Alangari et al. | J Pure Appl Microbiol | 16(1):540-548 | March 2022
Article 7481 | https://doi.org/10.22207/JPAM.16.1.51
Published Online February 21, 2022
Print ISSN: 0973-7510; E-ISSN: 2581-690X

RESEARCH ARTICLE OPEN ACCESS

Plasmid Replicon Diversity of Clinical Uropathogenic *Escherichia coli* Isolates from Riyadh, Saudi Arabia

Abdulaziz Alangari[1]*, Ahmad Abu Jaffal[2], Naif Almutairi[1] and Abdullah A. Alyousef[1]

[1]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, P.O. Box 10219, Riyadh, 11433, Saudi Arabia.
[2]Department of Clinical Laboratory Sciences, King Saud Bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia.

Abstract

The aim of this study was to identify and compare the plasmid replicons of clinical uropathogenic *Escherichia coli* (UPEC) isolates, involving extended spectrum β-lactamase (ESBL)-positive and ESBL-negative, *E. coli* ST131 and non-ST131 and various ST131 subclones. Plasmid replicon typing on 24 clinical UPEC isolates was carried out using polymerase chain reaction-based replicon typing. A statistical analysis was performed to assess the associations between plasmid replicon types and ESBL carriage, and to evaluate the link between ST131 isolates and high replicon carriage. Eight replicons, I1α, N2, Iγ, X1, FIIS, K, FIA, and FII were detected. The FII was the most common replicon identified here. ESBL-positive *E. coli* isolates were highly associated with I1α, N2, Iγ, X1, and FIIS replicons, while FIA was present only in ESBL-negative group. ST131 isolates were highly associated with I1α and N2 replicons compared to non-ST131. No link was found between replicon carriage and the number or type of ESBLs in *E. coli* isolates. The diversity observed in replicon patterns of our clinical *E. coli* isolates indicates that they might be originated from different sources. The presence of replicons reported previously in animal sources suggests a possible transfer of antimicrobial resistance between animal and human bacterial isolates.

Keywords: Plasmid, replicon typing, *E. coli,* ESBL, ST131, H30

***Correspondence:** aalangari2@ksu.edu.sa; +96 6114693711

(Received: December 9, 2021; accepted: January 15, 2022)

Citation: Alangari A, Jaffal AA, Almutairi N, Alyousef AA. Plasmid Replicon Diversity of Clinical Uropathogenic *Escherichia coli* Isolates from Riyadh, Saudi Arabia. *J Pure Appl Microbiol.* 2022;16(1):540-548. doi: 10.22207/JPAM.16.1.51

INTRODUCTION

Urinary tract infections (UTIs) are commonly caused by *Escherichia coli* (*E. coli*),[1] and it is shown that uropathogenic *E. coli* (UPEC) subset are responsible for approximately 80% of UTIs.[2] Over the past two decades, the levels of antibiotic resistance and extended spectrum β-lactamases (ESBLs) carriage of UPEC have increased markedly.[3,4]

ESBLs are enzymes capable of degrading β-lactams antibiotics, rendering these agents inactive.[5] ESBL genes have evolved into several hundreds of variants, complicating the fight against ESBL-producing bacterial isolates causing UTIs.[6] For instance, $bla_{CTX\text{-}M}$ gene has nearly 170 gene variants so far.[7] Among CTX-M gene variants, $bla_{CTX\text{-}M\text{-}15}$ is the most common and it belongs to the CTX-M-1 phylogroup.[8] Plasmids carry the vast majority of ESBL genes, and they are major players in the transfer and dissemination of ESBL-mediated resistance among clinical bacterial strains.[9] There has been a link between particular plasmid types and ESBL-encoding genes. For instance, IncFII plasmids frequently carry $bla_{CTX\text{-}M\text{-}15}$, while IncK plasmids commonly harbor $bla_{CTX\text{-}M\text{-}14}$.[10]

