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Research Article

Horizontal Transference of Antimicrobial Resistance Genes between a Non Pathogenic *Escherichia coli* and a Pathogenic Shiga Toxin-Producing *E. coli* Strain

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Abstract

A consequence of an inappropriate use of antimicrobials in veterinary medicine is the spreading of antibiotic resistance, which implies a problem for public health due to the potential risk of resistance transference through the food chain by bacteria such as Shiga Toxin- producing *Escherichia coli* (STEC). Bacteria can acquire resistance genes by horizontal transference of integrons. Integrons are genetic elements which carry an integrase (IntI). Those carrying the IntI1 (Class 1) are the most common and are related to antibiotic resistance. In the present work, a conjugation assay was performed with two *E. coli* strains to determine the horizontal transference of *intI1* from an antibiotic resistant bacteria isolated from a pig farm, to a pathogenic bacteria. At 4h of assay, initially negative *intI1* bacteria had acquired the integrase and they had probably become resistant to antimicrobials. Animals and farm environment can act as reservoirs for potential spread of resistant bacteria by means of integrons, allowing negative *intI1* bacteria (probably pathogenic) to become resistant to antibiotics in a very short period of time, constituting a problem for the future treatment of such bacterial infections.

Keywords: *E. coli; Integrons; Antimicrobial Resistance; Horizontal Transference*

Introduction

For more than 50 years, antibiotics have been an essential component in the treatment of infectious diseases. However, a non-rational use has been given to them over the years. The spreading of antibiotic resistance is a consequence of this intensive and inappropriate use, being currently a global problem in human and veterinary medicine. Both antibiotics for human use and those for animal or botanical use can promote the spreading of resistance genes and the emergence of resistant bacteria [1] (Newell., *et al.* 2010; Bush., *et al.* 2011). There is a close relationship between the amounts of antimicrobials used and the rate of resistance development [2-4]. The use of antimicrobial

agents in animal production, including the prescription of antibiotics as growth promoters, is the main cause of the development of resistance. This fact is important since it can lead to the selection of resistance genes in non-pathogenic bacteria, which can, subsequently, be transmitted to different species of pathogenic or zoonotic bacteria [5-9]. In addition, bacteria that carry these resistance genes can transfer them to humans through the food chain [10] (White and McDermott, 2006; Deng., *et al.* 2015), constituting a problem for public health by increasing the risk of failure in the usual treatments against infections [11-13]. Bacteria are able to acquire antibiotic resistance genes by horizontal transfer, a mechanism that has led to the rapid emergence of antibiotic resistance [14] (Carattoli, 2013). At present, the role of plasmids and transposons in the multi-resistance of bacteria and in the natural dissemination of resistance determinants is well-known [15]. However, the participation of integrons and resistance gene cassettes has only been studied in recent years [10,16]. Integrons play a fundamental role in the obtention and spreading of antibiotic resistance genes [17] (Hall and Collis, 1995). They are genetic elements identified in multiple resistant bacteria that, due to the action of an integrase (IntI), can incorporate new DNA in cassette gene forms, by a specific site recombination mechanism. They can associate themselves with insertion sequences present in conjugative plasmids or transposons, which serve as a vehicle for their intra or inter-species transmission, although they are not capable to self-transfer [18] (Fluit and Schmitz, 1999; Waldor, 2010). The 5' conserved segment (5'CS) is a fundamental part of an integron. It is composed of the *intIl* gene, the *att* and a promoter. The *intIl* gene encodes a site-specific recombinase and the receptor site (*att)* is adjacent to it [17] (Hall and Collis, 1995). There are two types of integrons: group I, also called 'mobile integrons', related to antibiotic resistance cassettes and group II or 'superintegrons', which are present, unlike the former, at the chromosomal level. Integrons have also been termed 'a genetic construction kit for bacteria' [19]. Integrons are classified according to their integrase (*intI*) gene sequence. Integrase is a tyrosine recombinase enzyme however; it has been found that integrase differs from the other tyrosine recombinase family members in a certain motif that is unique for integrase and is essential for its activity [20]. Integrons are classified in classes based on the aminoacid sequence of the *intI* protein (Cambray., *et al.* 2010). Those carrying *intI1* are defined as 'class I' and those carrying *intI2* as 'class II'. Classes 1 and 2 are the most prevalent and are largely implicated in the spreading of antibiotic resistance, though widespread studies have been the focus of these integron classes [21]. Among them, integrons carrying *intI1* represent the most common structure [22] (Stokes and Hall, 1989) and more than 100 different arrangements of resistance cassettes have been recorded [23]. On the other hand, *intI2* gene is frequently interrupted prematurely by a stop codon, constituting a pseudogene, which would explain the few arrangements that are described in the databases, compared to class I integrons [24] (Recchia and Hall, 1995). According to the previously exposed, isolates carrying integrons, can survive if exposed to antibiotics [25]. This mostly happens in clinical and veterinary isolates and it is responsible for resistance spreading and failure of treatments. In the enteric faecal flora of both animals and humans the occurrence of integrons and gene cassettes has been reported [26-27]. In this regard, the gastrointestinal tract of food animals is the main reservoir of zoonotic agents, site of bacterial propagation [28-29] and hot spot for the exchange of genetic information [6,30-34]. Strains of *Escherichia coli* (*E. coli*) are members of the intestinal microflora of humans and animals. As mentioned, many *E. coli* are harmless and they occur naturally in the gut of animals and humans. One of these, *E. coli*O157, is a harmless germ in cattle which can cause a serious disease in humans. In this regard, Shiga toxin-producing *E. coli* (STEC) cause hemolytic-uremic syndrome and hemorrhagic colitis. The transmission of STEC from animals (reservoirs) to humans occurs mainly through contaminated food and water [35]. Thus, the potential risk of the antibiotic resistance transference through the food chain by *E. coli*, including food borne pathogens such as STEC, implies a huge problem for public health [36-37]. In addition, exposure to resistant bacteria via the food chain has gained increased attention because the presence of resistant bacteria in food and water might have an impact on the development and spreading of antibiotic resistance among human bacterial pathogens [27]. Multiple antibiotic-resistant phenotypes in *E. coli* isolates has been previously reported [38]. It has been suggested that in bacterial populations, resistance may spread from one ecosystem to another by lateral gene transfer of integrons (horizontal transference) [26]. It is important to remark that the presence of integrase is potentially indicative of strains capable of recruiting antibiotic resistance genes [23]. Initially, STEC O157:H7 was found to be susceptible to many antibiotics [30,39]. However, several studies have documented antibiotic resistance among O157 STEC isolates [40-42]. Non-O157 STEC strains isolated from humans and animals have also developed antibiotic resistance, and many are resistant to multiple antimicrobials commonly used in human and veterinary medicine [40,42-43]. Thus, the study and monitoring of commensal bacteria, such as strains of *E. coli*, is a good indicator of the pattern of resistance within a population because

