International Journal of Food Microbiology 270 (2018) 39–43

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Short communication

Evaluation of resistance gene transfer from heat-treated *Escherichia coli*

Laëtitia Le Devendec, Eric Jouy, Isabelle Kempf*

ANSES, Laboratoire de Ploufragan-Plouzané, Ploufragan, France
Université Bretagne Loire, France

ARTICLE INFO

Keywords:
Antimicrobial resistance
Gene transfer
Heat-treated *E. coli*

ABSTRACT

Antimicrobial-resistant *Escherichia coli* may be present in various foods. The aim of this study was to evaluate the impact of heat treatment, simulating food preparation, on the possibility of antimicrobial resistance genes being transferred from *E. coli* cells. The study was performed on antimicrobial-resistant *E. coli* cells in suspension in a sterile saline solution. The stability of resistance genes and the possibility of their transfer by transformation or conjugation were analyzed. Results showed that antimicrobial-resistant *E. coli* cells managing to survive after a few minutes at 60 °C retained their antimicrobial resistance. No plasmid could be transferred by conjugation from antimicrobial-resistant *E. coli* cells heated to 60 °C for ten or more minutes. Twelve electroporation experiments were performed using a bacterial suspension heated to 70 °C for 30 min. Genes coding for resistance to extended-spectrum cephalosporins, tetracycline or sulfonamides were transferred to an *E. coli* DH5α recipient on two occasions. In conclusion we showed that heat-treated *E. coli* may occasionally transfer resistance genes.

1. Introduction

Resistance to antimicrobials in general, and to extended-spectrum cephalosporins (ESCs) in particular, is a global health concern. ESC-resistant *Enterobacteriaceae* may be harbored in the microbiota of healthy animals arriving at the slaughterhouse and spoil the meat (Aarestrup et al., 2008). Retail vegetables have also been shown to carry ESBL- and AmpC-producing *Enterobacteriaceae* (van Hoek et al., 2015). ESC-resistant *Enterobacteriaceae* may be responsible for human infections such as community-acquired urinary tract infections. Humans may be contaminated via the food chain, by direct contact or via the environment (Evers et al., 2017). Thus, the consumption or handling of contaminated food may result in the transmission to humans of ESBL- or pAmpC-producing bacteria, or genes coding for resistance to ESCs and other antimicrobials (EFSA-Panel-on-Biological-Hazards, 2011). These ESC resistance genes are usually borne by conjugative plasmids, which can be transferred from donor to recipient bacteria in vitro. Conjugative transfer has also been evidenced in an in situ continuous flow culture system simulating the human cecum and the ascending colon, inoculated with an ESC-resistant *E. coli* of avian origin (Smet et al., 2011). Such a transfer of resistance plasmids has also been evidenced in environments as varied as on a hand towel or a cutting board (Kruse and Sorum, 1994). Transformation, consisting in the uptake of naked DNA by competent cells, has also been described for a long time in Gram-positive bacteria, but the importance of this mechanism of genetic transfer between *Enterobacteriaceae* under natural conditions has received little attention (Baur et al., 1996). The aim of this study was to better understand the hazards associated with contaminated food. Therefore we decided to evaluate the possibility of ESC-resistant genes being transferred from *Enterobacteriaceae* having undergone various heat treatments simulating food preparation. The stability of resistance genes and the possibility of their transfer by conjugation or transformation were then analyzed.

2. Material and methods

2.1. Bacterial strains

Five different ESC-resistant *E. coli* were chosen from our collection. Four strains (Nos. 2, 40, 174 and 241) were from pullets or layers of different ages and from different regions (Chauvin et al., 2013), and one strain (05-M63-1) was from a healthy pig at a slaughterhouse (Fleury et al., 2015). All five strains had previously been shown to carry either a $bla_{CTX\text{-}M\text{-}1}$ or a $bla_{CMY\text{-}2}$ gene on a conjugative plasmid (data not shown). The $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CMY\text{-}2}$ genes are the two most prevalent genes in ESC-resistant *E. coli* of animal origin in France (Touzain et al., in press). The main characteristics of the strains, determined previously, are reported in Table 1.