The worldwide dissemination of the multidrug resistant (MDR) *E. coli* sequence type 131 (*E. coli* ST131) clone represents a major challenge to public health.[11] *E. coli* ST131 is often fluoroquinolone (FQ) insusceptible and commonly carry CTX-M-15 ESBL on IncFII plasmids.[12,13] ST131 isolates are subdivided into different subclones, and *H30* is among the most common ST131 subclone. *H30* comprises two subsets, *H30*R and H30Rx.[11]

We previously determined the antimicrobial sensitivity, ESBL production, ST131 clonal status, virulence associated gene carriage and metabolic potential of a panel of clinical UPEC isolates.[14-16] The identification of plasmid replicon types is important in understanding the epidemiological perspective of prevalence and transmission of ESBL encoding genes in *E. coli*, and this can help in tracking their origin by comparing them to environmental or animal isolates and in the diagnosis of clinically important ESBL-producing *E. coli*.[17] Given that information on plasmid replicon diversity of clinical *E. coli* isolates is very scarce in Saudi Arabia, this study sought to describe and compare the replicon diversity of clinical *E. coli* urine isolates, including ST131 and non-ST131, ESBL-positive and ESBL-negative, and different ST131 subclones. The link between plasmid replicon and ESBL carriage was also evaluated.

MATERIALS AND METHODS

Bacterial isolates

This study involved a total of 24 *E. coli* isolates that obtained from urine specimens of hospitalized in-patients at a tertiary hospital in Riyadh, Saudi Arabia. Details on these *E. coli* isolates are shown in Table 1.

Plasmid replicon typing assays

Plasmid replicon typing was performed by polymerase chain reaction (PCR)-based assay using 2.0 kit (Diatheva, Italy) following the manufacturer's instructions. Replicon typing was done through eight multiplex PCR reactions that can detect up to 30 replicon types (Table 2). Each reaction mix has 25µl containing 1U DNA polymerase. The setup of the PCR reactions included: 1 denaturation cycle for 10 minutes at 95°C; 25 denaturation cycles for 60 sec at 95°C, annealing for 30 sec at 60°C, and extension for 60 sec at 72°C; and 1 cycle of final extension for 5 minutes at 72°C. PCR products were visualized after being run on 2.5% agarose gel containing ethidium bromide. The assays were performed in triplicate showing fully concordant results.

Statistical analysis

Prism GraphPad (version 9.3.0) was used for statistical analysis. Fisher's exact test was used for comparisons of various isolate groups, while Mann-Whitney U test was used to determine the mean replicon scores. The threshold for statistical significance was a P-value ≤ 0.05.

RESULTS

Plasmid replicon carriage of all isolates

Of the 30 replicons tested in this study, a total of 8 (26.6%) replicons, I1α, N2, 1γ, X1, FIIS, K, FIA and FII, were detected for the 24 *E. coli* isolates (Table 3). Some isolates, such as U24, U46 and U3, carried only one replicon type, while others, such as U71, U9 and U68, concomitantly harbored 2, 3 and 4 plasmid replicons, respectively. The FII was the most common replicon that was found either solely or concomitantly in 20 (83.3%) of the 24 isolates, however, 1γ was the least common

replicon and it was present in a single isolate (4.2%) (Fig. 1). The remaining 6 replicons were variably distributed between isolates (Table 3).