they are frequently found and readily acquire resistance [29,38,44-46]. Therefore, it must be highlighted the potential risk that commensal animal and human bacteria strains may become true latent niches of resistance [7,39,47]. Likewise, there are well-founded suspicions that the excretion of antibiotics and/or their active metabolites exert selection pressure on the environmental bacteria, generating a "reservoir" of resistance-coding genes, which transfer from bacteria to bacteria and can reach, eventually, the human population [7,47-50]. According to some authors, saprophytic bacterial strains of the same genus and species, subjected to different degrees of antibiotic pressure, share resistance to the same antibiotics [28,37,47,51-55]. As shown, there is currently a great deal of speculation regarding the role that the therapeutic and subtherapeutic use of antimicrobials in animals has played in accelerating the development and spreading of antimicrobial-resistant bacterial pathogens [5,7,56]. Thus, the aim of this work was to determine the horizontal transference of *intI1* from an antibiotic resistant commensal *E. coli* (both sorbitol and *intI1* positive), isolated from a pig farm, to a pathogenic STEC 0157:H7 (both sorbitol and *intI1* negative) by a conjugation assay.

Materials and Methods

Each *E. coli* strain was cultured separately for 18 h at 37°C and shaked at100 rpm in 30 mL of LB broth. To perform the co-culture, 10 µL of each strain were placed at 37°C and shaked at 100 rpm in 20 mL of LB broth. At 0.5; 2 and 4h, aliquots of the co-culture were seeded on McConkey sorbitol agar. At the different time-points dilutions of the cultures were performed in order to distinguish colonies without difficulty. Sorbitol positive (red) and sorbitol negative (white) colonies were identified, spiked into tubes and allowed to grow in 800 µL of LB broth. Then, DNA was extracted to perform PCR to evaluate the presence or absence of *intI1*. The amplified products were visualized in a 2% agarose gel in TBE buffer containing SyBR Safe. Samples (10 µL) were run for 30 minutes, at 90V and visualized with UV illuminator. Molecular weight markers were included in each gel.

