



HAL
open science

Distribution of virulence factors associated with *Escherichia coli* under the One Health concept

Sandra Martínez-Álvarez, Soraya Herrera-Espejo, Myriam Zarazaga, Jean-Yves Madec, Marisa Haenni, Maria Eugenia Pachon Ibañez, Carmen Torres

► **To cite this version:**

Sandra Martínez-Álvarez, Soraya Herrera-Espejo, Myriam Zarazaga, Jean-Yves Madec, Marisa Haenni, et al.. Distribution of virulence factors associated with *Escherichia coli* under the One Health concept. Congress of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID Global), Apr 2025, Vienna, Austria. <10.13039/501100011033>. <ances-05047165>

HAL Id: ances-05047165

<https://ances.hal.science/ances-05047165v1>

Submitted on 25 Apr 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC 4.0 - Attribution - Non-commercial use - International License



Sandra A. Martínez-Álvarez¹, Soraya Herrera-Espejo², Myriam Zarazaga¹, Jean-Yves Madedec³, Marisa Haenni³, M^a Eugenia Pachón-Ibáñez^{2,4}, Carmen Torres¹

¹Area of Biochemistry and Molecular Biology, OneHealth-UR Research Group, University of La Rioja, Logroño, Spain; ²Clinical Unit of Infectious Diseases, Microbiology and Parasitology, Institute of Biomedicine of Seville (IBIS), Virgen del Rocío University Hospital/CSIC/University of Seville, 41013 Seville, Spain; ³National Health Security Agency (ANSES), Antimicrobial Resistance and Bacterial Virulence Unit-University of Lyon, France; ⁴CIBER de Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III, 28029 Madrid, Spain

Contact: Sandra A. Martínez-Álvarez, sandra.martinezal@unirioja.es

PURPOSE AND OBJECTIVES

Escherichia coli is a highly adaptable bacterium with a commensal role in the intestines of humans and animals. However, certain strains have evolved into pathogenic variants, known as pathotypes, each characterized by specific virulence factors. These pathotypes include Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), and Enterohemorrhagic *E. coli* (EHEC). Additionally, Extraintestinal Pathogenic *E. coli* (ExPEC), encompassing Uropathogenic *E. coli* (UPEC), are distinguished by their ability to cause diseases beyond the intestinal tract. All these pathotypes are primarily associated with infantile and traveller's diarrhoea as well as in the case of EHEC enhanced ability to cause infections outside of the intestine (ExPEC) like causing of urinary tract infection (UTI) associated with UPEC pathotype. The dissemination of pathogenic *E. coli* strains within the environment constitutes a significant public health challenge.

The main objectives were to thoroughly investigate the pathotypes of primary clinical significance detected in both livestock and wildlife. *In vitro* studies followed by a phylogenomic approach was employed to examine the genetic frameworks involved in the mobilization of clinically relevant resistance genes that have not previously been considered, as well as the associated virulence factors.

MATERIAL & METHODS

Bacterial strains. Thirty-six isolates from wild birds (n=22), poultry (n=3), pigs (n=7), and chicken meat (n=4) previously characterized by Whole-Genome Sequencing (WGS) and analysed with tools from the Centre of Genomic Epidemiology were included.

Biofilm. An abiotic solid surface biofilm formation assay was performed for all strains using an overnight culture diluted 1:100 in Luria-Bertani broth (~7.5 log CFU/ml) in 96 well flat-bottom microplates and incubated 24 h at 37°C. Biofilm was stained with crystal violet 0.4% (v/v) and quantified at 580 nm after solubilization with ethanol 95%.

Bacterial adherence and invasion in cell culture. Bacterial adherence and invasion assays in A293 cell cultures were carried out with all the strains. To measure the number of adherent bacteria, cells were infected using a 1:1000 dilution of the overnight culture (~6.5 log₁₀ CFU/ml) during 2 h and, after washing five times with PBS 200 µl of trypsin-EDTA was added for 10 min at 37°C. Then, 200 µl of 0.5% Triton X-100 was added for 2 min. The invasion protocol had a 2 h-infection and a treatment with gentamicin 256 µg/ml for 1 h before the addition of trypsin-EDTA. Serial dilutions were plated on sheep blood agar plated to determine the attached and internalized bacteria by A293 cells. All *in vitro* studies described were performed thrice in different days.

Surface motility essays. Overnight cultures of each strain were adjusted to an optic density (O.D.) at 600 nm of 0.6 in PBS. Three µL of the bacterial suspension were placed in the centre of a LB plate containing 0.3% agarose. Plates were incubated at 37 °C with 80% of humidity, and the radius of surface extension was measured at 24 hours of incubation.

