Genetic Diversity of Uropathogenic Escherichia coli Isolated from Human T Lymphotropic Virus Type I (HTLV-I) Infected Individuals

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Abstract

Urinary tract infection (UTI) is a frequent pathology among HTLV-I+ individuals being capable of severely compromising the kidneys and bladder. Molecular characteristics of uropathogenic Escherichia coli (UPEC) from HTLV-I+ infected individuals are unknown. UPEC isolates from HTLV-I+ individuals, with and without clinical symptoms of myelopathy, were submitted to genetic typing seeking to infer bacterial diversity and potential virulence. 71 bacterial isolates were characterized according to random amplified polymorphic DNA and phylotypes. Phylotyping classified E. coli into four phylogenetic groups: A (18.3%), B1 (16.9%), B2 (39.4%), and D (25.3%) and 8 phylotypes according to the presence of the genetic sequences chuA, yjaA and the DNA fragment TSPE4.C2: −−− (5.6%) and −+− (12.6%) in phylogroup A, −−+ (7.0%) and −++ (9.8%) in B1, +++ (32.3%) and ++− (7.0%) in B2, +++ (15.4%) and ++− (9.8%) in D. The B2 phylogroup was the most prevalent among HTLV- associated myelopathy and asymptomatic individuals. RAPD-PCR typing revealed a high degree of bacterial polymorphism indicating a non-clonal origin. Genotypes were not found to be distributed according to clinical status or epidemiological features. Our results lead us to suggest that the neurological impairment in HTLV-I+ individuals can be a risk factor for urinary infections due E. coli which are caused by distinct bacterial lineages.

Keywords

Uropathogenic Escherichia coli, Genetic Diversity, Phylotype, HTLV-I+
1. Introduction

The Human T-lymphotropic type I (HTLV-I) is a retrovirus associated with a chronic myelopathy known as HTLV-I-Associated Myelopathy or Tropical Spastic Paraparesis (HAM/TSP) [1]. HAM/TSP is a progressive neurologic disorder characterized by leg weakness, diffuse hyperreflexia, clonus, loss of vibration sense, and detrusor insufficiency leading to bladder dysfunction. Central Africa, Japan, the Caribbean, South America and Melanesia are the principal endemic areas for HTLV-I [2]. The majority of HTLV-I-infected individuals are clinically asymptomatic and less than 5% of seropositive subjects develop HAM/TSP [1] [3] [4]. Urologic manifestations are present in up to 90% of patients with HAM/TSP and are characterized by frequency, urgency and urge incontinence [1] [5] [6] [7]. Epidemiological studies have described that HTLV-I+ individuals have a higher prevalence and incidence of urinary symptoms than seronegative controls [5] [6] [8] [9]. Urinary tract infection (UTI) is an extremely frequent pathology among HTLV-I+ individuals due to the occurrence of bladder dysfunctions favoring colonization by bacteria [6] [8] [9] [10]. These urinary infections can be clinically relevant, being capable of severely compromising the kidneys and bladder [11]. However, bladder impairment depends on the stage of development of the illness [10]. With the advancement of the degree of motor impairment, the exacerbation of urinary symptoms has been observed, with the development of systematic complications even being possible [5] [6] [9]. Studies which investigate the etiology of UTIs in HTLV-I+ patients found that Escherichia coli is a bacterial species relevant in urinary infections; however, the genetic characteristics of these microorganisms isolated in this group of individuals are little known [9]. Recent studies have shown a great variety of molecular methodologies utilized as diagnostic tools, as well as for typing purposes, allowing the detection of diversity, inference of genetic interrelation, and correlation with the clinical-epidemiological context. Among the molecular techniques used for the study of diversity in E. coli populations, the technique of random amplification of polymorphic DNA (RAPD-PCR) has received special attention as a typing method due to its simplicity of execution, low cost, flexibility, discriminatory power, and reproducibility under standardized experimental conditions. These studies have allowed the detection of the circulation of particular genetic lineages and revealed that E. coli epidemiologically correlated share urovirulence properties which are relevant for the determination and evolution of the clinical conditions [12] [13]. Phylogenetic analysis has significantly contributed to the elucidation of the pathogenic potential of E. coli populations. This molecular characterization has allowed the designation of the main phylogenetic groups: A, B1, B2, and D [14]. The microorganisms belonging to these distinct phylogroups are genetically diverse and differ according to metabolic properties, ecological niches, and the capacity to cause disease [15] [16]. E. coli belonging to group B2, and to a lesser extent from D, have frequently been associated with extra-intestinal infections, while commensal E. coli and diarrheagenic strains are normally found as members from groups A, B1, and D [15] [16] [17] [18].

