BRIEF REPORT

PRESENCE OF *fim*H AND *afa* GENES IN URINARY ISOLATES OF EXTENDED-SPECTRUM BETA-LACTAMASES PRODUCING *Escherichia coli* IN LIMA, PERU

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This study is part of Matta Chuquisapon J.' s undergraduate thesis: Frequency of *fimH* and *afa* genes in extended-spectrum beta-lactamases producing *Escherichia coli* isolated from urine cultures, Faculty of Medicine, Universidad Nacional Mayor de San Marcos, presented in 2018 in Lima.

ABSTRACT

Descriptive study conducted in order to determine the presence of the *fimH* and *afa* genes in urinary isolates of extended-spectrum beta-lactamases (ESBL) producing *Escherichia coli*. Isolates from project TO-06/09 of the Instituto Nacional de Salud del Niño in Lima, Peru were used. A total of 75 urinary isolates of *Escherichia coli* were included. Gene identification was performed by polymerase chain reaction. From the 75 isolates, 74 (98.7%) were positive for the fimH gene and 6 (8.0%) were positive for the afa gene. Virulence factors produced by the fimH and afa genes were evident in urinary isolates of ESBL producing *Escherichia coli*.

Keywords: Uropathogenic *Escherichia coli;* Virulence Factors; beta-Lactamases; Peru (source: MeSH NLM).

INTRODUCTION

Urinary tract infections (UTIs) in children can affect both the upper and lower urinary tract, causing several urinary disorders, such as cystitis and pyelonephritis. During childhood, approximately 6% to 8% of pediatric patients with urinary symptoms have a UTI [1,2]. Frequency varies according to several factors, such as age and gender. It is more common in girls and uncircumcised boys. UTIs in children with urinary tract abnormalities such as neurogenic bladder or vesicoureteral reflux may result in irreversible kidney damage [3,4].

UTIs are caused by a group of microorganisms known as uropathogens, which can minimize the host's immune response and invade the urinary system with uropathogenic *Escherichia coli* (UPEC), causing 85% of episodes of acute cystitis in humans. This pathotype has virulence factors that allow it to adhere to and invade tissues, besides, these factors determine the capacity for infection, chronicity, recurrence and the possibility of dissemination to other tissues [5,6].

Among the most frequent virulence factors of UPEC are fimbriae (P and type 1); adhesins, such as *fimH*, *S, M FIC, Dr/afa, Sfa;* and systems for the uptake of iron (aerobactins),

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alpha hemolysin and other enzymes with protease activity [5]. *fimH* adhesin is present in more than 80% of *Escherichia coli* strains that cause UTIs. This adhesin is responsible for generating the adhesion of the bacteria to the urinary tissue, thus favoring colonization and subsequent invasion of the urothelium [6]. *afa* adhesin appears in no more than 40% of UPEC but is a key element in the development of infections in children and pregnant women because of its ability to cause complications [7]. In addition, antimicrobial susceptibility profiles of UPECs need to be constantly updated to provide adequate empirical treatment for urinary tract infections, as these may vary according to origin, geographical region or institution [8].

The main mechanism of resistance to beta-lactams in enterobacteria is the production of extended-spectrum beta-lactamase (ESBL). Since ESBL have the capacity to hydrolyze most of the beta-lactams (except carbapenemics and cephamycins), it is a pattern of multi-resistance, which causes a serious therapeutic problem. This explains its association with higher mortality, hospital stay and increased economic cost [9]. Therefore, the objective of this study was to determine the presence of the *fimH* and *afa* genes in urinary isolates of ESBL-producing *Escherichia coli*.

KEY MESSAGES

Motivation for the study: Adhesins (such as *fimH* and *afa*) are responsible for the colonization, invasion and chronicity of uropathogenic Escherichia coli infections.

Main findings: In urinary isolates of ESBL producing *Escherichia coli*, the *fimH* gene was present in 98.7%, and the *afa* gene, in 8.0% of the samples.

Implications: The presence of *fimH* and *afa* in isolates of ESBL-producing *Escherichia coli* from pediatric patients could indicate a relationship between adhesins and age group. A possible relationship was found between being not sensitive to amikacin and the *afa* gene.

