_{TEM}, bla_{CTXM}, bla_{OXA-I}, bla_{OXA-II}, $bla_{OXA-III}$, bla_{NDM}, bla_{OXA-48} either alone or in varied combinations. The numbers on the right side indicate the sample ID of each isolate. Colour key represents the variation in colours from red to white, illustrating the absence of a particular gene to its presence.

women similar to other recent studies across the globe[18,21]. Nonetheless, few studies also indicated a high prevalence of bla_{CTX-M} amongst pregnant women[20,21]. Moreover, our study also indicated the highest co-occurrence of bla_{TEM} and bla_{CTX-M} akin to a study reported from Lebanon[20].

The role of plasmid in the dissemination of antibiotic-resistant genes (ARGs) by either integrating into the chromosomal DNA, or transmission by conjugation has been demonstrated in non-pregnant population[8,23], but there was a paucity of information regarding the prevalence of MGEs and/or on the prevalence of the *Inc* types of resistant plasmids in uropathogenic *E. coli* (UPEC) isolated from the pregnant population. In our study, we identified the replicon types of the resistant plasmids that harbored the β-lactamase genes (bla_{OXA-I}, bla_{OXA-II}, $bla_{OXA-III}$, bla_{TEM}, bla_{CTX-M}, bla_{NDM}, bla_{OXA-48}) and associated mobile genetic elements (MGEs; int*1*, int*2*, IS*Ecp1*, IS*5*, IS*26*) to gain an insight into the acquisition and risk of dissemination of AMR mediated by these resistant determinants identified in UPECs collected from pregnant women. Our study also showed significant distribution of multiple replicon plasmids, akin to other studies in UPECs from non-pregnant populations[15,24]. The multiple-replicon plasmids were better agents that disseminated resistance genes than non-multiple-replicon plasmids, which resulted in the selection of the antibiotic-resistant pathogen. In this study, a statistically significant distribution of MGEs

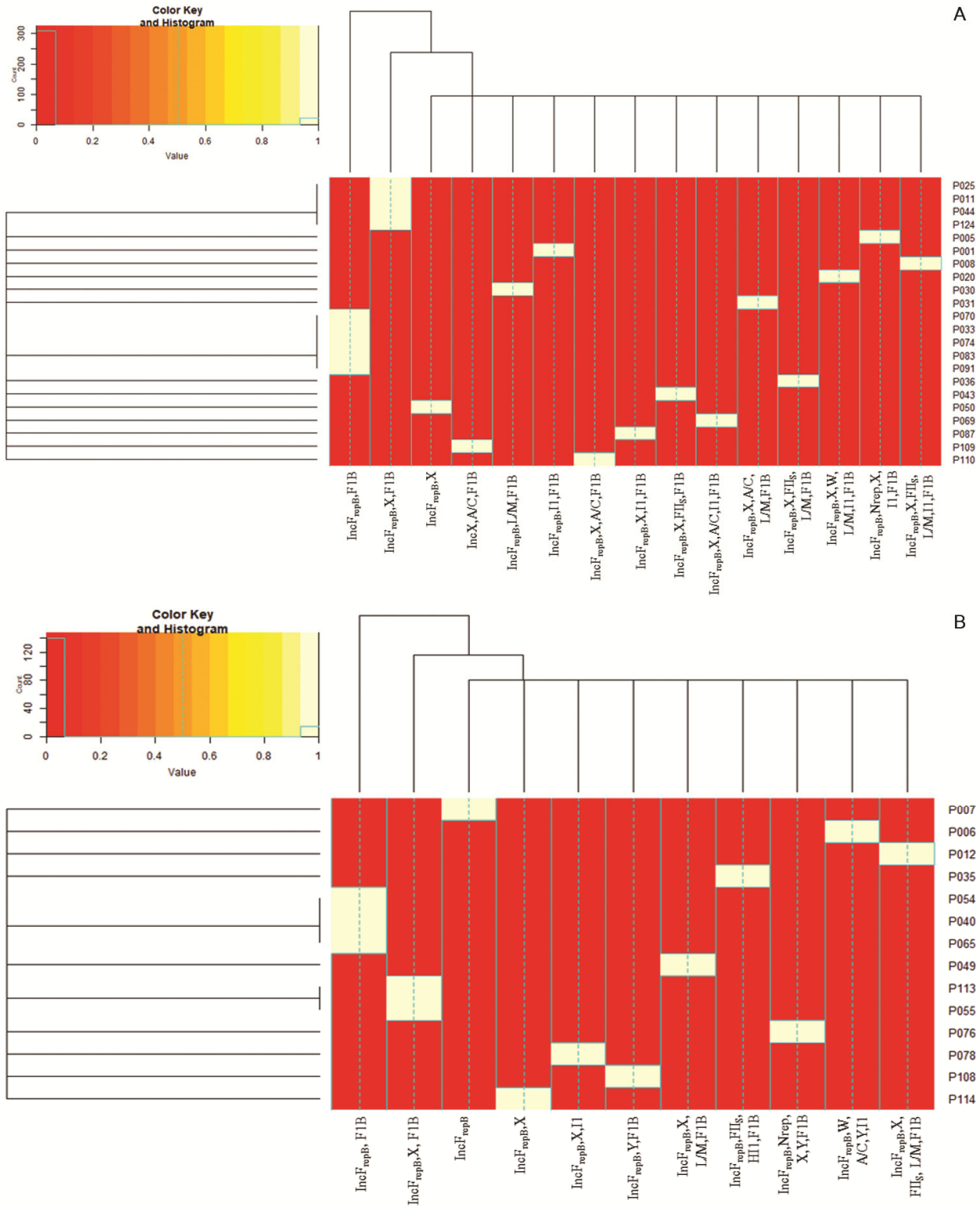

Fig. 3 — Heatmap representation of multiple-replicon plasmids in (A) *bla*$_{TEM}$ positive; and (B) *bla*$_{TEM}$ negative UPEC isolates collected from pregnant women. The numbers on right side indicate the sample ID of each UPEC isolates. The colour key represents the variation in colours from red to white, illustrating the absence of a specific combination of multiple-replicon plasmids to the presence of that combination respectively.

