Development and validation of an *in vitro* HTS assay that mimics urine conditions to identify new compounds active against multidrug-resistant uropathogenic enterobacteria

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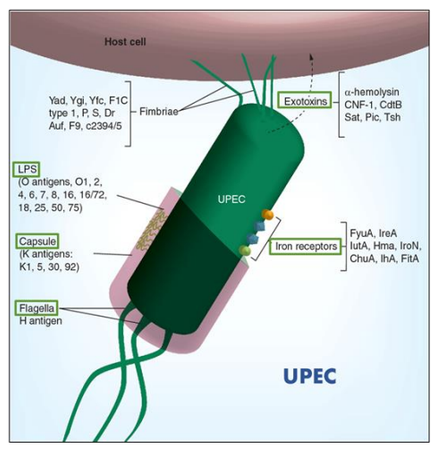
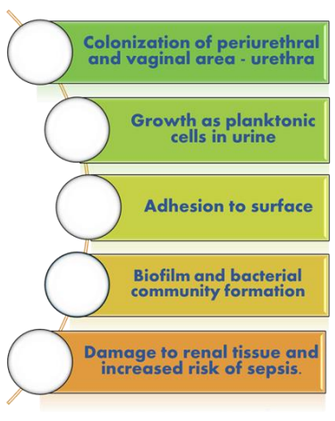
INTRODUCTION

- The increase in antibiotic resistance is limiting the therapeutic options for bacterial infections, especially those involving the most prevalent Gram-negative pathogens.
- The expression pattern of essential genes is not only strain-dependent but is also influenced by the environment of infection, which conditions typically do not correlate with the conditions used *in invitro* assays.
- We hypothesize that a differential assay that mimics the infection microenvironment may lead to the discovery of new antibiotics.
- This project uses urinary tract infection in combination with natural products of microbial origin to identify new potential antibiotics.

MECHANISMS OF VIRULENCE

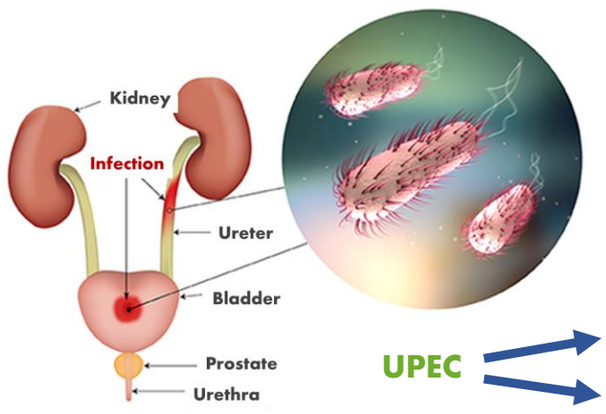


(Sánchez García J. *et al*, 2019); (Alós, 2005)



MATERIAL AND METHODS

1.- Uropathogenic *Escherichia coli* strains (UPEC) for *in vitro* HTS assays



(Maria E. Terlizzi, Giorgio Gribaudo and Massimo E. Maffei, Front. Microbiol., 2017)

UPEC → ***E. coli* ATCC 700928** (CFT073)

UPEC → ***E. coli* extraintestinal 536** (O6:K15:H31)

3.- Fundación MEDINA's Natural Products Libraries

Culture Collection

Bacteria & Fungi
(190K strains)

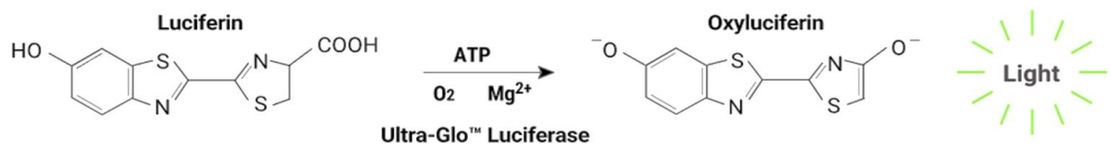
Natural Product Libraries
(200K extracts)

2.- Liquid Culture media for *in vitro* HTS assays

MHII: (Muller Hinton II) non-selective growth medium commonly used in research laboratories.

AUM: Artificial Urine Medium (Brooks *et al*. 1997) that mimics the physiological environment of urinary pathogens.

