Plasmid-mediated transformation of extended spectrum β-lactamase (ESBL)-producer phenotype to *E.coli* DH5α competent cells

Introduction

Salmonellosis is the leading foodborne bacterial infection in Armenia. Among *Salmonella* isolates circulating in the region for two decades, the most prevalent multidrug-resistant(MDR) serotype was *S*. ser. Typhimurium. Of note, the majority of clinical MDR *S*. Typhimurium isolates were extended-spectrum β -lactamase (ESBL)-producers and were assigned to ST328[1]. The $bla_{CTX\text{-}M\text{-}5}$ gene predominantly located on the pCTXM5 plasmids was *in silico* identified in all ESBL-producing *S*. Typhimurium isolates from Armenia.

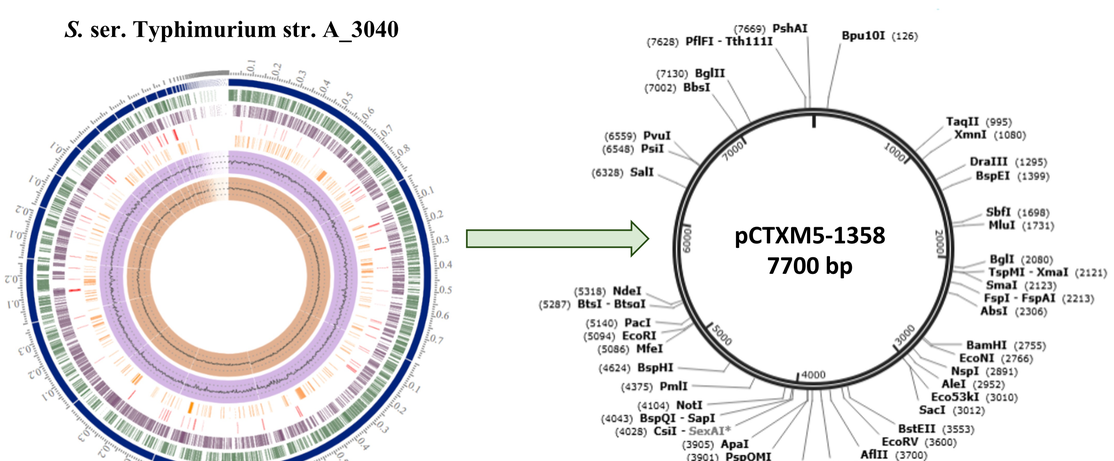
Aim

To assess efficiency of bacterial transformation with the plasmid carrying the $bla_{CTX\text{-}M\text{-}5}$ gene encoding extended spectrum β-lactamase.Introduction

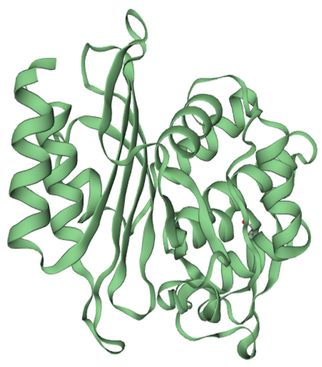
Metodology

Bacterial Strains.
Plasmid DNA donor strain: human *S.* ser. Typhimurium str. A_3040 (ST328; whole genome sequence: ENA Project PRJEB36290, SAMEA6488673).
Antimicrobial resistance phenotype: ampicillin, amoxicillin-clavulanic acid, ceftazidime, ceftriaxone, naidixic acid, chloramhenicol, streptomycin, tetracycline, trimethoprim-sulfamethoxazole; multidrug-resistant; ESBL-producer.

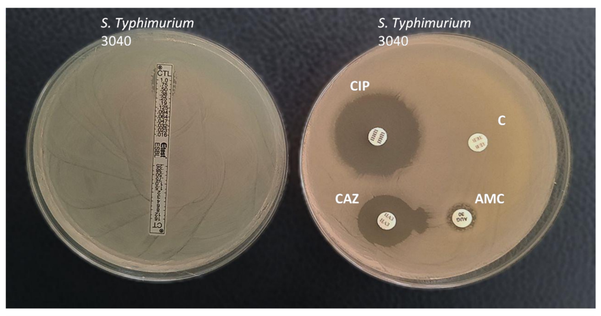
***S.* ser. Typhimurium str. A_3040**



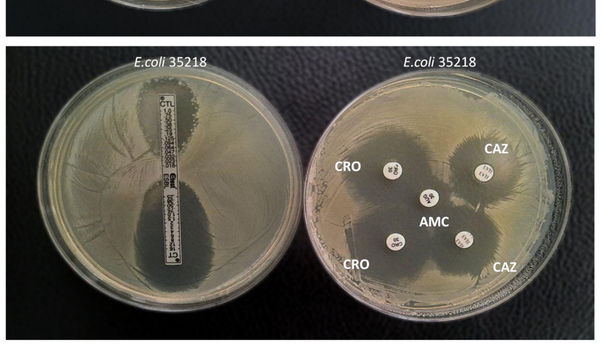
CTX-M-5 β-lactamase



ESBL-producer phenotype of donor strain was confirmed by double-disk synergism test and E-strip-test.