In this study, we characterized uropathogenic E. coli (UPEC) isolates from HTLV-I+
individuals, with and without clinical myelopathy symptoms, by the random amplification of polymorphic DNA (RAPD) and PCR-multiplex seeking to infer bacterial diversity, genetic interrelations and potential virulence.

2. Methods

2.1. Bacterial Samples and Participants

A total of 71 _Escherichia coli_ isolates from adult individuals treated at the Laboratório de Pesquisa em Neuroinfecções do Instituto Nacional de Infectologia (INI/Fiocruz), with positive urine culture, and HTLV-I+ serology were included in this study. _E. coli_ was isolated in the Laboratório de Bacteriologia e Bioensaios (INI/Fiocruz). For patients with clinical symptoms of myelopathy, neurological impairment was determined based on the Expanded Scale of the State of Incapacity (ESSI) [19]. Out of all of the _E. coli_ isolates, 35 were obtained from HTLV-I associated myelopathy patients (HAM/TSP), presenting different degrees of neurological impairment (EDSS >0 to EDSS < 7) and, 36 isolates were recovered from individuals without clinical myelopathy symptoms (EDSS = 0, asymptomatic) (Table 1). Clinical-epidemiological data were obtained from the electronic records. The study protocol was approved by the Instituto Nacional de Infectologia and Escola Nacional de Saúde Pública Sergio Arouca Ethics Committee on Human Research, Fundação Oswaldo Cruz (CAEE 0071.0.009.031-07/ CAAE 0022.0.031.000-10).

2.2. Random Amplification of Polymorphic DNA

RAPD-PCR was used as the molecular typing technique with the primers A04 (AATCGGGCTG), 1254 (CCGCAGCCAA), and M13 (GAGGGTGGCGCTTCT) [20]. RAPD profiles were inspected visually and defined according to the presence or absence and intensity of polymorphic bands. A 1 Kb DNA ladder was used as a molecular weight marker (Invitrogen). Semi automated analysis used the UVI Soft Image Acquisition and Analysis Software, program UVIPrBandmap, version 11.9 (Uvitec, Cambridge). Cluster analysis was done by using the unweighted pair group method with arithmetic averages (UPGMA) of the Image Analysis System. The percentages of similarity were estimated by Dice coefficient. The reproducibility of RAPD amplifications was assessed using the selected primers with different DNA samples isolated independently from the same strain and amplified at different times. Numerical index of the discriminatory ability of RAPD primers was calculated by applying Simpson’s Index of Diversity equation [21].

2.3. PCR-Triplex for _E. coli_ Phylotyping

PCR assay was performed using phylogenetic group specific primers for the genes _chuA1/chuA2_, _yjaA1/yjaA2_ and the DNA fragment TSPE4C2.1/TSPE4C2.2 [14]. A, B1, B2 and D phylogroups and respective allelic variants (phylotypes) were determined based on the presence or absence of bands according to previously defined criteria in the triplex amplification assay. To estimate the size of the fragments a 100 bp DNA
Table 1. *Escherichia coli* identification and clinical aspects of HTLV-I seropositive individuals.

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*aHAM: HTLV-I-Associated Myelopathy; bSex: F (female), M (male); cEDSS: Expanded disability status scale; dUTI: Urinary tract infection; S*: symptomatic; A**: asymptomatic.*
ladder was used. Molecular assays included a non-template reaction and the clinical
UPEC strain 119C (UPEC chuA+, yjaA+ and TSPE4.C2+) as the positive control [16].