Comparison of plasmid replicon carriage between ESBL-positive and ESBL-negative isolates

Our plasmid replicon typing results

Table 1. Information on the E. coli isolates used in this study

Isolate ID	MDR[a]	ESBL	ESBL type(s)	ST131	ST131 subclone	Ref.
U9	MDR	+	CTX-M-15	+	*H30*-nonRx	
U10	MDR	+	CTX-M-15	+	*H30*-nonRx	
U12	MDR	+	CTX-M-15	+	*H30*-nonRx	
U16	MDR	+	CTX-M-15	-	NAb	
U24	MDR	+	CTX-M-15	+	*H30*-Rx	
U27	MDR	+	CTX-M-15	+	*H30*-Rx	
U46	MDR	+	CTX-M-15 & OXA	+	*H30*-nonRx	
U57	MDR	+	CTX-M-15	+	*H30*-Rx	
U68	MDR	+	CTX-M-15	-	NA	
U71	MDR	+	CTX-M-15	+	Non-*H30*	
U78	MDR	+	CTX-M-15	+	*H30*-nonRx	
U82	MDR	+	CTX-M-15, OXA & TEM	+	*H30*-Rx	
U3	Non- MDR	-	NA	-	NA	14,15
U6	Non- MDR	-	NA	-	NA	
U19	Non- MDR	-	NA	+	*H30*-Rx	
U25	MDR	-	NA	-	NA	
U30	Non- MDR	-	NA	-	NA	
U32	Non- MDR	-	NA	-	NA	
U34	MDR	-	NA	-	NA	
U35	Non- MDR	-	NA	-	NA	
U37	Non- MDR	-	NA	-	NA	
U45	MDR	-	NA	-	NA	
U79	MDR	-	NA	-	NA	
U99	MDR	-	NA	+	Non-*H30*	

[a] MDR phenotype refers to displaying resistance to at least 1 antibiotic in ≥3 antibiotic groups.[43] [b] NA: Not applicable.

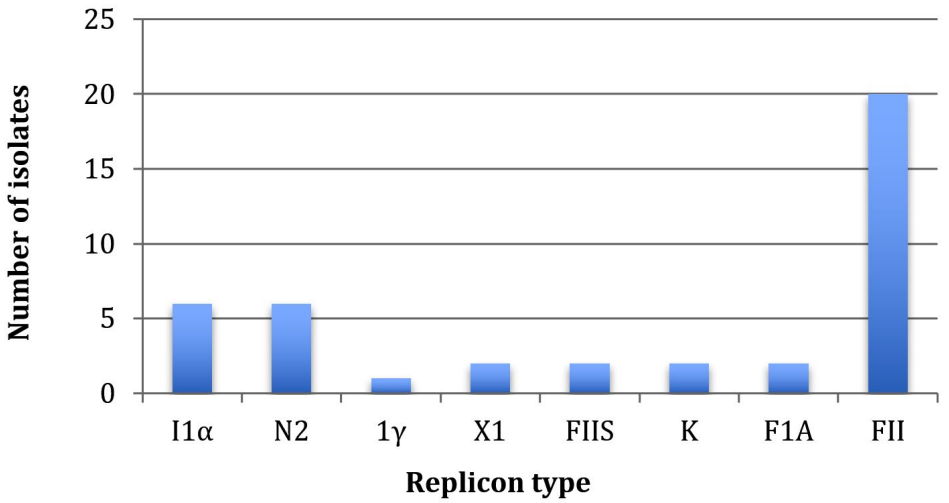

Fig. 1. The overall distribution of plasmid replicons detected in this study.

demonstrated a similarity between these isolate groups in their capability of harboring two replicon types: K and FII (Fig. 2). However, some differences in their replicon carriage were identified. Four replicons: N2, 1γ, X1 and FIIS were only carried by ESBL-positive isolates, while ESBL-negative isolates were exclusively carried the FIA replicon (Fig. 2). The ability of ESBL-positive isolates to harbor I1α replicon was more than that of ESBL-negative *E. coli* isolates. The difference between ESBL-positive and ESBL-negative isolates in carrying the N2 replicon was significant (*P* = 0.01) (Fig. 2).