Statistical analysis. Variations in bacterial adherence, invasion, biofilm formation, and motility were assessed utilizing the Mann-Whitney U test provided by the software package SPSS (Version 26.0; Armonk, NY: IBM Corp.). The graphical representations depicted were produced using GraphPad Prism 10. All P-values less than 0.05 were regarded as statistically significant.

RESULTS

We identified 47 virulence-related genes, of which 13 were primarily associated with relevant pathotypes. Within the EAEC strains, we observed the pathotypes ExPEC (n=8; *pap*, *sfa/focDE*, *afa/draBC*, *kpsMII*, *iutA*), UPEC (n=9; *chuA*, *fyuA*, *vat* or *yfcV*), and a combination of ExPEC and UPEC (n=10; characterized by a minimum of two of the previously described pathotypes) (Table 1).

In vitro adherence and invasion assays demonstrated that the pathotypes exhibiting the highest colonization rates corresponded to strains carrying genes associated with ExPEC and UPEC (Figure 1a). Furthermore, significant differences were noted in both adherence and invasion when comparing ExPEC with UPEC, as well as the comparison between STEC and EPEC, among other pathotypes (Figure 1b).

Due to the elevated incidence of ExPEC+UPEC and their potential chromosomal localization of the encoding genes, we decided to compare our results regarding genetic lineages:

- ST117 exhibited significant differences in their capacity for cellular invasion, with UPEC demonstrating a higher level of invasiveness (Figure 2b). Secondly, in reference to the comparison of pathotypes and genetic lineages, within the ExPEC and UPEC pathotypes, the high-risk genetic lineages identified in ST117 and ST131 exhibited significant differences in their invasiveness (Figure 2 and Figure 3).

Consequently, the insights derived from the genomic data enabled us to establish a correlation between the *in vitro* results and specific virulence factors. Our approach involved: i) identifying which genes associated with each pathotype were evidently linked based on our findings, and ii) investigating statistically significant relationships between virulence genes and bacterial pathotypes, particularly emphasizing those genes that displayed an exclusive correlation with a single pathotype.

The outcomes of this investigation are encapsulated in a heat map, wherein the associations between pathotypes and genes are illustrated; exclusivity is denoted in pink, while genes detected across multiple pathotypes are highlighted in yellow. Initially, it was observed that the *chuA* gene exhibited a significant association with the UPEC pathotype. Additionally, the *kpsMII* and *pap* genes were linked to both ExPEC and UPEC, the *eae* gene was associated with EPEC, while the *stx1/2* genes corresponded to STEC and the toxins *eltAB-4* and *stap-Sta1* were related to ETEC, as it was well described previously (Figure 4a). Regarding the complementary virulence factors identified, it has been observed that the ExPEC pathotype exhibited a notable specificity for the *hly* gene. In contrast, UPEC demonstrated specificity for the *tsh* gene, both of which play significant roles in cell adhesion. Additionally, the *tia* gene in UPEC is implicated in cell invasion (Figure 4b).

The lack of significant associations for certain pathotypes (EPEC/STEC/ETEC) in this analysis may primarily be attributed to the minimal sample sizes, with totals of only 3/1/1, respectively.

ACKNOWLEDGEMENTS

S.A.M.A. received a predoctoral fellowship FPI from the Government of La Rioja (FPI-CAR-2021-3567). S.H.-E. is supported by the program PFIS, Instituto de Salud Carlos III, (ISCIII, FI21/00280). M.E.P.-I. is a "Nicolás Monardes" researcher (RC1-0006-2023, Servicio Andaluz de Salud, Junta de Andalucía, Spain). This project was partially financed by PID2022-139591OB-I00 (MICIU/AEI/10.13039/501100011033 and EFRD/EU) and by internal funding of ANSES. M.E.P.-I. also received support from the CIBER de K(CIBERINFEC, CB21/13/00006), ISCIII, Ministerio de Ciencia e Innovación, co-financed by the European Development Regional Fund.