4.- Measurement of bacterial grwoth by luminiscence (BacTiter-Glo ™)



5.- Dereplication of known compounds by LCMS-HR (High Resolution Liquid Chromatography Mass Spectrometry)

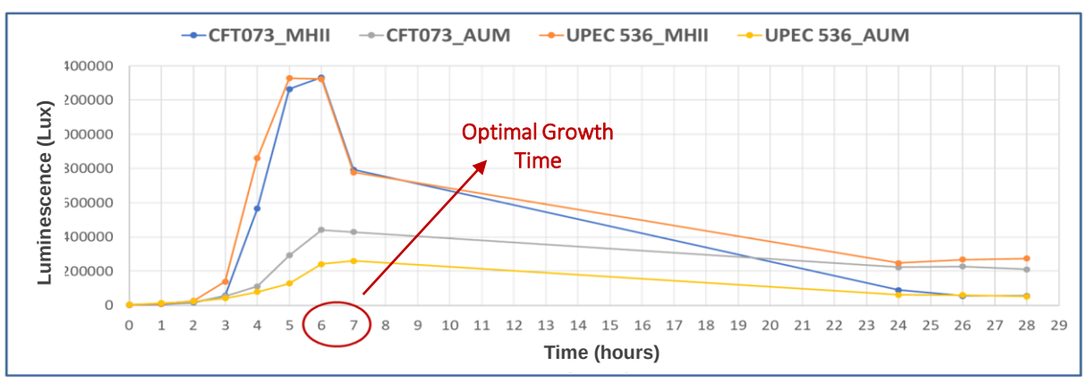
RESULTS

1.- Set Up and Validation of *in vitro* HTS assay (BacTiter-Glo ™)

1.1- Absorbance as a measure of bacterial growth is discarded

AUM medium creates crystals that precipitate and obstruct the correct reading of optical density (OD).

Low growth of both UPEC pathogens in AUM medium that hinders a good assay window (S/B).



UPEC strains Growth Curves measured with luminescence in MHII and AUM culture media using BacTiter-Glo ™.

1.2- Optimal HTS assay Conditions

Conc. inoculum: 5 x 10^5 CFU/mL

$V_{inoculum}$: 25 µL $V_{extracts}$: 2.5 µL

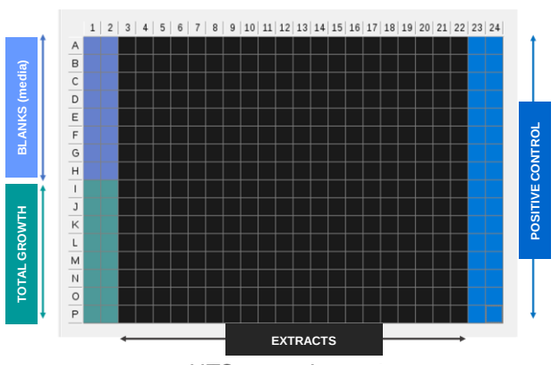
$V_{assay\ final}$: 27.5 µL $V_{Bactiter\ Glo}$: 25 µL

Incubation Time: T_{AUM} **7h**; T_{MHII} **6h**

Higher accuracy and sensitivity

Improved QC parameters: RZ', CV and S/B

Better minimized assay costs and times



HTS assay Layout

1.3- Antibiotic Minimal Inhibiting Concentration MIC (µg/mL) Validation

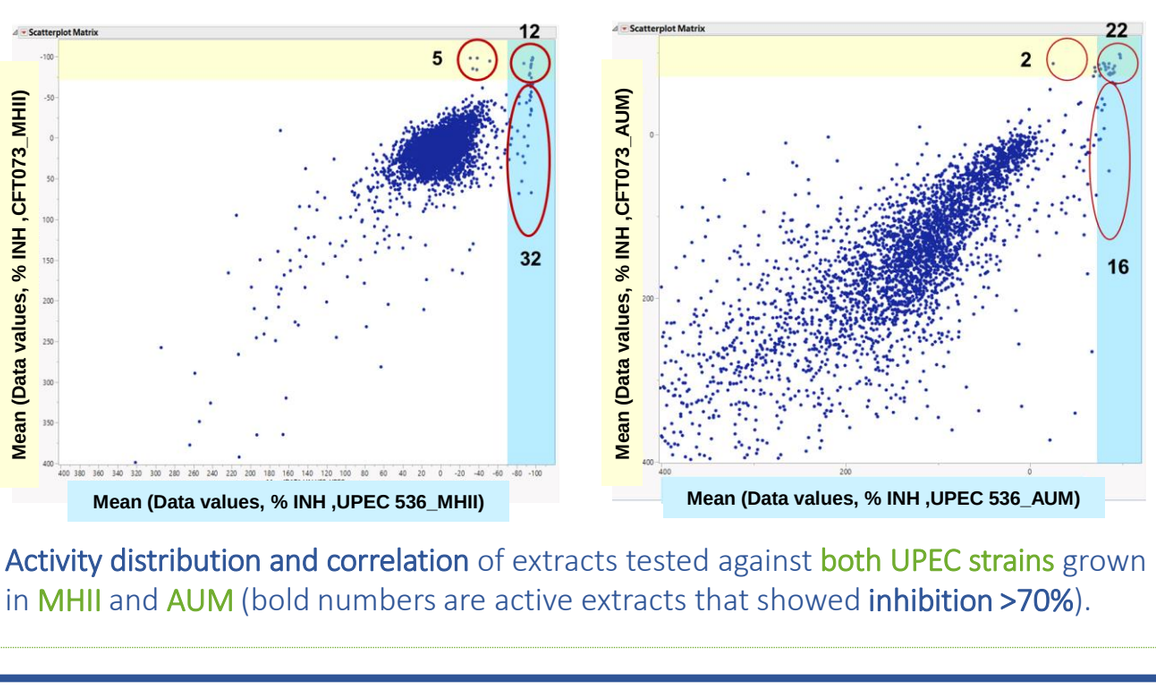
MIC (µg/mL) values against both UPEC strains grown in MHII and AUM for 6 and 7 hours.