Results and Discussion

At 4 h, six out of nine repetitions corresponding to initially *intI1* negative *E. coli* colonies acquired the *intI1* gene (892 bp).

Figure 1 shows the gel corresponding to 14 samples (0.5h, 2h, 4h) of initially *intI1* negative *E. coli* colonies.

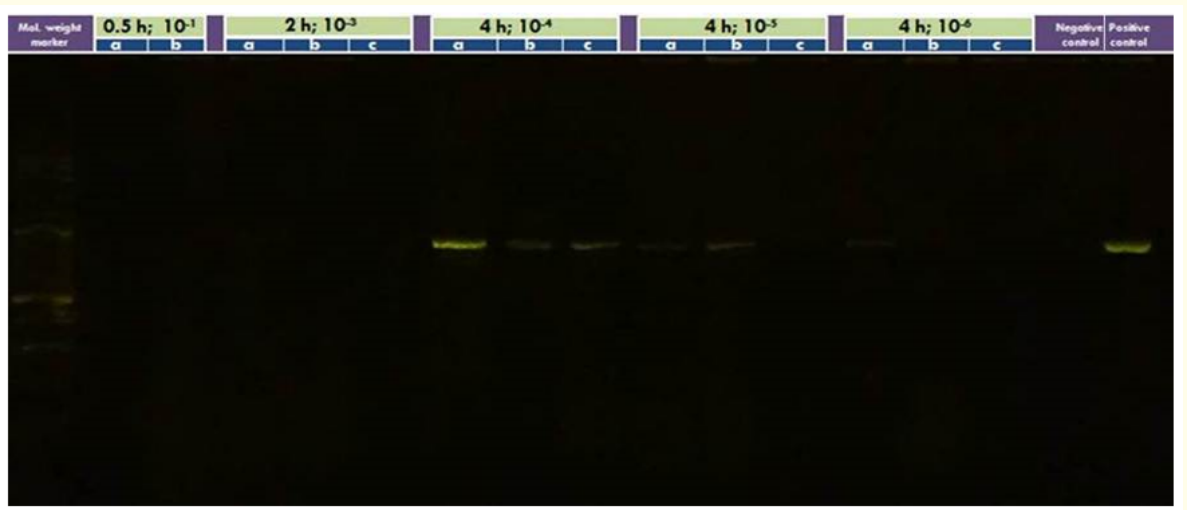

Figure 1: *Electrophoretic gel showing the fourteen samples of initially int1 negative E. coli colonies (Times: 0.5h, 2h and 4h; Dilutions: 10-1, 10-3, 10-4, 10-5 and 10-6; Repetitions: a, b and c).*

On a previous study by our research group it was demonstrated that commensal *E. coli* isolated from farm sows harbored the genes for *IntI1* and/or *IntI2*. It was also confirmed the presence of integrons in strains isolated from piglets younger than 12h of age, suggesting the importance of the transmission of resistant strains at birth [57]. On this work we prove the transference of integrons from an *E. coli* resistant strain isolated from a swine farm, to a negative and pathogenic isolate in a period of only 4h. This fact is extremely important considering our previous findings on pig farms. In this regard, Shin., *et al.* [58], similarly to us, performed conjugation assays between a sensitive *E. coli* strain and tetracycline-resistant isolates from beef cattle. They found that a high percentage (82.9%) of the isolates could transfer a tetracycline resistance gene to a recipient. However, they have not determined at which moment of the conjugation assay the resistance genes transference occurs. Anyway, these findings suggests that the high prevalence of antimicrobial resistant *E. coli* isolates is due to the transferability of resistance genes between *E. coli* populations which have survived the selective pressure caused by the use of antimicrobial agents [49,50].

Conclusion

As it was previously shown by conjugation assay, animals and farm environment can act as reservoirs for potential spread of resistant bacteria by means of integrons, allowing negative *intI1* bacteria to become resistant to antibiotics in a very short period of time, constituting a problem for public health due to the concern that it means for the future treatment of such bacterial infections.

The transmission of resistance elements from commensal to pathogenic bacteria remains a viable source of resistance gene transmission and a significant cause for concern. This work is a first approach to this type of transmission of resistance genes and serves as a trigger for future research. Whilst the transference of the *intIl* element itself is an important observation, it is imperative understanding the frequency dynamics of *intI1* transfer in the broader context which includes the transfer into different pathogenic strains and the transfer into a mixed microbial population. Furthermore, details around the location of the *intIl* element in the commensal bacteria and how that relates to its location following conjugation into the pathogenic strain and, critically, if this transfer also produces an alteration in drug susceptibility, have to be investigated.

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