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Original article

Relationship between virulence factors, resistance to antibiotics and phylogenetic groups of uropathogenic *Escherichia coli* in two locations in Mexico☆

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ABSTRACT

Introduction: *Escherichia coli* is the major causative agent of urinary tract infections (UTI), and virulence factors are responsible for the severity of these emerging infections. The aim of this study was to evaluate the relationship between virulence determinants and antibiotic susceptibility with phylogenetic groups of *E. coli* isolates of UTI in two locations in Mexico.
Methods: An analysis was performed on 50 isolates of *E. coli* from the centre of the country and 57 from a town in the southwest. The isolates were characterised by phenotype (serotyping assays, *in vitro* adhesion, biofilm formation, production of haemolysin, and antibiotic susceptibility) and genotype (phylogenetic groups and virulence genes).
Results: In the centre of the country location the phylogenetic group B2 (60%) and F (12%) were significantly more prevalent and had a higher frequency of genes, *fimH* (96%), *iutA* (66%), *sat* (36%), compared to the southwest location, where the group A (35%) and B1 (21%) were significantly predominant and had fewer virulence genes. About one-fifth (21.5%) of all isolates belonged to the O25-ST131 group. Haemolysin and biofilm producing strains were significantly higher in the southwest location. Resistance to ampicillin (92.5%), tetracycline (76.6%), and trimethoprim/sulfamethoxazole (70.1%) were the most common in both groups.
Conclusion: The phylogenetic group, virulence factors, and antibiotic susceptibility of the *E. coli* that causes UTI in the community, varies significantly among the Mexican populations studied. Phylogenetic groups A and B1 may be multidrug resistant and have the ability to produce UTI.

Relación entre factores de virulencia, resistencia a antibióticos y los grupos filogenéticos de *Escherichia coli* uropatógena en dos localidades de México

Palabras clave:
Escherichia coli uropatógena
Infección del tracto urinario
Grupos filogenéticos
Factores de virulencia
Resistencia a antibióticos

RESUMEN

Introducción: *Escherichia coli* es el principal agente causal de infecciones del tracto urinario (ITU), y sus factores de virulencia son los responsables de la gravedad de estas infecciones emergentes. El objetivo de este estudio fue evaluar la relación entre los determinantes de virulencia y susceptibilidad a antibióticos con los grupos filogenéticos de *E. coli* aisladas de ITU en 2 localidades de México.

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Métodos: Se analizaron 50 aislamientos de *E. coli* de una localidad en el centro del país y 57 provenientes de una localidad al suroeste. Los aislamientos fueron caracterizados fenotípica (serotipificación, ensayos de adherencia, formación de biopelícula, producción de hemolisina y susceptibilidad antibióticos) y genotípicamente (grupos filogenéticos y genes de virulencia).
Resultados: Los grupos filogenéticos B2 (60%) y F (12%) fueron significativamente predominantes en la localidad del centro con mayor frecuencia de los genes *fimH* (96%), *iutA* (66%) y *sat* (36%) en comparación con la localidad en el suroeste, donde los grupos A (35%) y B1 (21%) fueron más frecuentes y presentaron menor cantidad de genes de virulencia. El 21,5% del total de aislamientos pertenecieron al grupo O25-ST131. La producción de hemolisina y biopelícula fue significativamente mayor en cepas de la localidad del sureste. La resistencia a ampicilina (92,5%), tetraciclina (76,6%) y trimetoprim/sulfametoxazol (70,1%) fueron las más comunes en ambos grupos.
Conclusión: El grupo filogenético, los factores de virulencia y la susceptibilidad a antibióticas de *E. coli* causante de ITU en la comunidad varían significativamente entre las poblaciones mexicanas estudiadas. Los grupos filogenéticos A y B1 pueden ser multirresistentes y tienen la capacidad de producir infecciones urinarias.

