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Molecular Characterization of *Escherichia coli* Strains That Cause Symptomatic and Asymptomatic Urinary Tract Infections

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The differences between *Escherichia coli* strains associated with symptomatic and asymptomatic urinary tract infections (UTIs) remain to be properly determined. Here we examined the prevalence of plasmid types and bacteriocins, as well as genetic relatedness, in a defined collection of *E. coli* strains that cause UTIs. Comparative analysis identified a subgroup of strains with a high number of virulence genes (VGs) and microcins M/H47. We also identified associations between microcin genes, VGs, and specific plasmid types.

Urinary tract infections (UTIs) are among the most common infectious diseases of humans (17). Uropathogenic *Escherichia coli* (UPEC) is the major etiological agent of UTIs and can cause both symptomatic infection and asymptomatic bacteriuria (ABU) in catheterized (CA-ABU) and noncatheterized patients with ABU. Two recent studies identified and compared the virulence gene (VG) carriage of *E. coli* strains isolated from patients with ABU, CA-ABU, and symptomatic UTIs (13, 21). However, the prevalence of plasmid types and bacteriocins and the genetic relatedness of these strains were not elucidated. Here we have examined these features in the same set of *E. coli* strains that cause UTIs by determining the prevalence of different plasmid incompatibility types and sequence types (STs), the distribution of colicin and microcin genes, and their random amplified polymorphic DNA (RAPD) profile.

A collection of 159 UTI-causing *E. coli* strains consisting of 51 ABU, 62 CA-ABU, and 44 symptomatic UTI-causing strains (comprising 25 pyelonephritis and 19 cystitis strains) were used in this study (13, 21). The ABU prototype strain 83972 (2, 11) and the ABU *E. coli* reference strain (ECOR) 71 were also used in this study. All strains have been previously characterized for the prevalence of 18 VGs and Clermont phylogenetic groups (13, 21). These data were used for the comparative analysis with plasmid types and bacteriocins and RAPD analysis in this study. DNA extraction from the isolates was performed as previously described (1).

Plasmid replicon typing was carried out to investigate the presence of 18 replicons using three multiplex panels (5, 8). The presence of the following 16 bacteriocins was assessed by multiplex PCRs: colicins A, B, D, E1, E2, E3, E6, E7, Ia/Ib, K, M, and V and microcins B17, H47, J25, and M. The PCR primers and pools are described in Table S1 in the supplemental material. The genetic relatedness of the UTI-causing strains was assessed by RAPD analysis using primer 1254 (CCGCAGCCAA) (14). The prevalence of the three most common STs (ST69, ST95, and ST131) associated with *E. coli* UTIs were also investigated using previously described methods (3, 7). Comparisons of prevalence among the different groups of *E. coli* were analyzed using the χ^2 test ($n > 5$) and Fisher's exact test ($n \leq 5$) using SPSS (version 17) software. The likelihood ratio χ^2 and Kappa (κ) index was used to quantify the degree of coassociation between genes.

Plasmid typing revealed 14 different plasmid replicons, with 84% of the strains carrying at least one plasmid replicon (Table 1 and Fig. 1). The most common plasmid replicons identified were Inc FIB (present in 103/159 strains [65%]) and Frep FII, FIII, FIV, and FV variants of IncF (present in 108/159 strains [68%]). The B/O plasmid replicon was more prevalent in symptomatic UTI strains (23%) than in ABU strains (8%; $P = 0.0342$), while the FIB replicon was more prevalent in CA-ABU strains (74%) than in ABU strains (51%) ($P = 0.0098$). None of the strains contained the Inc A/C, T, K/B, and H12 plasmid replicons.

No significant difference was observed in the prevalence of bacteriocin genes between ABU, CA-ABU, and symptomatic UTI strains. Bacteriocin genes were detected in 56% of strains; 29% of strains contained colicin genes, and 38% contained microcin genes. Microcin M (31%) and H47 (30%) and colicin Ia/Ib (14%), E1 (12%), and V (11%) were the most common bacteriocin genes. Colicins A, D, E2, and E6 were not detected in any of the strains.