ANTIBIOTIC	UPEC536 AUM (7hr)	UPEC536 MHII (6hr)
Ác.Nalidixic	≤128	≤64
Ceftazidime	≤128	16
Levofloxacin	0.25	0.5
Meropenem	4	1
Nitrofurantoin	64	4

ANTIBIOTIC	CFT073 AUM (7hr)	CFT073 MHII (6hr)
Ác.Nalidixic	>128	64
Ceftazidime	128	32
Levofloxacin	4	0.5
Meropenem	16	4
Nitrofurantoin	128	64

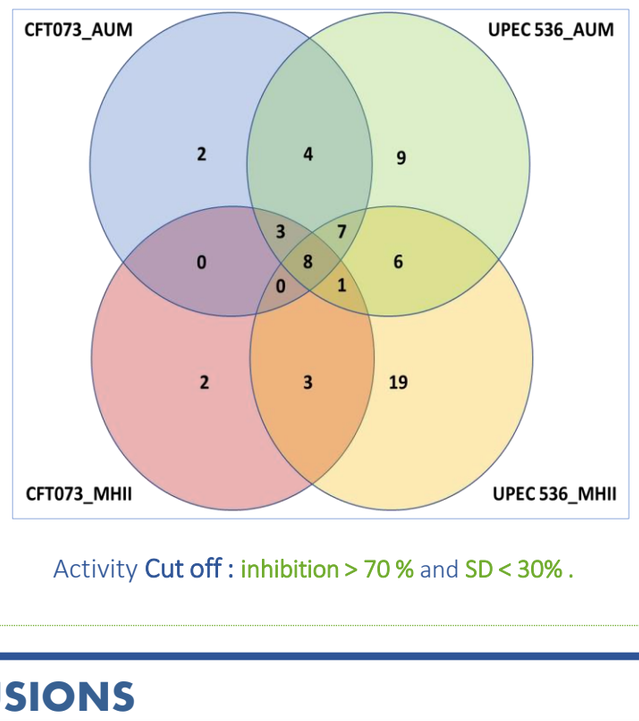
2.- Data Analysis from Pre-Screening HTS assay

2.1- Screening of 1520 extracts tested in duplicate against both UPEC strains in both MHII and AUM



Activity distribution and correlation of extracts tested against both UPEC strains grown in MHII and AUM (bold numbers are active extracts that showed inhibition >70%).

2.2- Distribution of 64 active extracts

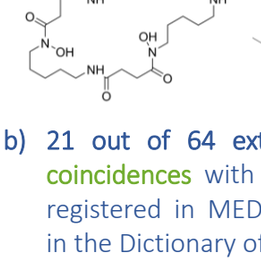


Activity Cut off : inhibition > 70 % and SD < 30% .

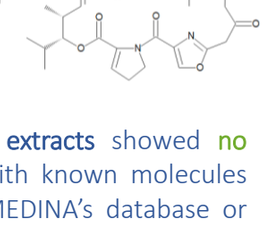
2.2- LCMS-HR analysis of 64 active extracts

a) 43 out of 64 extracts contain known compounds such us oxytetracycline, illudin, virginiamycin, nocardamine and griseofulvin, among others.

Nocardamie



Virginiamycin



b) 21 out of 64 extracts showed no coincidences with known molecules registered in MEDINA's database or in the Dictionary of Natural Products.

CONCLUSIONS

- ✓ UPEC_536 is more sensitive than ATCC700928 (CFT073) in both HTS assay conditions (MH II and AUM).
- ✓ The 21 extracts with no match in the chromatographic profile have been selected for further studies to purify and elucidate the structure of the active compounds.
- ✓ Our next goal is to determine the mode of action of the specific extracts that are active in the urine-mimicking growth conditions (AUM).

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