Abbreviations: ESC, Extended-spectrum cephalosporin; MC, MacConkey; MH, Mueller Hinton

* Corresponding author at: ANSES, Ploufragan Laboratory, Mycoplasmology Bacteriology Unit, 22440 Ploufragan, France.
E-mail address: isabelle.kempf@anses.fr (I. Kempf).

https://doi.org/10.1016/j.ijfoodmicro.2018.02.019
Received 24 August 2017; Received in revised form 8 February 2018; Accepted 16 February 2018
Available online 18 February 2018
0168-1605/

Table 1
Characteristics of the ESC-resistant strains and transformants.

E. coli strain	Origin	Resistances of the strain	Replicon type of the ESC plasmid	$bla_{CTX-M-1}$	bla_{CMY-2}	*tetA*	*sul2*	Phylogenetic group
2	Hen	ESC, TET, KAN	Inc I1	+	−	+	+	A
2-TF-T1	Transformant from strain 2 heated to 70 °C for 30 min	TET, NAL[a]	NA	−	−	+	+	A
40	Hen	ESC, TET, SXT	Inc I1	+	−	+	+	B1
174	Hen	ESC, TET, STR	Inc A/C	−	+	+	+	A
241	Hen	ESC, TET	Inc I1	+	−	+	+	B2
05-M-63-1	Pig	ESC, TET, SXT	IncI1	+	−	+	+	B1
05-M-63-1-TF-C1 to 4	Transformants from strain 05-M-63-1 heated to 70 °C for 30 min	ESC, SXT, NAL[a]	NA	+	−	+	+	A

NA: not applicable.
ESC: extended-spectrum cephalosporins, KAN: kanamycin, NAL: nalidixic acid, STR: streptomycin, SXT: trimethoprim-sulfamethoxazole, TET: tetracycline.
[a] The recipient *E. coli* is resistant to NAL and belongs to phylogenetic group A.

2.2. Heating and titration of bacterial suspensions

For the initial trial, bacteria were grown overnight at 37 °C on Mueller Hinton (MH) agar, then 0.5 McFarland suspensions of each strain were prepared from plates in a sterile saline solution. For each strain, 28 tubes containing 2 mL of the suspension were prepared and each vial was submitted to one of eight different temperatures in a water bath (40 °C, 50 °C, 60 °C, 70 °C, 80 °C, 90 °C or 100 °C) for different durations (5 min, 10 min, 20 min, 30 min or 60 min). Tubes were placed in a water bath, allowed to reach the desired temperature, and then kept at that temperature for the desired time. At the end of the heat treatment, the suspensions were left to cool at room temperature and the numbers of CFU/mL were determined on MacConkey (MC) agar plates incubated overnight at 37 °C as described below. No attempt was made to recover sub-lethally injured cells. Antibiotic resistance genes were detected by PCR and the possible transfer of resistance genes to recipient *E. coli* by conjugation or transformation was analyzed.

To determine the number of colony-forming units in the heat-treated suspensions, three decimal dilutions (10^{-3} to 10^{-5}) for tubes heated to 40 °C or 50 °C were prepared and 50 µL of each dilution was inoculated onto agar plates using an EasySpiralDilute inoculator (Interscience, Saint-Nom-la-Bretèche, France). The undiluted suspension was used for tubes heated to 60 °C or more (limit of detection: 20 CFU/mL). After incubation, the colonies observed in predefined segments of the plate were counted and the population was calculated according to the manufacturer's instructions. Moreover, colonies from plates inoculated from the suspensions heated to the highest temperature for the longest time (Table 2) were stored at 4 °C for 5 days before analysis.