2.4. Statistical Analysis
Results were compared and analyzed using the Fisher exact test, Epi Info program, ver-
sion 3.5.1. P-value ≤ 0.05 was considered statistically significant.

3. Results and Discussion
Our study investigated the genetic diversity and the phylogenetic groups of 71 E. coli
uropathogenic isolates recovered from HTLV-I+ individuals, with and without clinical
myelopathy symptoms. RAPD typing revealed high levels of polymorphism that are
consistent with previously reported observations for E. coli [22]. Escherichia coli has
been recognized as a genetically diverse species with a complex phylogeny, broad viru-
ulence factors armament and significant genomic plasticity, comprising non-pathogenic
gut commensals and strains responsible for intestinal and extra-intestinal disease [22]
[23] [24]. The diversity is especially observed among extra-intestinal E. coli (ExPEC)
strains. Unlike commensal E. coli, ExPEC have the ability to cause diseases once outside
the host gut reservoir due to the possession of a wide range of pathogenic virulence
factors and horizontal gene transfer [24] [25]. RAPD clustering showed a diverse bacte-
rial population constituted by small clonal groups, with indexes of similarity varying
from 31% - 89%, 9% - 80%, and 23% - 95% for the primers A04, 1254, and M13, re-
spectively. The overall chromosomal analysis of E. coli isolates from HTLV-associated
myelopathy and asymptomatic individuals, showed distinct genetic background which
is consistent with previous observations with nonrelated epidemiological strains and
thus characterizing distinct evolutionary lineages. The reactions employing the primers
A04, M13, and 1254 resulted in 62, 58, and 56 reproductive profiles respectively (Figure
1, Figure 2). The discriminatory power of the primers assessed by the diversity equa-
tion revealed indexes of 97.9% (primer A04), 96.7% (M13), and 94.9% (1254). The
number of polymorphic bands varied from 4 - 10 to 1 - 11 for the primers A04 and
1254, respectively. The primer M13 generated more complex electrophoretic patterns.
For the E. coli isolates in the HAM/TSP group (n = 35), 35, 29, and 28 electrophoretic
profiles were detected for the primers A04, 1254, and M13, respectively, with the nu-
mer of polymorphic bands varying from 4 - 9 (A04), 1 - 9 (1254), and 4 - 25 (M13). Us-
ing the primer 1254, 17 RAPD patterns were observed for isolates recovered from the
patients presenting high degree of neurological impairment (score 7). From the total 22
isolates in this HAM/TSP group, 8 profiles were detected in patients with symptomatic
urinary infection (n = 10) and 9 in the asymptomatic UTI group (n = 9). In the
HAM/TSP group EDSS score 6 (n = 6), 5 electrophoretic profiles were observed dis-
tributed among patients with UTI symptomatic (n = 5) and asymptomatic infection (n =
1). For the HAM/TSP groups scores 5 (n = 1) and 4 (n = 4), 1 and 4 electrophoretic
patterns were found mainly distributed among the asymptomatic patients, respectively.
The different primers used to investigate the overall chromosomal diversity among E.
coli from HAM/TSP group were strongly correlated. No common RAPD pattern was observed between *E. coli* isolates recovered from individuals with distinct EDSS scores. RAPD patterns generated by primers A04 and M13 also exhibited similar distribution according to the urinary symptomatology and EDSS score. In general, identical RAPD profiles were mainly observed in *E. coli* isolates recovered from the same patient at distinct times, suggesting the occurrence of urinary tract infection recurrence. In the group of asymptomatic individuals (n = 36), RAPD reactions performed with primers M13, A04, and 1254 resulted in 30, 27, and 26 different profiles, respectively. The total number of polymorphic bands was 4 - 10 (A04), 5 - 11 (1254), and 6 - 15 (M13). In this group of individuals, common RAPD types were mainly observed in *E. coli* isolates recovered from the same individual.