The cooccurrence of bacteriocin genes was also examined (see Table S2 in the supplemental material). A significant coassociation of microcin M and H47 genes was identified (likelihood ratio $\chi^2 = 122.254$ and $P < 0.0001$). Out of the 49 strains that harbored the microcin M gene, 43 strains (88%) also carried microcin H47 gene. A similar coassociation was observed between colicin V and Ia/Ib (likelihood ratio $\chi^2 = 84.545$ and $P < 0.0001$). Microcin genes were more prevalent in strains belonging to phylogenetic group B2 (53% [$P = 0.003$]) compared to strains in groups A and B1 (33%) and D (22%).

RAPD analysis resolved 3 major clusters with 60% band similarity and 7 independent branches with 70% band similarity (see Fig. S1 in the supplemental material). There was a strong correlation between RAPD profiling and phylogenetic group typing. The likelihood of each phylogenetic group clustering together on the

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TABLE 1 Distribution of bacteriocins, plasmid replicons, and phylogenetic groups among *E. coli* strains causing ABU, CA-ABU, and symptomatic UTIs

Gene or replicon	No. of strains (%) causing:			Statistical significance of prevalence[a]		
	ABU ($n = 53$)	CA-ABU ($n = 62$)	Symptomatic UTI ($n = 44$)	ABU vs CA-ABU	ABU vs symptomatic UTI	CA-ABU vs symptomatic UTI
Plasmid replicons						
B/O	4 (8)	10 (16)	10 (23)	NS	0.0342	NS
FIC	1 (2)	0 (0)	0 (0)	NS	NS	NS
P	0 (0)	1 (2)	0 (0)	NS	NS	NS
W	2 (4)	0 (0)	0 (0)	NS	NS	NS
FIIA	1 (2)	2 (3)	0 (0)	NS	NS	NS
FIA	6 (11)	7 (11)	4 (9)	NS	NS	NS
FIB	27 (51)	46 (74)	30 (68)	0.0098	NS	NS
Y	5 (9)	0 (0)	1 (2)	0.0187	NS	NS
I1	3 (6)	5 (8)	4 (9)	NS	NS	NS
Frep	30 (57)	49 (79)	29 (66)	0.0097	NS	NS
X	0 (0)	2 (3)	0 (0)	NS	NS	NS
HI1	0 (0)	3 (5)	2 (5)	NS	NS	NS
N	2 (4)	2 (3)	1 (2)	NS	NS	NS
L/M	0 (0)	0 (0)	1 (2)	NS	NS	NS
Bacteriocins						
Colicins						
B	2 (4)	1 (2)	0 (0)	NS	NS	NS
E1	6 (11)	10 (16)	3 (7)	NS	NS	NS
E3	1 (2)	0 (0)	0 (0)	NS	NS	NS
E7	2 (4)	0 (0)	0 (0)	NS	NS	NS
K	1 (2)	2 (3)	4 (9)	NS	NS	NS
Ia/Ib	6 (11)	9 (15)	7 (16)	NS	NS	NS
M	1 (2)	1 (2)	0 (0)	NS	NS	NS
Microcins						
V	6 (11)	5 (8)	6 (14)	NS	NS	NS
B17	2 (4)	4 (6)	1 (2)	NS	NS	NS
H47	12 (23)	23 (37)	12 (27)	NS	NS	NS
M	15 (28)	22 (35)	12 (27)	NS	NS	NS
J25	0 (0)	2 (3)	0 (0)	NS	NS	NS
Sequence types						
ST95	4 (8)	6 (10)	12 (27)	NS	0.0090	0.0174
ST131	2 (4)	4 (7)	4 (9)	NS	NS	NS
ST69	0 (0)	0 (0)	0 (0)	NS	NS	NS
Phylogenetic groups						
A	5 (9)	6 (10)	1 (2)	NS	NS	NS
B1	3 (6)	2 (3)	1 (2)	NS	NS	NS
B2	37 (70)	44 (71)	34 (77)	NS	NS	NS
D	8 (15)	10 (16)	8 (18)	NS	NS	NS

[a]NS, not statistically significant.

basis of RAPD profiles was as follows: B2, $\chi^2 = 49.5$ and $P < 0.0001$; D, $\chi^2 = 41.1$ and $P < 0.0001$; A, $\chi^2 = 9.0$ and $P = 0.041$; B1, $\chi^2 = 9.15$ and $P = 0.076$.