Comparison of plasmid replicon carriage of ST131 and non-ST131 isolates

Our data demonstrated a similarity between both isolate groups to carry three replicon types: FIA, FIIS and FII (Fig. 3). Nonetheless, few insignificant differences in their replicon carriage were observed. Non-ST131 isolates exclusively harbored 1γ, X1 and K replicons, with at least one isolate showing capability of carrying these replicons. However, ST131 isolates were more able to carry I1α and N2 compared to non-ST131. No statistical difference in the median replicon carriage score between these two isolate groups was detected (P = 0.60) (Fig. 3).

Comparison of plasmid replicon carriage of *E. coli* ST131 subclones

All ST131 isolates were similar in their incapability of carrying three replicons: 1γ, X1 and K, however there was a slight variability between ST131 subclones in carrying five replicons: I1α, FIA, N2, FIIS and FII. In general, the of replicon carriage of *H30* isolates were nearly comparable to non-*H30* isolates, and there was no specific replicon profile of a particular ST131 subclone (Fig. 4). The replicon carriage of *H30* non-Rx subclone was higher than *H30*Rx, and a significant difference

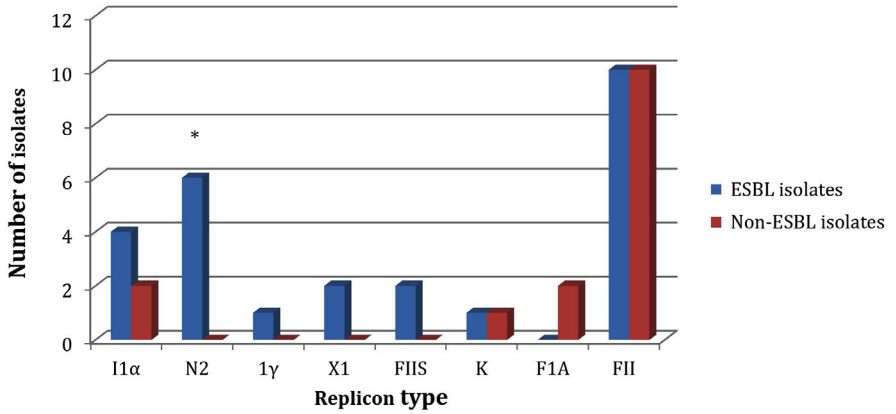

Fig. 2. The plasmid replicon types detected for ESBL-producing and non-ESBL-producing isolates. The asterisk refers to presence of significant difference between the two isolate groups for N2 replicon carriage (*P* = 0.01).

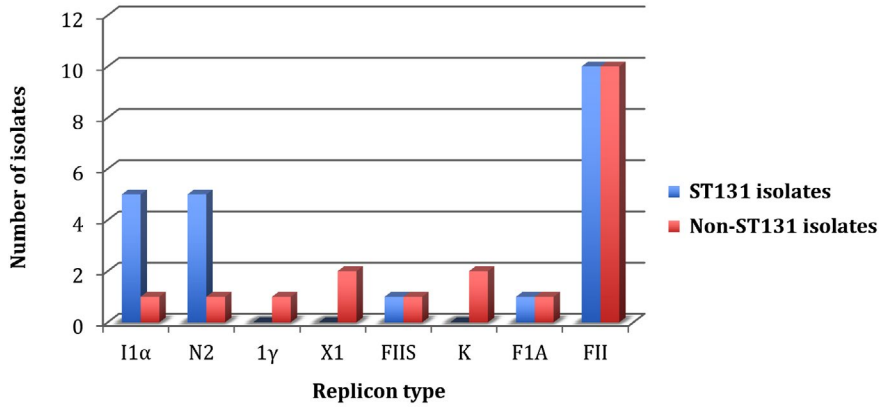

Fig. 3. The plasmid replicon types detected for ST131 and non-ST131 isolates.

between these subclades in their carriage of I1α and N2 replicons was found (P = 0.05) (Fig. 4). Non-*H30* isolates was not significantly associated with a specific replicon type compared to *H30* isolates (Fig. 4).