Clinical-epidemiological parameters of the HTLV-I+ individuals such as sex, age, degree of neurological impairment, and clinical UTI condition, were not considered re-

![Dendrogram generated by the Dice coefficient and clustering by unweighted pair group method with arithmetic averages and respective RAPD numeric profiles (primer 1254) of *E. coli* isolates from HTLV-I seropositive individuals with myelopathy.](image-url)
Lane 1: 1 Kb DNA Ladder; Lane 2: I01; Lane 3: I02; Lane 4: I07; Lane 5: I09; Lane 6: I12; Lane 7: I16; Lane 8: I24; Lane 9: I25; Lane 10: I27.

**Figure 2.** RAPD-PCR profiles (primer A04) of representative *E.coli* isolates from HTLV-I sero-positive individuals with myelopathy.

levant for the RAPD-PCR types distribution and the genetic relatedness of *E. coli* isolates. The overall chromosomal analysis of UPEC isolates from HTLV-associated myelopathy and asymptomatic individuals, showed distinct genetic background which is consistent with previous observations with nonrelated epidemiological strains and thus characterizing distinct evolutionary lineages [22].

Clermont genotyping was used to assign the diversity and to characterize the potential virulence within *E. coli* isolates (**Table 2, Figure 3**). The results obtained classified the *E. coli* into four phylogenetic groups: A (18.3%), B1 (16.9%), B2 (39.4%), and D (25.3%). The B2 phylogroup was the most common among the bacterial isolates as much in the HAM/TSP group of patients as with asymptomatic patients, corresponding to 31.5% (n = 11/35) and 47.2% (n = 17/36), respectively. The distribution of the phylogroups A, B1, and D corresponded to 21% (n = 8/35), 20% (n = 7/35), and 25.8% (n = 9/35) in the HAM/TSP group and to 13.9% (n = 5/36), 13.9% (n = 5/36), 25% (n = 9/36), in the asymptomatic patient group. Studies on molecular epidemiology have shown that the occurrence of *E. coli* phylogroups varies according to the characteristics of the population, associated pathologies, and the geographical region. However, the B2 phylogroup is the most prevalent among extra-intestinal infections caused by these microorganisms and is particularly prominent among more virulent isolates associated with urinary tract infections. The other phylogroups are also seen in urinary tract infections, however at a lower frequency and with a distribution associated with particular epidemiological contexts. Considering the degree of neurological impairment, the *E. coli* isolates belonging to the B2 phylogroup were recovered from patients with HAM/TSP presenting EDSS > 0 and <7. The bacterial isolates belonging to the phylogroups A and B1 were obtained from patients with a greater degree of neurological impairment (EDSS > 6). The distribution of phylogroups according to the clinical states of
Table 2. Phylotype distribution among *E. coli* isolates recovered from individuals with and without HTLV-associated myelopathy.

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<tr>
<td>B2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>10 (28.6)</td>
<td>13 (36.1)</td>
<td>23 (32.3; 0.68)</td>
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<tr>
<td>B2</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>1 (2.80)</td>
<td>4 (11.1)</td>
<td>5 (7.0; 0.27)</td>
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<td>D</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>7 (20.0)</td>
<td>4 (11.1)</td>
<td>11 (15.4; 0.29)</td>
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<tr>
<td>D</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>2 (5.70)</td>
<td>5 (13.9)</td>
<td>7 (9.8; 0.31)</td>
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*aHAM/TSP, HTLV-I-Associated Myelopathy or Tropical Spastic Paraparesis.

Figure 3. Representative electrophoretic profiles of *E. coli* carrying phylogenetic markers.