The analysis of the three common STs revealed that ST95 (14%) was more common than ST131 (6%) and ST69 (not detected). All of the ST95 and ST131 isolates were identified as B2 strains. The ST95 strains were significantly associated with strains causing symptomatic UTIs (27%) compared to strains causing ABU (8%; $P = 0.009$) and CA-ABU (10%; $P = 0.0174$). A significant coassociation of colicin V and Ia/Ib genes was also observed in the ST95 strains (likelihood ratio $\chi^2 = 28.4$ and $P < 0.0001$) (see Table S2 in the supplemental material). None of the ST131 strains contained any bacteriocin genes tested for in this study (Fig. 1).

Cluster analysis using VGs, bacteriocin genes, and plasmid replicon types separated a group of strains (cluster 1) harboring a large number of VGs (median score of 9 VGs). Cluster 1 contained 55 strains with a substantial mix from all the three UTI groups (27% of ABU, 42% CA-ABU, and 31% of symptomatic UTI strains).The strains in this cluster belonged predominantly to phylogenetic group B2 (95%). All of the strains in cluster 1 contained the *iroN* gene, while the majority of strains contained the toxin genes *hlyA* (93%) and *cnf1* (91%) and F1C fimbrial gene *focG* (97%). Forty-seven strains in cluster 1 contained the microcin M gene; 87% of these strains also contained the microcin H47 gene. A significant coassociation of the microcin M/H47 genes with *hlyA*, *cnf1*, *focG*, and *iroN* was also observed (see Table S2 in the supplemental material). Overall, the major difference between