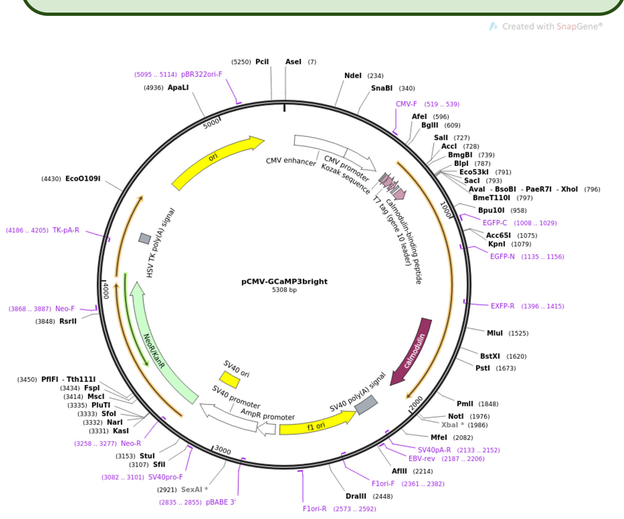


ESBL-positive phenotype



Quality control strain:
***E.coli* ATCC 35218**
(negative)

Recipient strain: *E.coli* DH5a cells carrying pCMV-GCaMP3 plasmid encoding resistance to kanamycin (KM).



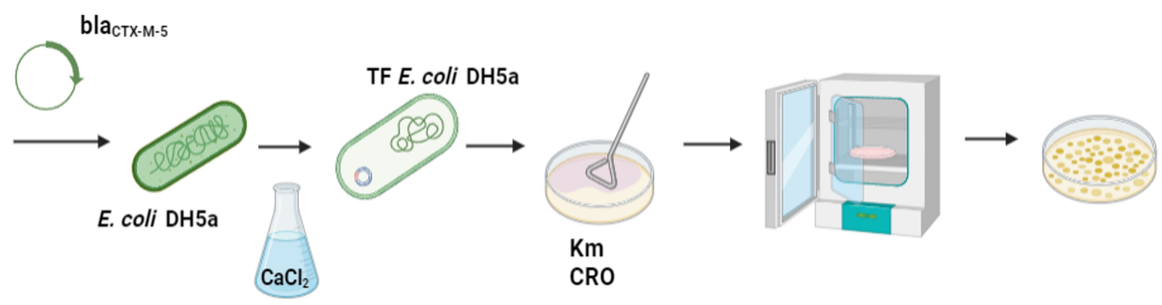
Quality control strain:
***E.coli* ATCC 35218**
(negative)

Conclusions

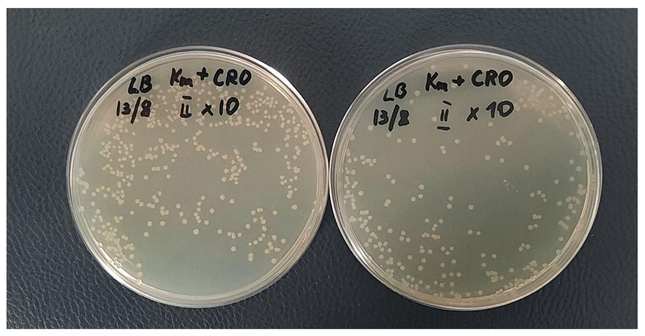
The efficiency of bacterial transformation with the plasmid carrying the $bla_{CTX\text{-}M\text{-}5}$ gene encoding ESBL was assessed. In plasmid transfer experiments, ceftriaxone-resistant transformants carrying the low-molecular-weight plasmids of 8.5 kb (from donor strain) and 5.3 kb (recipient strain) were readily obtained.
The results indicated the possibility of spreading the clinically problematic ESBL-producer phenotype among Enterobacteriaceae.

Plasmid-mediated transformation:

Preparation of competent cells by $CaCl_2$ method was done as described previously [2]. Plasmid DNA extracted from ESBL-producing *S*. Typhimurium A_3040 isolate was transferred by transformation into *E. coli* DH5α cells as described previously [3]. Transformed colonies were selected on agar plates containing ceftriaxone (CRO, 4 mg/liter) and kanamycin (Km, 16 mg/liter). Transformation efficiency was calculated as the number of colony forming units(cfu) per microgram of plasmid DNA used.