2.3. PCR tests

Cellular lysates were prepared (Kellog and Kwok, 1990) from the different bacterial suspensions before and after heat treatment, and from the colonies obtained after heat treatment. The lysates were used to detect *E. coli* DNA by PCR (Perrin-Guyomard et al., 2008) and resistance genes bla_{CTX-M} (Woodford et al., 2006) and bla_{CMY-2} using the primers recommended by the LRUE (http://www.crl-ar.eu/201-resources.htm#primer), *tetA* (Nolvak et al., 2013) or *strA* (Popowska et al., 2012). The amplified products were 133 bp (*E. coli*), 415 bp ($bla_{CTX-M-1)}$, 113 bp ($bla_{CMY-2)}$, 96 bp (*tetA*) or 126 bp (*strA*) long.

2.4. Conjugation

Conjugative plasmids were sought in representative heat-treated suspensions according to a previously described capture method (Le Devendec et al., 2016). Briefly, heated suspensions containing fewer than 20 CFU/mL (eight suspensions heated to 60 °C for 10 min, 30 min or 60 min), or where few cultivable *E. coli* (*E. coli* 174 heated to 60 °C for 10 min) could be detected (Table 2) were mixed with a sterile saline solution or with the rifampicin- and kanamycin-resistant receiving strain, *E. coli* K-12 CV601gfp (Rif^R Thr_Leu_Thi_miniTn5::gfp-nptII), kindly contributed by K. Smalla (Institute for Epidemiology and Pathogen Diagnostics, Federal Research Center for Cultivated Plants, Braunschweig, Germany). Controls with non-heated suspensions mixed with the recipient strains were also prepared. One hundred microliters of the re-suspended pellet obtained from this mixture was deposited via a 0.22 µm filter onto MH agar plates. After overnight incubation at 37 °C, the filter was re-suspended in 1 mL of sterile saline solution and 100 µL of each mixture was inoculated onto MC agar media; MC containing rifampicin (50 mg/L) and kanamycin (50 mg/L); MC containing cefotaxime (8 mg/L); MC containing tetracycline (8 mg/L); MC containing rifampicin (50 mg/L), kanamycin (50 mg/L), and cefotaxime (8 mg/L); and MC containing rifampicin (50 mg/L), kanamycin (50 mg/L), and tetracycline (8 mg/L). The plates were read after 48 h at 37 °C.

2.5. Transformation

We assessed whether resistance genes from heated suspensions could be transferred to the recipient *E. coli* DH5α strain. In the first transformation assay, suspensions from which no bacteria could be isolated (i.e. strains 2 and 40, heated to 70 °C for 30 min; strain 174, heated to 80 °C for 30 min; and strains 241 and M63, heated to 90 °C for 10 min) were used to prepare plasmid DNA with the KingFisher DUO apparatus (Thermo Scientific) and King FisherPure Plasmid kits (Thermo Scientific) according to the manufacturer's instructions. The DNA was used to transform *E. coli* DH5α by electroporation using the Gene Pulser (Bio-Rad, Marnes-la-Coquette) according to standard procedures. The *E. coli* DH5α cultures were then inoculated onto MH agar media containing tetracycline (8 mg/L), sulfonamide (32 mg/L), cefotaxime (8 mg/L) or cefoxitin (8 mg/L).

To confirm the results of the first transformation assay, two further trials were performed. The protocol was modified to take into account the presence of injured cells in the heated suspension by inoculation of 1 mL of each heated suspension into 49 mL of brain heart infusion broth for overnight culture at 37 °C before plating 100 µL onto MC plates. Thus the second transformation assay was performed with two strains (strains 2 and 40). The bacterial populations (12 mL of a 0.5 MacFarland suspension) were enumerated before heating to 70 °C for 30 min. Then, for each strain, 0.5 mL of the heated suspension was used to prepare a cellular lysate; 9 mL was used to prepare plasmid DNA using the KingFisher kit with the DUO apparatus, and 1 mL was used to prepare total DNA using the QIAmp DNA Mini kit (Qiagen, Courtaboeuf, France). The different genes were detected by PCR on the cellular lysates. The plasmid and total DNA were used for *E. coli* DH5α electroporation, and the *E. coli* DH5α suspensions were then inoculated