RESULTS

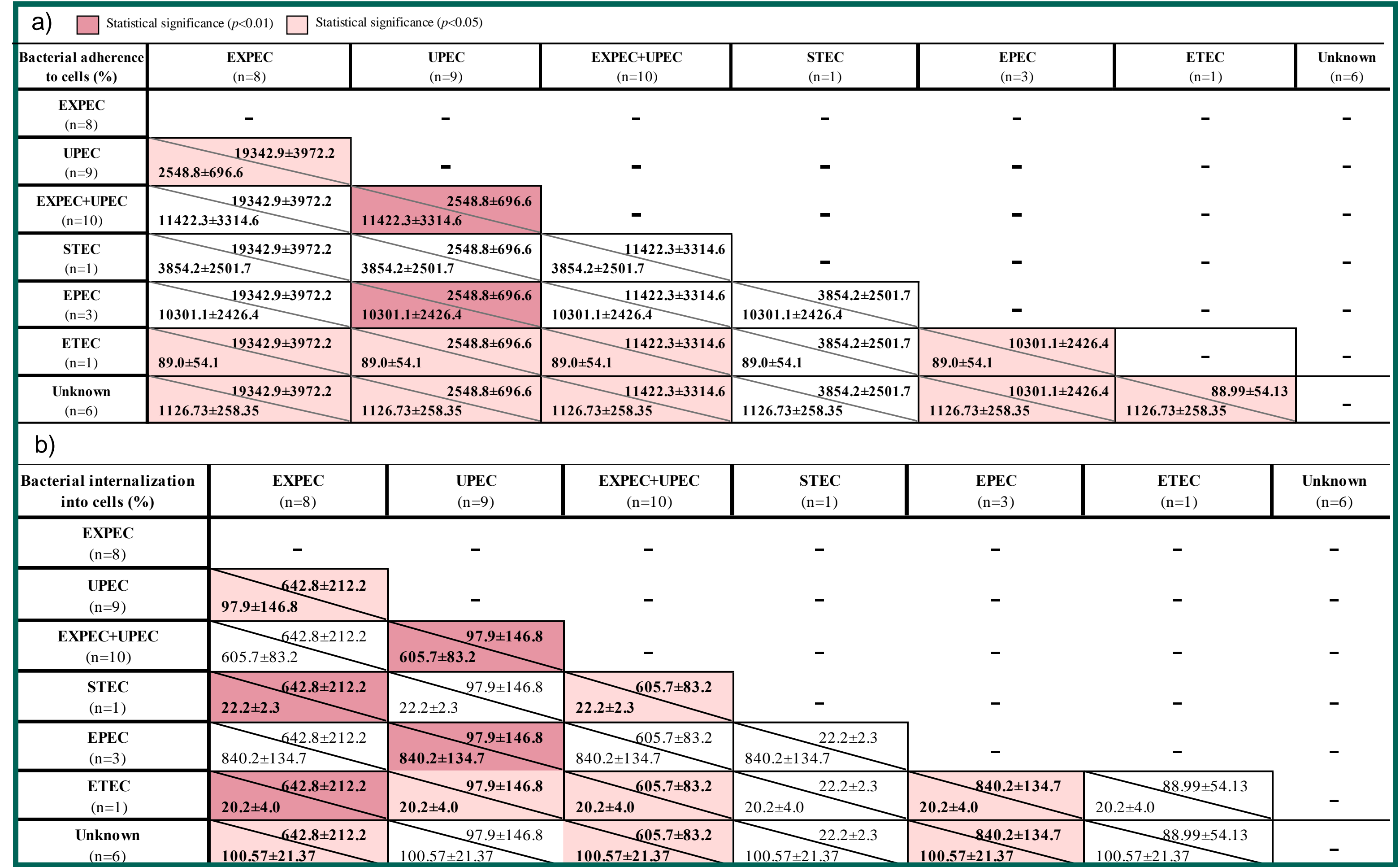


Figure 1. Mann-Whitney U analysis for the 38 *E. coli* isolates grouped by pathotypes for the interpretation of adherence and invasion to epithelial kidney cells (HEK-293 line).