Relating the plasmid replicon carriage to ESBL carriage

The results of relating the plasmid replicon carriage of the twenty-four *E. coli* isolates to their ESBL carriage are shown in Table 4. In general, there was no specific relationship between replicon carriage and the number or type of ESBLs carried by *E. coli* isolates. Our data showed that FII was found in 10 (83.3%) CTX-M-15-producing isolates, however this replicon type was not exclusively harbored by ESBL-positive isolates, as it was also detected in 10 (83.3%) ESBL-negative isolates. Additionally, isolates carrying more than one ESBL type, such as U46 and U82, did not carry more replicons in comparison to those carrying single ESBL type (Table 4).

Table 2. Information on replicon types provided by PBRT kit

Multiplex	Target	Amplicon size (bp)
M1	HI1	534
	HI2	298–308
	I1-α	159
M2	M	741
	N	514
	I2	316
	B/O	159
M3	FIB	683
	FIA	462
	P1	345
	W	242
M4	L	854
	X3	284
	I1-γ	161
M5	T	750
	A/C	418
	FIIS	259–260
	N2	177
M6	U	843
	X1	370
	R	248
	FIIK	142–148
M7	FIB KN	631
	X2	376
	FIB KQ	258
	K	190
M8	HIB-M	570
	FIB-M	440
	FII	288–292
	X4	172

DISCUSSION

Characterizing bacterial plasmids in MDR bacteria, such as ExPEC, is essential in elucidating the role these plasmids play in the global spread of antimicrobial resistance. Several techniques have successfully been used to characterize plasmids with variable degrees of applicability. Among these techniques, PCR-based replicon typing is an easy useful tool with proven specificity and sensitivity.[18,19] In *Enterobacteriaceae*, IncF, IncI, IncA/C, IncL, IncN and IncH are among the most frequently reported plasmids associated with carrying antibacterial resistance genes.[20]

In our analysis, eight replicons were detected in the 24 isolates, and there was a high degree of plasmid replicon diversity among these isolates. This is common for *E. coli* isolates and

Table 3. Plasmid replicon types detected for all tested *E. coli* isolates

Isolate ID	Number of replicons	Replicon type(s)
U9	3	I1α, N2 & FII
U10	3	I1α, N2 & FII
U12	3	I1α, N2 & FII
U16	3	1γ, X1 & FII
U24	1	FII
U27	1	FII
U46	1	FIIS
U57	1	FII
U68	4	N2, FIIS, X1 & K
U71	2	N2 & FII
U78	3	I1α, N2 & FII
U82	1	FII
U3	1	FII
U6	1	FII
U19	2	FIA & FII
U25	1	I1α
U30	1	FII
U32	2	K & FII
U34	2	FIA & FII
U35	1	FII
U37	1	FII
U45	1	FII
U79	1	FII
U99	1	I1α

Table 4. Relating the plasmid replicon type(s) to ESBL carriage

Isolate ID	No. of replicons	Replicon type(s)	ESBL type(s)
U9	3	I1α, N2 & FII	CTX-M-15
U10	3	I1α, N2 & FII	CTX-M-15
U12	3	I1α, N2 & FII	CTX-M-15
U16	3	1γ, X1 & FII	CTX-M-15
U24	1	FII	CTX-M-15
U27	1	FII	CTX-M-15
U46	1	FIIS	CTX-M-15 & OXA
U57	1	FII	CTX-M-15
U68	4	N2, FIIS, X1 & K	CTX-M-15
U71	2	N2 & FII	CTX-M-15
U78	3	I1α, N2 & FII	CTX-M-15
U82	1	FII	CTX-M-15, OXA & TEM
U3	1	FII	NA
U6	1	FII	NA
U19	2	FIA & FII	NA
U25	1	I1α	NA
U30	1	FII	NA
U32	2	K & FII	NA
U34	2	FIA & FII	NA
U35	1	FII	NA
U37	1	FII	NA
U45	1	FII	NA
U79	1	FII	NA
U99	1	I1α	NA