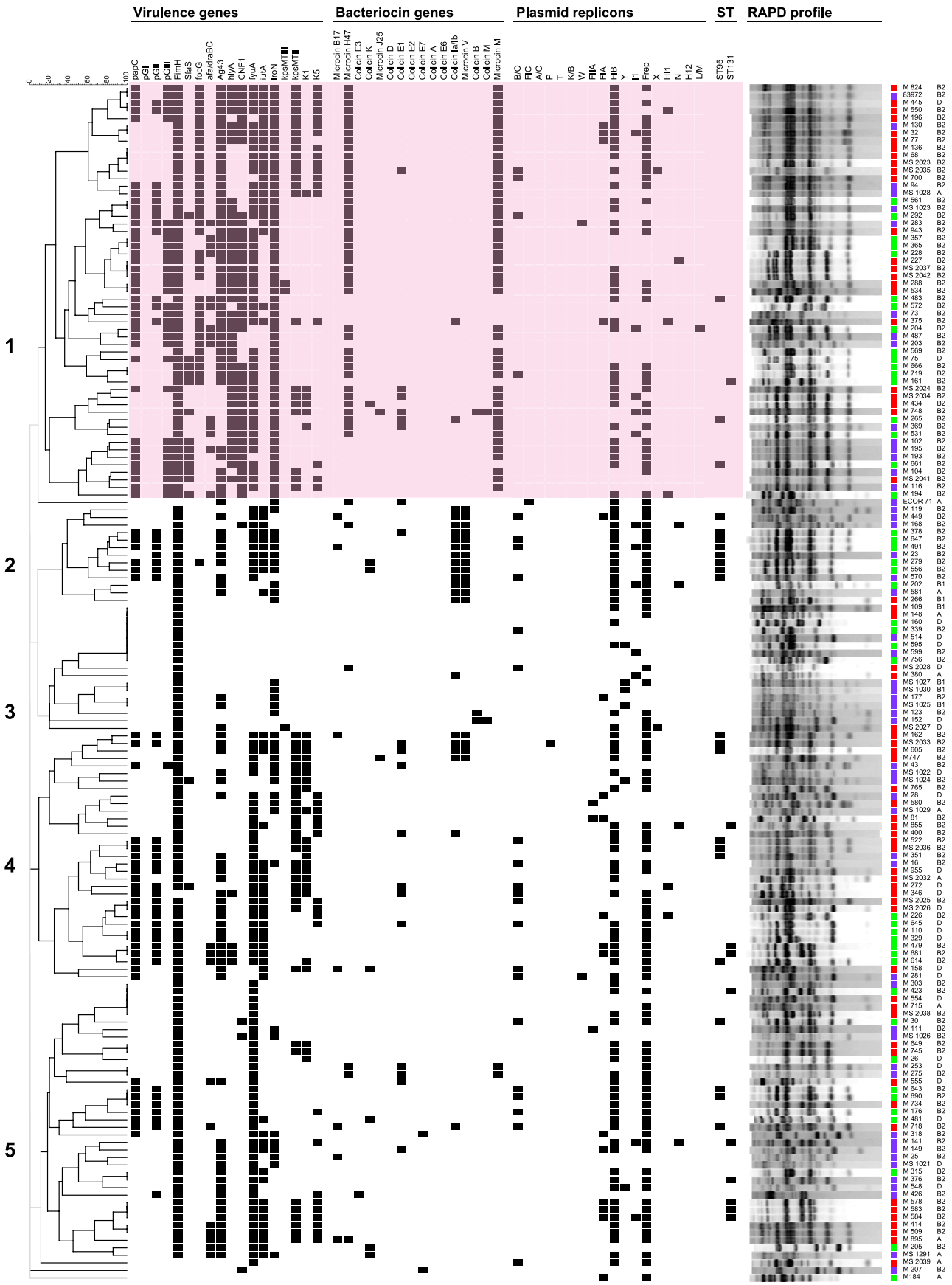

FIG 1 Cluster analysis based on VGs, bacteriocins, plasmid types, and sequence types created using Jaccard correlation and complete linkage. The dendrogram shows the RAPD profiles of each isolate. Green, purple, and red represent strains causing symptomatic UTI, ABU, and CA-ABU, respectively. Bacterial strains and their respective phylogenetic groups are also indicaated.

strains in cluster 1 and clusters 2 to 5 was the presence of the microcin M/H47 genes.

Bacteriocin gene analysis revealed that microcin genes were more prevalent than colicin genes. A coassociation of microcin M/H47 and colicin V and Ia/Ib was observed. The isolates that carried microcin M/H47 predominantly belonged to phylogenetic group B2. The isolates that possessed microcin M/H47 genes were also associated with the carriage of a greater number of VGs (median VG score of 9) compared to the isolates that did not carry M/H47 genes (median score of 5 VGs). Cluster analysis using all three markers (VG, bacteriocins, and plasmid replicons) (Fig. 1) demonstrated that microcin M/H47 carrying isolates shared a VG profile that was distinct from all other strains. This was further supported by the RAPD analysis, which revealed that there was minimal genetic diversity within this cluster in comparison to strains that lacked the M/H47 microcin genes (Fig. 1). One isolate in this cluster belonged to the ST131 lineage, and three isolates belonged to the ST95 lineage (7, 15, 18, 20). Taken together, the data suggest that the group of strains that formed cluster 1 are genetically distinct from other strains examined in this study.

A highly significant coassociation between the M/H47 microcin genes and *hlyA*, *cnf1*, *focG*, and *iroN* was observed among the strains in cluster 1. Previous reports have shown that these genes are often located on the same pathogenicity island (PAI) (12, 16, 19), and this would also explain our data. In addition, PAI-CFT073-*serX* contains the microcin M/H47 gene cluster, F1C fimbrial genes, and the *iro* gene cluster (4). The genetic arrangement of these genes in the strains examined in this study remains to be determined.

The comprehensive genotyping employed in this study could not clearly differentiate the *E. coli* strains based on clinical UTI types. Many of the ABU strains were similar to CA-ABU and symptomatic UTI strains based on RAPD patterns, VG profiling, bacteriocins, and plasmid types. Interestingly, we identified ABU and CA-ABU strains that possessed similar properties to the prototype ABU *E. coli* strain 83972 (2, 9, 11). This suggests that attenuation of ABU and CA-ABU strains through gene loss and/or mutation may be a common occurrence (9, 10, 22).

Plasmid typing revealed differences in the prevalence of plasmids among the *E. coli* strains based on UTI severity. There was a high prevalence of FIB plasmids in the CA-ABU and symptomatic UTI strains; these conjugative plasmids have also been shown to contribute to biofilm formation (6). Further investigation into the dissemination of specific plasmid types and their involvement in virulence and antibiotic resistance is necessary to understand the epidemiology of UTI-causing *E. coli*.

In summary, this study identified a cluster of similar UTI-causing *E. coli* strains associated with symptomatic and asymptomatic UTIs. The association between microcin and VGs, as well as the high prevalence of specific plasmid types, might reflect characteristics associated with an enhanced ability to colonize the urinary tract.

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