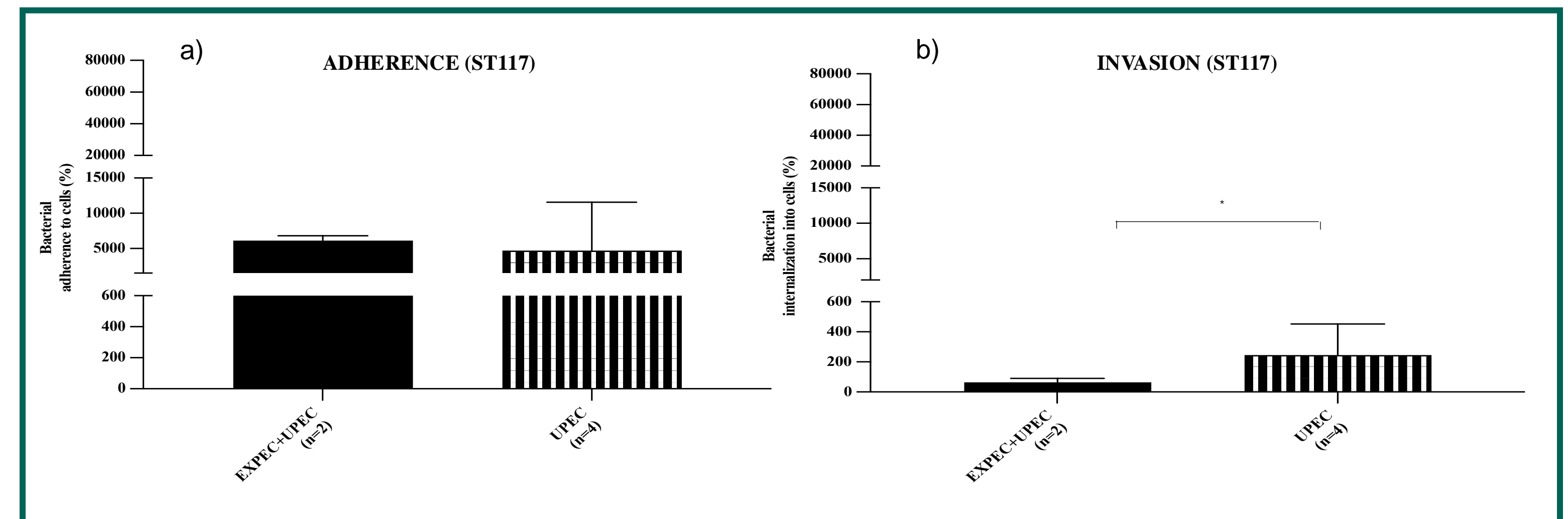


Figure 2. ST117 *E. coli* strains analysed in this study associated with ExPEC+UPEC or UPEC pathotypes to compare the genetic lineage with pathotypes in a) adherence to cells and b) internalization into cells A293.

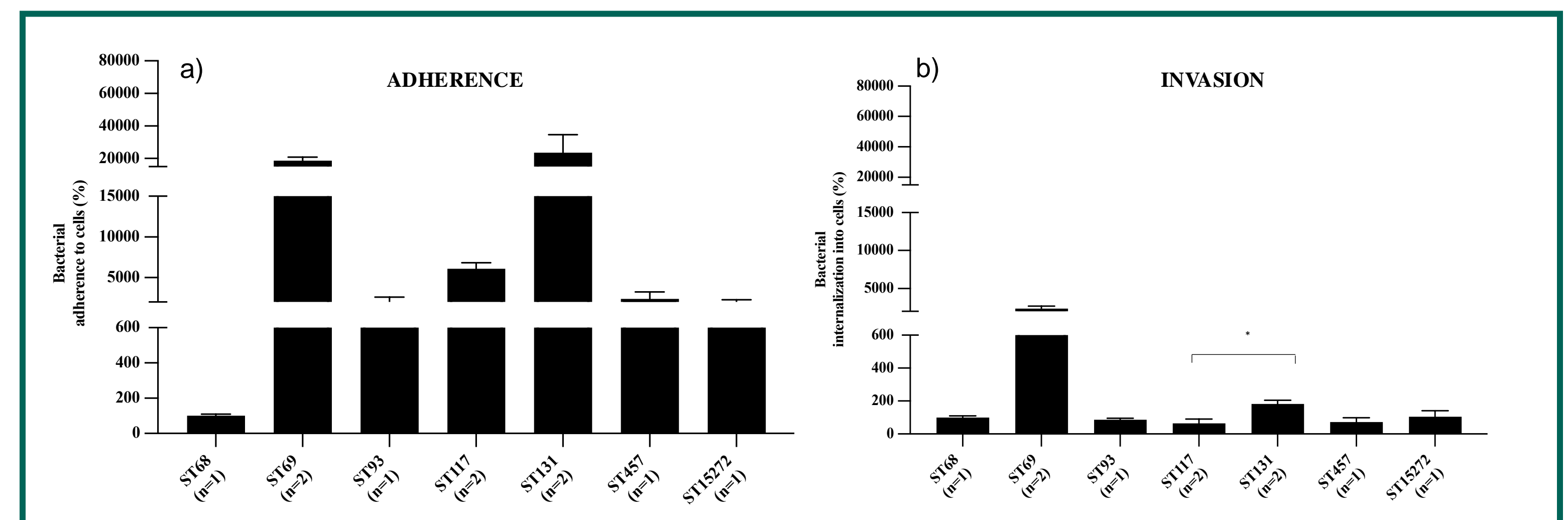


Figure 3. Statistical analysis of *Ec* isolates using the Mann-Whitney U Test grouped by genetic lineage across ExPEC+UPEC pathotype according to a) adherence to cells and b) internalization into cells A293.