Lane 1: 100 bp DNA Ladder; Lane 2: L119C; Lane 3: I01; Lane 4: I07; Lane 5: I68; Lane 6: I49; Lane 7: I56; Lane 8: I59; Lane 9: I62; Lane 10: I19; Lane 11: I41.

urinary infection, as much in the HAM/TSP patients as in the asymptomatic patients, showed a wide distribution of the groups. The B2 group however, was the most prevalent. In the HAM/TSP patients with symptomatic UTI, 33.3% belonged to the B2 group, 23.8% to the A and B1 groups, and 19.1% to the D group. Among bacterial isolates from individuals with asymptomatic UTI, the B2 and D groups were the highest represented, corresponding to 33.3%, followed by groups A and B1, with 25%, and 8.3% respectively. Epidemiological studies have shown that in patients with classic myelopathy, the greater majority present urinary symptomatology [5] [6] [26]. In patients without clinical symptoms for myelopathy (asymptomatic) the phylogenetic groups B2, B1, D, and A were detected in 50%, 25%, 18.6%, and 6.3% from the isolates recovered from patients with symptomatic UTI, respectively. Among patients without UTI symp-
toms, the isolates showed the following distribution: B2 (45%), D (30%), A (20%), and B1 (5%). The *E. coli* isolates from recurrent infections with symptomatic and asymptomatic UTI conditions were characterized as belonging to the B2 and A/B1 groups, respectively. Our results confirm previous observations, highlighting the relevance of B2 phylogroups in urinary infections, reinforcing in this manner the role of this pathogenic lineage in the establishment of these infections. In the group of patients with more compromised neurological functioning (EDSS > 6), the higher frequency was for isolates belonging to the A and B1 groups. These findings lead us to suggest that the vulnerability of the host could contribute to the establishment of the infection, given that these phylogenetic groups do not constitute typical agents for urinary infection.

Different combinations in the genetic sequences *chuA*, *yjaA* and of the DNA fragment TSPE4.C2 allowed the characterization of the *E. coli* isolates in 8 phylotypes: −−− (5.6%) and −−+ (12.6%) in group A, −++ (7.0%) and −++ (9.8%) in group B1, +++ (32.3%) e ++− (7.0%) in group B2, −−− (15.4%) and −++ (9.8%) in group D (Table 2). In the HAM/TSP group, we found the following phylotypes: −−− (8.6%) and −−− (14.3%) in group A, −−− (11.4%) and −++ (8.6%) in B1, +++ (28.6%) and −−− (2.8%) in B2, −−− (20%) and +++ (5.7%) in D. In the group of asymptomatic individuals, the same genetic variants were found, however with different frequency percentages: −−− (2.8%) and −−− (11.1%) in group A, −−− (2.8%) and −++ (11.1%) in B1, +++ (36.1%) and ++− (11.1%) in B2, −−− (11.1%) and +−+ (13.9%) in group D (Table 2). In the whole population studied, the phylotype +++ was the most prevalent in the B2 group. In the phylogenetic D group, the phylotype +−− was the most prevalent among the HAM/TSP patients, and the +−+ allelic variant was the most common among the asymptomatic individuals. No statistical significance was observed in terms of the distribution of the phylotypes. The −++ variant of the B1 phylogroup had not been described in previous studies into pathogenic populations of *E. coli* [15] [16]. The phylogenetic characterization of the UPEC from HTLV-I+ patients was not previously reported; however, we mainly classified the isolates as belonging to the B2 phylogenetic group, where typically is classified uropathogenic populations of *E. coli*. The phylogenetic classification together with the diversity analysis permitted the elucidation of the clonal structure and the pathogenic potential of these microorganisms. Our results contributed to the molecular epidemiology of UPEC among HTLV-I+ individuals, showing that urinary infections due to *E. coli*, either recurrently manifested or not, are owed themselves to distinct bacterial lineages. Urinary symptoms have been described to negatively influence the quality of life of individuals infected by HTLV-I. Investments on therapeutic resources in order to decrease the risks of urinary infection, and preservation of a functional urinary system have been stimulated [8]. Studies seeking to investigate properties of virulence and resistance to antimicrobials of these uropathogens are currently under way in our laboratory. The data set obtained will be applicable to support epidemiological measures and public health monitoring, especially, together with therapeutic and preventative measures for these infections, which place at risk and compromise quality of life for these individuals.
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