THE STUDY

A descriptive study conducted to evaluate urinary isolates of ESBL-producing Escherichia coli (the bacteria were collected between August 2012 and January 2013) from the strain obtained from project TO-06/09 of the Instituto Nacional de Salud del Niño (Molecular detection and characterization of extended-spectrum beta lactamases in *E. coli* and *K. pneumoniae* isolated at the Instituto Nacional de Salud del Niño). A total of 75 consecutive non-repeated isolates were recovered from urine samples from pediatric patients in the inpatient and outpatient departments.

Molecular detection was performed at the Laboratory of Molecular Epidemiology and Genetics of the Instituto de Medicina Tropical Daniel A. Carrión - Universidad Nacional Mayor de San Marcos (UNMSM). Total DNA was used as a mold. The *fimH* gene was amplified by the polymerase chain reaction (PCR) method according to Tolentino's protocol [10]. For the *afa* gene, a protocol was standardized in this study considering the concentration of primers, Taq polymerase DNA, hybridization temperature and mold DNA concentration.

IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA) was used to report absolute and relative frequencies for the variables of interest that were obtained from the strain database.

The study protocol was approved by the UNMSM School of Medical Technology. The study follows the good practice and ethics in biomedical research guidelines.

RESULTS

Out of the 75 ESBL-producing *Escherichia coli* isolates from urine cultures, 74 (98.7%) were positive for the *fimH* gene, and 6 (8.0%) were positive for the *afa* gene. In the descriptive analysis of the variables (Table 1), the frequency of *fimH* gene-producing *Escherichia coli* according to its origin was 31.1% in a hospital setting and 68.9% in the community. For the afa gene, the frequency was 16.7% in a hospital setting and 83.3% in the community. Out of the total number of isolations, 54 (72%) were female and the median age was 3 years. No link was found between the virulence genes and the gender, age and location variables. The antibiotic susceptibility profile from ESBL-producing *Escherichia coli* isolates is shown in Figure 2. The relationship between the presence of virulence genes and non-sensitivity to antibiotics was evaluated. An association was found between non-sensitivity to amikacin and the presence of the *afa* gene (Table 2).

In the PCR standardization for the *afa* gene, temperature gradient was performed and the best hybridization temperature was obtained at 62 °C, with a minimum concentration of primers at 1 µM, and of Taq polymerase DNA at 0.5 U/rx. The minimum concentration of DNA detectable by the test was 400 pg/dL (Figure 1).

DISCUSSION

The results of this study show that 98.7% of ESBL producing *Escherichia coli* isolates recovered from a pediatric po-

Table 1. General distribution of *Escherichia coli* virulence genes

Characteristics	*afa* gene		*fimH* gene	
	Yes (%)	No (%)	Yes (%)	No (%)
Sex				
Male	2 (9.5)	90 (90.5)	21 (100)	-
Female	4 (7.4)	50 (92.6)	53 (98.2)	1 (1.8)
Age (years)	-			
<1	1 (5.0)	19 (95.0)	20 (100)	-
1	1 (7.7)	12 (92.3)	13 (100)	-
2	-	3 (100)	3 (100)	-
3	1 (16.7)	5 (83.3)	5 (83.3)	1 (16.7)
4	1 (20.0)	4 (80.0)	5 (100)	-
6	-	6 (100)	6 (100)	-
7	-	7 (100)	7 (100)	-
8	2 (40.0)	3 (60.0)	5 (100)	-
9	-	1 (100)	1 (100)	-
11	-	1 (100)	1 (100)	-
12	-	2 (100)	2 (100)	-
13	-	5 (100)	5 (100)	-
14	-	1 (100)	1 (100)	-
Localization				
Community				
Outpatient	4 (9.1)	40 (90.9)	44 (100)	-
Emergency	1 (12.5)	7 (87.5)	7 (87.5)	1 (12.5)
Hospital				
Internal medicine	-	5 (100)	5 (100)	-
Neonatology ICU	-	4 (100)	4 (100)	-
Orthopedics	-	2 (100)	2 (100)	-
Pneumology	-	1 (100)	1 (100)	-
ICU	-	2 (100)	2 (100)	-
Neurology	-	2 (100)	2 (100)	-
Nephrology	-	2 (100)	2 (100)	-
Cardiology	1 (50.0)	1 (50,0)	2 (100)	-
Surgery	-	1 (100)	1 (100)	-
Urology	-	1 (100)	1 (100)	-
Gynecology	-	1 (100)	1 (100)	-

ICU: Intensive Care Unit

Table 2. Virulence genes according to antibiotic susceptibility.