concurs with a previous report demonstrating high diversity in replicon types among UPEC isolates.[21] The diversity observed in replicon patterns of our clinical *E. coli* isolates indicates that they might be originated from different sources. With respect to the distribution of replicons, our data found that IncF plasmids were the most encountered group. FII replicons were found alone in 11 isolates and combined with FIA in two isolates. This is concordant with several reports showing that IncF plasmids are limited to *Enterobacteriaceae* and mainly present in *E. coli*,[22] and is also in agreement with many reports describing the epidemiology of IncF plasmids in *E. coli*.[23-25] However, it is in contrast to a previous study, showing that FIB and B/O were the most common replicon types among a collection of UPEC isolates.[21]

We also found four isolates showing the replicon combination I1α, N2 & FII. The presence of such a combination is not uncommon and the cointegration of IncF plasmids with IncI1 and IncN in *E. coli* was previously reported.[26,27]

Our study compared the replicon types of ESBL-positive and negative isolates, and demonstrated that K and FII replicons were detected in both isolate groups in similar frequency, and that ESBL-positive isolates were more probable to carry FIIS, I1α, N2, 1γ and X1 isolates in comparison to ESBL-negative isolates. It has been shown that ESBL genes are mostly found on IncF plasmids, that also encode for

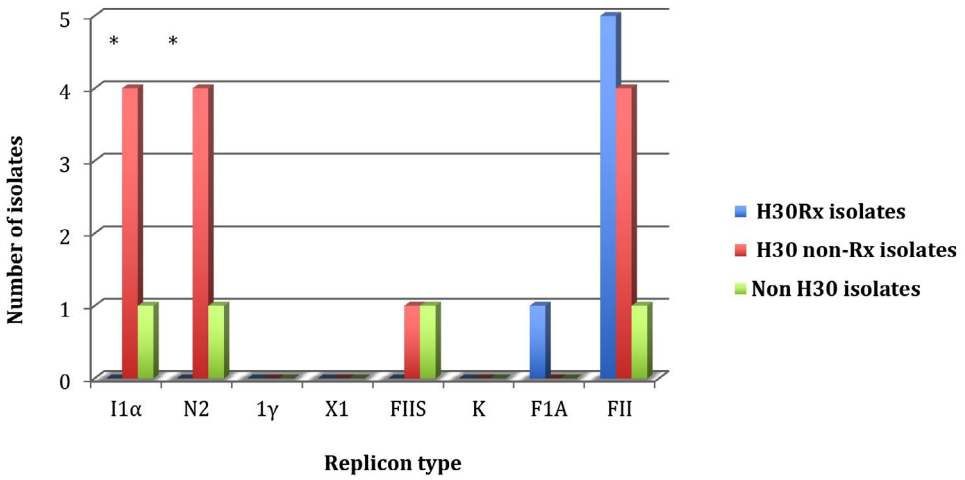

Fig. 4. The plasmid replicon types detected for isolates belonging to ST131 subclones. The asterisks refer to the presence of significant difference between the *H30*Rx and *H30* non-Rx isolate groups for I1α and N2 replicon carriage (P = 0.05).

carbapenemases, aminoglycoside-modifying enzymes, and quinolone resistance,[10] which agrees with our findings. However, we showed that FII carriage was comparable between ESBL-positive and negative isolates, suggesting that this replicon is not specific to ESBL-producing *E. coli*. Given the little focus towards characterizing plasmid replicon carriage of non-ESBL producing *E. coli* strains in the literature, it is crucial to perform comparative large-scale studies on the epidemiology of IncF plasmid carriage of ESBL-positive and ESBL-negative *E. coli* isolates.