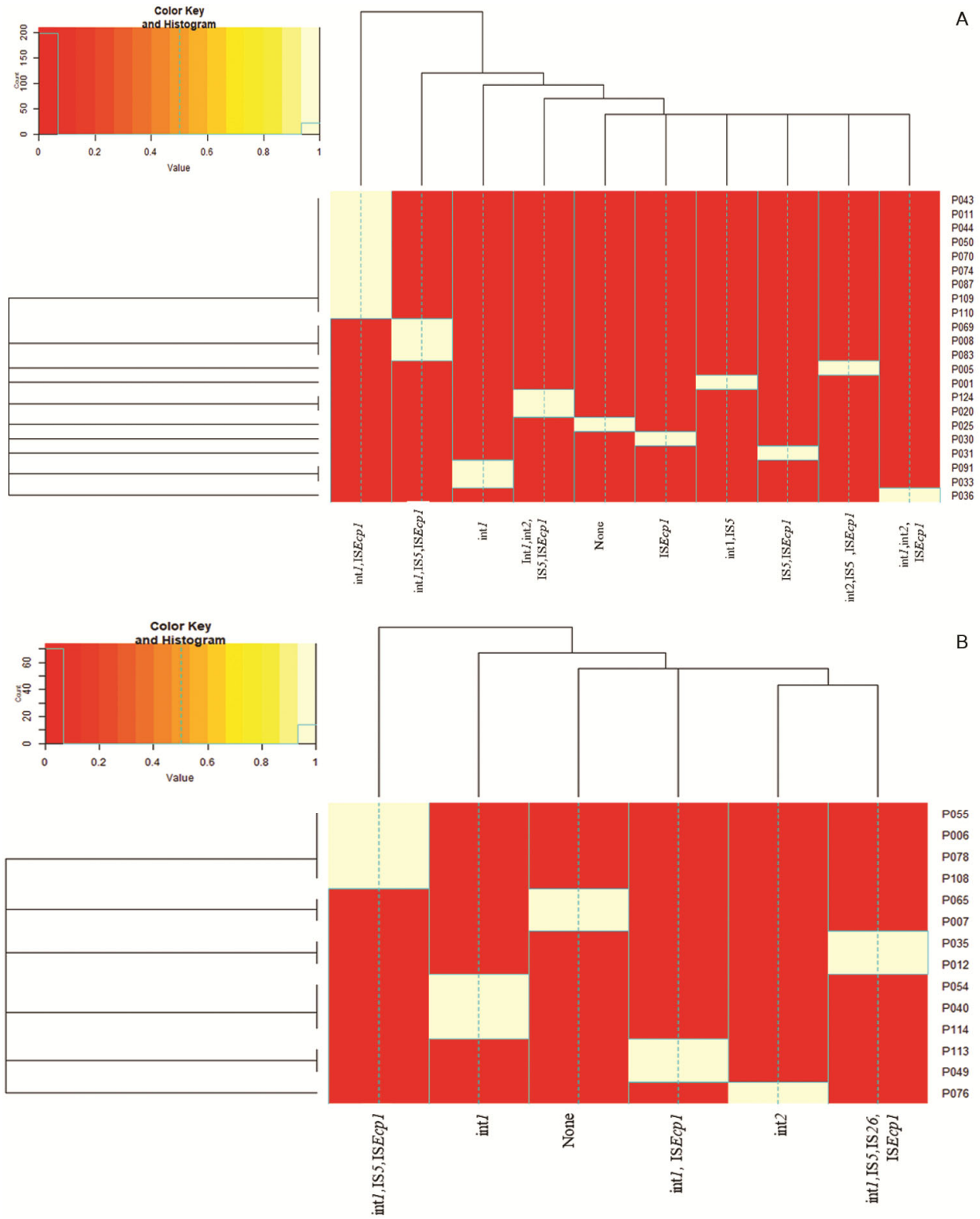

Fig. 4 — Heatmap representation of different combinations of MGEs; integrons (int*I1*, int*I2*) and insertion elements (IS*5*, IS*Ecp1*, IS*26*) in (A) bla_{TEM} positive; and (B) bla_{TEM} negative UPEC isolates collected from pregnant women. The numbers in the right side indicate the sample ID of each UPEC isolate. The colour key represented the variation in colours from red to white, illustrating the absence of a specific combination of MGEs to the presence of that particular combination.

in the bla_{TEM} negative isolates than bla_{TEM} positive ones indicated that these elements pose a risk to the horizontal transmission of resistant determinants from mother to the fetus or neonates after birth, if not addressed in mothers with appropriate therapeutics.

Conclusion

The findings of this study, revealed co-occurrence of potential β-lactamase (oxacillinase, ESBL carbapenemase) genes, prevalence of multiple-replicon plasmids and associated MGEs in UPEC isolated from pregnant women from the eastern region of India. In view of therapeutic limitations to treat UTI during pregnancy, this study necessitates detailed surveillance programs towards antibiotic prescription policies to treat UTI in pregnancy which will aid the clinicians to formulate appropriate antibiotic regimens to protect both maternal as well as fetal health in a resource poor country like India.

Conflict of Interest

Authors declare no competing interests.

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