Antibiotics	*afa* gene		*fimH* gene	
	Yes (%)	No (%)	Yes (%)	No (%)
Amikacin				
Not sensible	3 (33.3)	6 (66.7)*	9 (100)	-
Sensible	3 (4.5)	63 (95.5)	65 (98.5)	1 (1.5)
Gentamicin				
Not sensible	3 (6.7)	42 (93.3)	45 (100)	-
Sensible	3 (10.0)	27 (90.0)	29 (96.7)	1 (3.3)
Ciprofloxacin				
Not sensible	6 (8.8)	62 (91.2)	67 (98.5)	1 (1.5)
Sensible	-	7 (100)	7 (100)	
Imipenem				
Not sensible	-	-	-	-
Sensible	6 (8.0)	69 (92.0)	74 (98.7)	1 (1.3)
Meropenem				
Not sensible	-	-	-	-
Sensible	6 (8.0)	69 (92.0)	74 (98.7)	1 (1.3)
Trimethoprim-sulfamethoxazole				
Not sensible	5 (9.3)	49 (90.7)	53 (98.5)	-
Sensible	1 (4.8)	20 (95.2)	21 (100)	-
Nitrofurantoin				
Not sensible	1 (14.3)	6 (85.7)	7 (100)	-
Sensible	5 (7.4)	63 (92.6)	67 (98.5)	1 (1.5)

*p-value < 0.05 with Chi-square test

pulation presented the *fimH* gene. This finding is similar to that reported by Kim, *et al.* [11], who observed the presence of fimH adhesin in the total number of *Escherichia coli* isolates from pediatric urine cultures and found a relationship between this adhesin and the phylogroups B2 and D. Likewise, Tabasi, *et al.* [12] found, after studying isolates from adult patients with UTIs, that 100% of *Escherichia coli* isolates carried the *fimH* gene from isolates of UTI patients in the adult population. Similarly, Rahdar, *et al.* [13] detected the fimH gene in 95% of UPEC isolates and found no relationship between the presence of the *fimH* gene and *Escherichia coli* phylogroups. In 2009, Berry, *et al*. described the mechanism of action for *fimH* adhesin, which acts and interacts with urothelium, allowing UPEC to enter and form intracellular bacterial colonies (IBC) after the first 6 hours of infection [14]. IBCs are responsible for the recurrence, chronicity, and formation of bacterial reservoirs in the urothelium [15].

Regarding the *afa* gene, we reported a frequency of 8.0% in the studied UPEC; this is consistent with the findings of Ramirez [16], who reported a frequency of 8.2% of the *afa* gene in multiresistant UPEC strains. In contrast, Tabasi, *et al.* [12], reported a frequency of 29.5% for the *afa* gene, and a relationship between cystitis and recurrent infections with the presence of the afa gene. On the other hand, Servin considers that the presence of the afa gene (subtype *afaE*) is more frequent in *Escherichia coli* that causes pyelonephritis [7]. Tajbakhsh, *et*