Our analysis also showed the ability of some isolates to carry replicons that are harbored by *E. coli* isolates from animal sources. For example, IncIγ, the replicon detected in one isolate in this study, was also reported to carry bla_{CMY-2} gene in *E. coli* isolate from animal source.[28] Additionally, we detected IncK plasmid in two isolates, and several previous studies reported the presence of IncK plasmids in *E. coli* isolated from animal sources, mainly carrying bla_{CMY-2} and blaCTX-M-14 genes.[29-32] In this study, N2 replicon was found in six isolates, all of them also have FII replicons. This is in accordance with a previous study reported the colocalization of IncN with IncF plasmids in *E. coli*.[33] Several ESBL genes were carried on IncN plasmids in *E. coli* isolates including $bla_{CTX-M-1}$,[34] $bla_{CTX-M-14}$ and $bla_{CTX-M-27}$.[35] IncN2 plasmids were reported in *E. coli* from human hosts and carried bla_{NDM-1} gene in Thailand,[36] Australia,[37] and China.[38] IncX1 plasmid was present in two isolates, both are ESBL-producing non-ST131 isolates. IncX1 encoding qnrS1 gene was identified in a previous study in a quinolone-resistant *E. coli* isolate from animal source.[39]

Currently, *E. coli* ST131 is considered as the main contributor for the spread of multidrug resistance and certain genes coding for CTX-M ESBLs on IncFII plasmids. Surprisingly, our data has not found higher plasmid replicon carriage of ST131 compared to non-ST131, although almost all our ST131 isolates were MDR and CTX-M-15 producing. Moreover, FII replicon was detected in similar frequencies in both ST131 and non-ST131 isolates. Despite our sample size is low, this finding is important and highlights the importance of performing comparative studies on different successful ExPEC clones to evaluate the difference in their plasmid replicon carriage.

Two fluoroquinolone-resistance clades are believed to lead the global expansion of ST131. The two clades are *H30*R (Clade 1), and *H30*Rx (Clade 2), both have *fimH30* allele.[40,41] A recent plasmidome meta-analysis-based evidence has indicated that CTX-M-encoding ST131 plasmids are clade-specific, meaning that clonal expansion is the cause of the global expansion of ST131 rather than horizontal gene transfer. The analysis found that IncF plasmids coding for CTX-M-27 are found only in clade 1, whereas IncF plasmids coding for CTX-M-15 are found only in clade 2.[42] Our analysis showed that, among the 12 ST131 isolates included in the study, five are *H30*R, five are *H30*Rx and two are non-*H30*. All *H30*R isolates have identical replicons (I1α, N2 and FII) except one isolate that possessed only FIIS replicon. All *H30*Rx isolates posses FII replicon except one of them that had both FII and FIA. However, in contrast to the recent evidence, most of these subclones were having CTX-M-15 regardless of the subclone.

With respect to the limitations of this study; it examined a small number of UPEC isolates, therefore the associations observed in the current analysis might not be fully definitive. It also provided a description and a comparison of the replicon diversity of *E. coli* urine isolates collected from Riyadh city, which does not necessarily reflect the plasmid replicon diversity of *E. coli* isolates collected from other geographical parts in Saudi Arabia.

CONCLUSION

This study is the first to identify and compare the plasmid replicon diversity of different UPEC isolate groups from Saudi Arabia. This diversity observed in replicon patterns of our isolates indicates that they might be originated from different sources. The presence of replicons reported previously in animal sources suggests a possible transfer of antibiotic resistance between animal and human bacterial isolates. The lack of specific replicon carriage of the globally disseminated MDR ExPEC clone, ST131, or its subclones was also demonstrated here. In future, studying the plasmid replicon diversity of major MDR *E. coli* clones is essential to define the role of these plasmids in making MDR ExPEC clones such successful pathogens.

ACKNOWLEDGMENTS

The authors extend their appreciation to the College of Applied Medical Sciences Research Centre at King Saud University, Saudi Arabia for their support of this research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the Research Ethics Committee at College of Applied Medical Sciences, King Saud University, Saudi Arabia. (CAMS 042-3839).

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