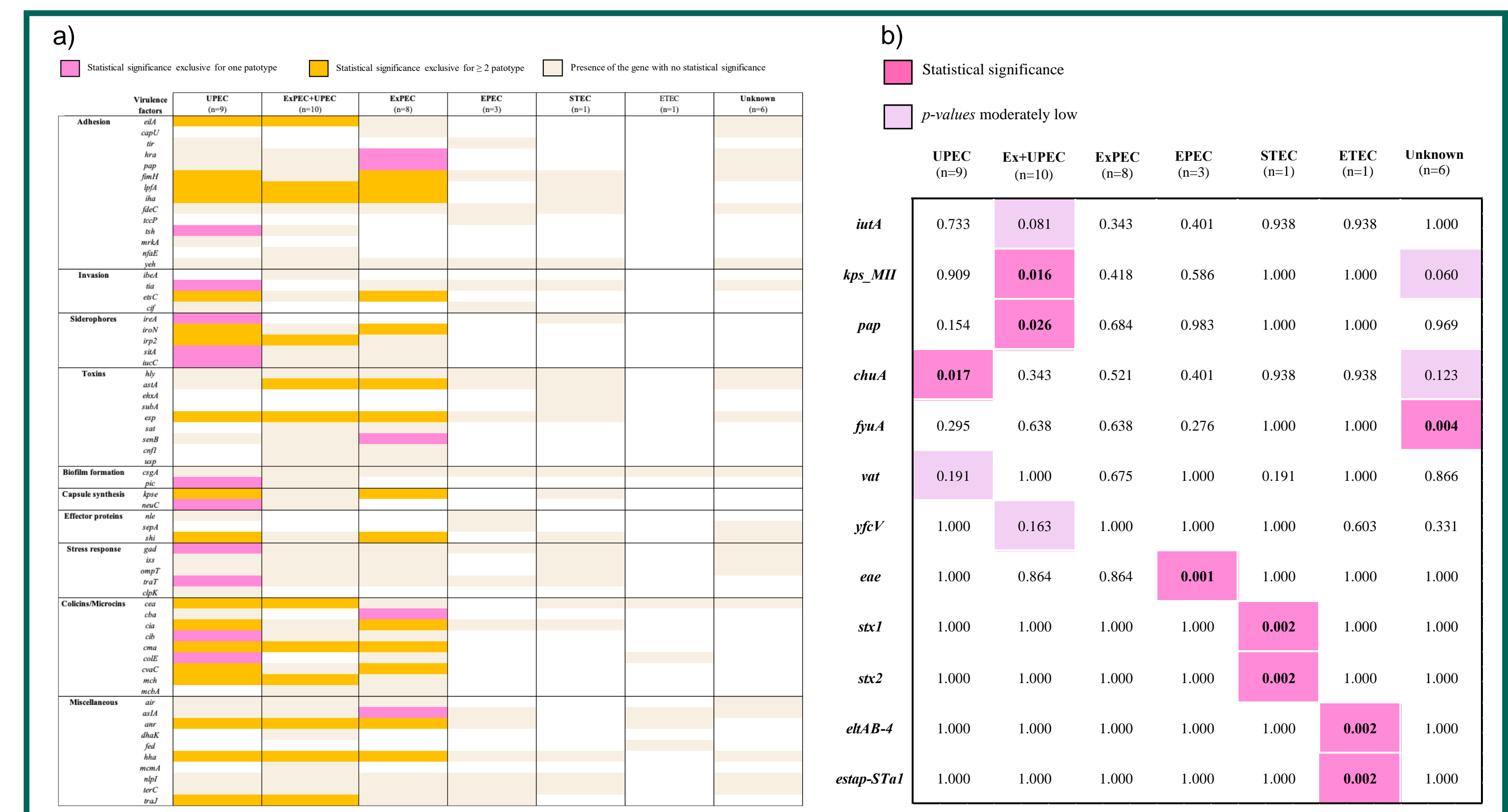


Figure 4. a) Statistical analysis of the distribution within the virulence profiles encoding the different pathotypes detected in our study. b) Overview among pathogenic *E. coli* of the additional virulence determinants encountered and their correlation between the different pathotypes analysed.

SUMMARY/CONCLUSION

ExPEC and UPEC strains exhibited the highest colonization rates in adherence and invasion assays, with UPEC displaying significantly greater invasiveness, potentially due to additional virulence factors such as iron acquisition systems and toxins. Moreover, a strong correlation was observed between the ST117 lineage and elevated invasive capacity, reinforcing its pathogenic potential, consistent with similar findings for the ST131 lineage.