Introduction

Escherichia coli is the most common causal agent of urinary tract infections (UTIs), including acute cystitis, pyelonephritis and urosepsis – the three most common and clinically different syndromes. Normally, *E. coli* establishes a symbiotic relationship with its host and plays an important role in promoting the stability of the normal intestinal microbiota.[1] However, infections caused by extraintestinal *E. coli* are the main cause of morbidity, mortality and high health-related costs. *E. coli* has to adapt to the host's environment (bladder, kidney and bloodstream) and virulence factors play an important role in the initial stages of interaction with the host.[2] There are primarily two types of virulence factors: those expressed on the cell surface (which perform tissue adhesion and invasion functions, as well as forming biofilms and inducing cytosines) and those produced within the bacteria cell, which are exported to the infection site.[3]

The main factors that facilitate the invasion of bladder epithelial cells are type 1 pili, which are expressed in over 90% of all *E. coli* isolates, including both pathogenic and commensal strains. Type 1 pili are composed of repetitions of FimA pilin subunits; the distal part of the pilus is formed from two adapters (FimF and FimG proteins) and the mannose-bound FimH adhesin. This FimH adhesin mediates the adherence of the bacteria to a series of glycoproteins and non-glycosylated peptide epitopes in the epithelium of the bladder, which lead to the internalisation of the bacteria, forming intracellular bacterial communities.[4–6] While the P pilus (pilus associated with pyelonephritis) has been found in approximately 80% of the isolates causing upper UTIs,[7] the different structural subunits of the P fimbriae are coded by the pap operon.[8]

Three types of toxins are produced by uropathogenic *E. coli* (UPEC): α-haemolysin, cytotoxic necrotising factor type 1 (CNF1) and the secreted autotransporter toxin (Sat). α-Haemolysin (HlyA), also known as the "pore-forming toxin", enters the cell membrane of the host, causing cell lysis, thereby facilitating the release of iron and nutrients that are essential for bacterial growth.[9] CNF1 leads to constitutive activation of members of the Rho family, resulting in the rearrangement of the host cell cytoskeleton and causing apoptosis of the bladder cells, stimulating their *in vivo* exfoliation.[10] The Sat toxin is a serine protease that is classified within the family of serine protease autotransporters of *Enterobacteriaceae* (SPATE), which are primarily found in strains of UPEC and are characterised by their cytopathic effects on the kidney and bladder; this toxin may induce vacuolisation in the cytoplasm of the uroepithelial cells.[11]

Based on the relationships of similarity assessed using electrophoresis techniques for different enzymes and gene sequencing (MLST), phylogenetic groups have been determined.[12] Clermont et al.[13] have developed a quadruple PCR assay that recognises seven phylogenetic groups (A, B1, B2, C, D, E, F), one of which is called clade I. Moreover, there is evidence that certain *E. coli* serotypes are associated with UTIs: O1, O2, O4, O6, O7, O8, O16, O18, O25 and O75.[14,15]

The clinical management of UTIs is complicated due to the increasing incidence of infections caused by *E. coli* strains that are resistant to commonly-used antibiotics and which produce biofilms. Recently, a multidrug-resistant extended-spectrum beta-lactamase-producing *E. coli* clone (O25-ST131) with a high virulence has emerged around the world as a significant cause behind community-acquired UTIs.[16]

Despite the identification of multiple virulence-associated genes in UPEC strains, it has not been possible to determine a urovirulence profile, given that half of all UPEC isolates do not contain any or only one of the virulence factors identified. The objective of this study was to evaluate the relationship between virulence factors (serotyping, adherence capacity, biofilm and toxin production) and the resistance profile with phylogenetic groups of *E. coli* in UTI isolates of outpatients in two Mexican localities.

Methodology

Bacterial isolates

In the period between September 2010 and August 2011, clinical isolates of *E. coli* were recovered from the urine samples of patients with community-acquired UTIs. Only one strain was worked on per patient, and they were from acute uncomplicated cystitis (outpatients, non-pregnant women and no other concomitant disease). The urine cultures were processed using conventional methods and included samples with a viable count of $>10^5$ CFU/ml. Clinical isolates were identified using the semi-automated API20E system (BioMérieux) and biochemical tests. Fifty strains were recovered from clinic number 61 of the Mexican Institute of Social Security (IMSS) and 57 from the ISSSTE hospital in Chilpancingo, Guerrero, located in central and south-western Mexico, respectively.

Susceptibility tests

Antibiotic susceptibility tests were carried out using the disc diffusion method (Oxoid Ltd, Basingstoke, UK) as per the guidelines of the Clinical and Laboratory Standards Institute.[17] Isolates with a resistance to three or more classes of antibiotics were considered to be multidrug-resistant (MDR).