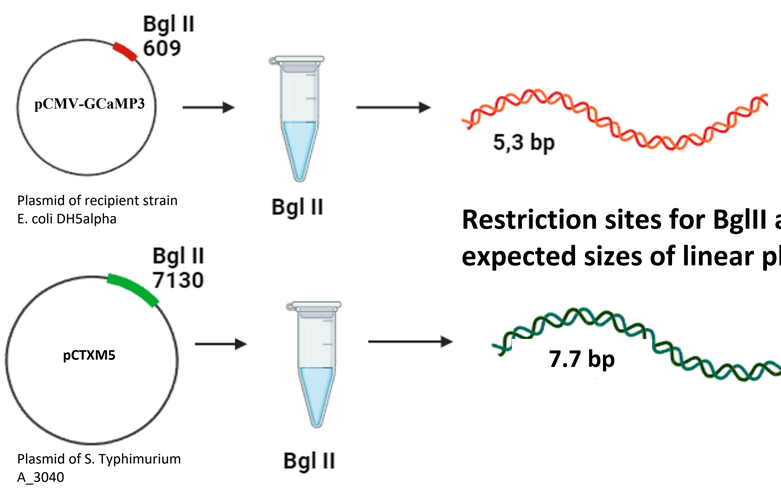
The ceftriaxone-resistant transformants were obtained and transformation efficiency was calculated:



1.6 x 10^4 cfu/µg

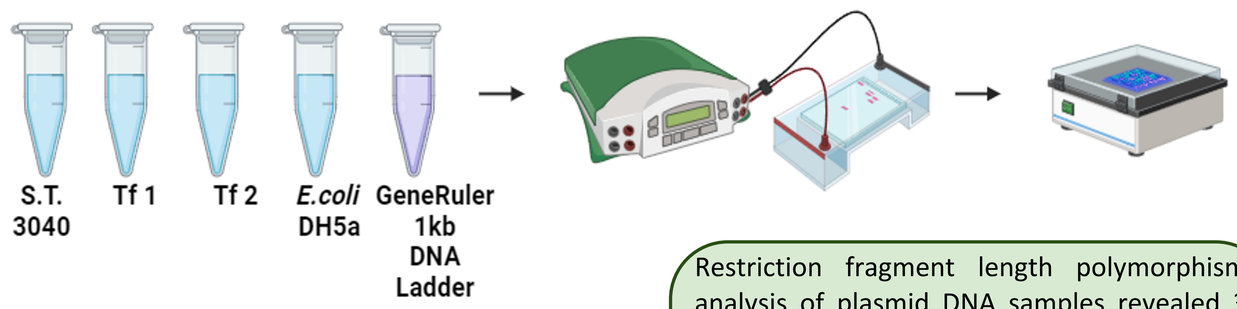
(shown as mean results of two repeated experiments)

Plasmid DNA restriction. Plasmid DNA samples extracted from two transformants (Tf1 and Tf2), as well as from donor and recipient strains, were digested with BglII endonuclease (Thermo Fisher Scientific) according to the manufacturer's recommendations.



Restriction sites for BglII and expected sizes of linear plasmids

The digest products were separated by performing gel electrophoresis in 1% agarose. The 1 KB DNA Ladder (GenRuller) was used as a molecular weight marker.



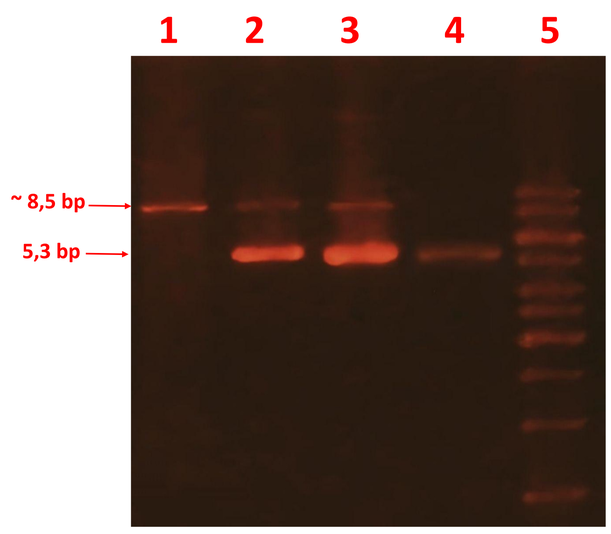


Fig. Restriction fragments: 1 – Donor, 2 – Tf1, 3 – Tf2, 4 – Recipient, 5 - Marker

Restriction fragment length polymorphism analysis of plasmid DNA samples revealed 3 types of restriction profiles that are shown in Fig.

The type I profile is a single band of a linear donor plasmid of ~8.5 kb (line 1), which is larger than predicted by the genome annotation (7.7 kb, **pCTXM5-1358, Genbank JX017308).**

Type III profile is a single band of linear plasmid of recipient strain (line 4), which size is in good agreement with expected, 5.3 kb.

The same profile of restriction fragments (Type II, ~8.5 kb and 5.3 kb) was found in both transformant strains (lines 2 and 3), indicating the presence of both the donor and recipient plasmids.

Literature:
1] Sedrakyan, A.M.; Ktsoyan, Z.A.; Arakelova, et al. Extended-Spectrum β-Lactamases in Human Isolates of Multidrug-Resistant Non-typhoidal Salmonella enterica. Front. Microbiol.2020, 11, 592223.
2] Xiaowei Li, Xin Sui et al. – African J. of Biotechnology. 2010. V. 9 (50). P. 8549-8554.
3] Hanahan D., Jessee J., Bloom F. R. – Methods Enzymol. 1991. V. 20. P. 63-113.