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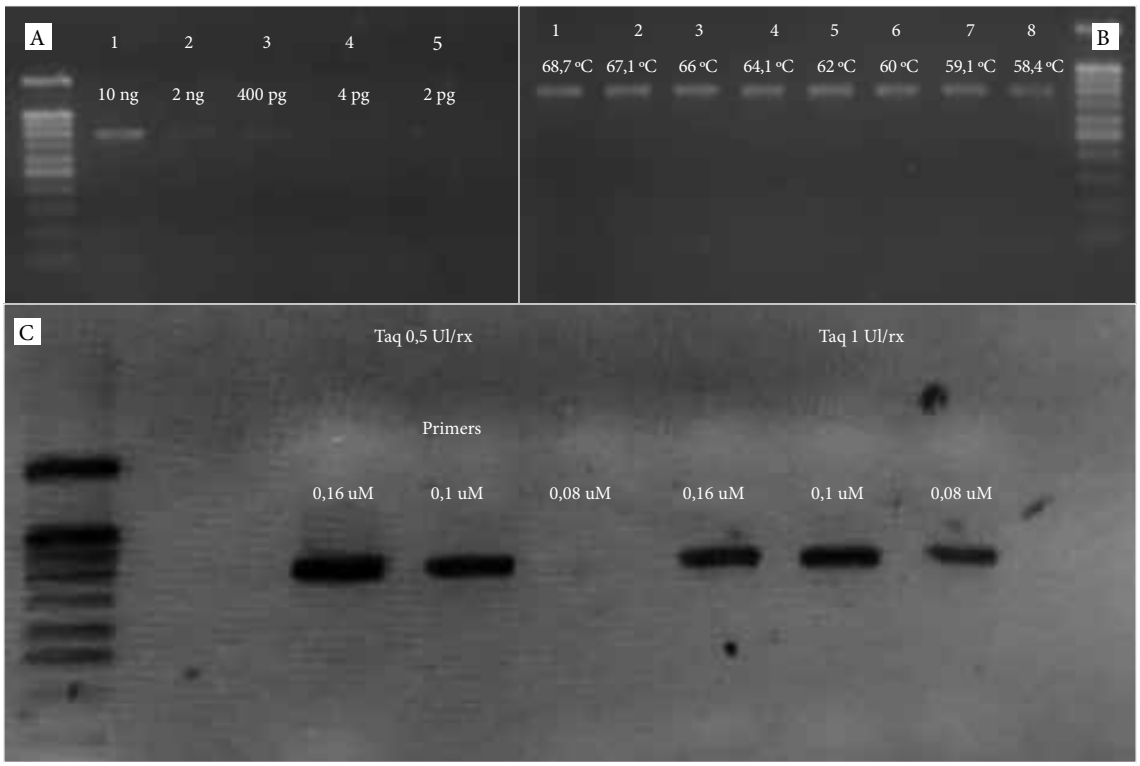

Figure 1. Standardization of the DNA polymerase chain reaction for the *afa* gene in a 2% ladder agarose gel of 100 bp. **A.** Concentration gradient to evaluate the PCR sensitivity for the *afa* gene (molecular weight of the *afa* gene 750 bp). The minimum concentration detected was 400 pg. **B.** Temperature gradient for the standardization of the hybridization temperature. **C.** Simultaneous standardization of the concentration of primers and Taq polymerase DNA. It was concluded that the optimal concentration of primers is 0.1 µM with 0.5 IU Taq concentration.

al. reported that 32% of UPEC isolates had the *afa* gene, and found a significant association between the presence of *afa* and biofilm production ($p<0.05$), a characteristic that was also associated with the presence of beta-lactamases [17].

In 2017 Souza, *et al.* reported a 9% frequency of the *afa* gene in UPEC isolates [18] but found no significant association between the gene and the phylogroup, nor with the gender of the patient from which it was isolated. Furthermore, they showed that the presence of the *afa* gene and being not sensitive to amikacin were significantly associated. Which differs from what was reported by Malekzadegan, *et al.* [19], who found no association between the *afa* gene and no being sensitive to amikacin, but did find an association between the presence of the *afa* gene and the production of ESBL.

The study has some limitations that are relevant to address. The results obtained correspond to a single center, which could differ according to each population and institution. Furthermore, no other virulence factors of importance in the pediatric population were sought, nor their possible relationship with resistance markers.

In conclusion, the presence of virulence factors produced by the genes *fimH* and *afa* was evidenced in urinary isolates of ESBL-producing *Escherichia coli*. Besides, the standardization and optimization of PCR for the detection of the *afa* gene performed satisfactorily.

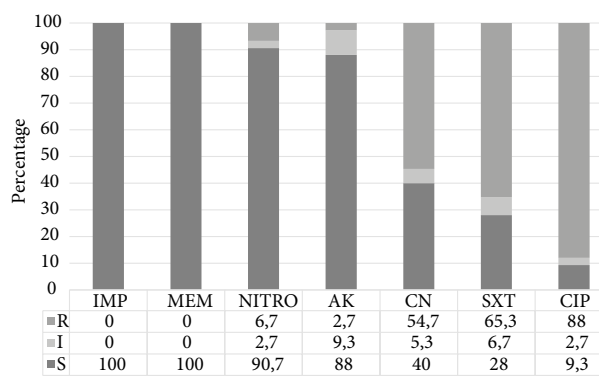

Figure 2. Antimicrobial Resistance Profile of ESBL-producing *Escherichia coli* isolates (n=75). R: resistant; I: intermediate; S: susceptible; IMP: imipenem; MEM: meropenem; NITRO: nitrofurantoin; AK: amikacin; CN: gentamicin; SXT: sulfamethoxazole-trimethoprim; CIP: ciprofloxacin.

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