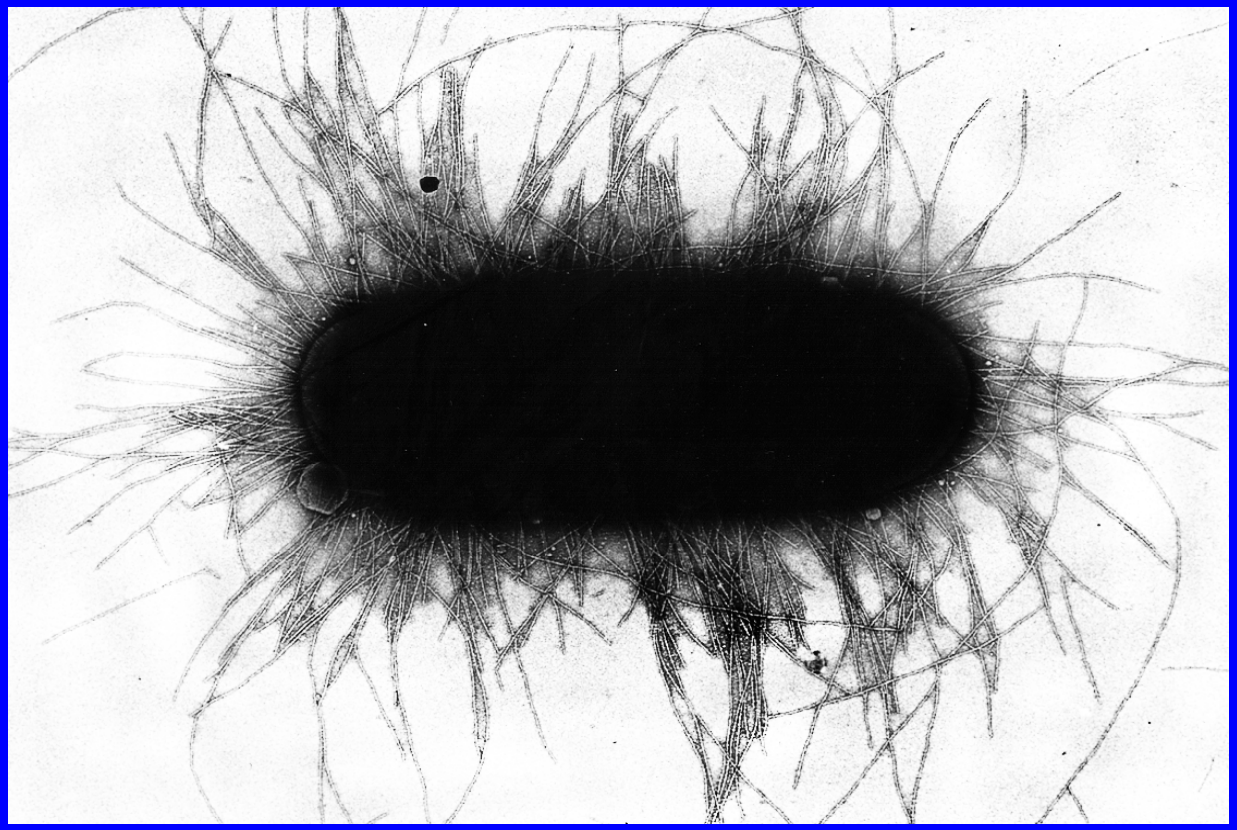
# Microbial Genomics Group (Center Bio Seqanl, DTU):

- Pet organism: E. coli
- Transcriptomics
- virulence factors
- Biofilm formation
- Anti-biofilm drug development/Efflux Pump inhibitors
- Probiotic strains

# *E. coli –a versatile pathogen*



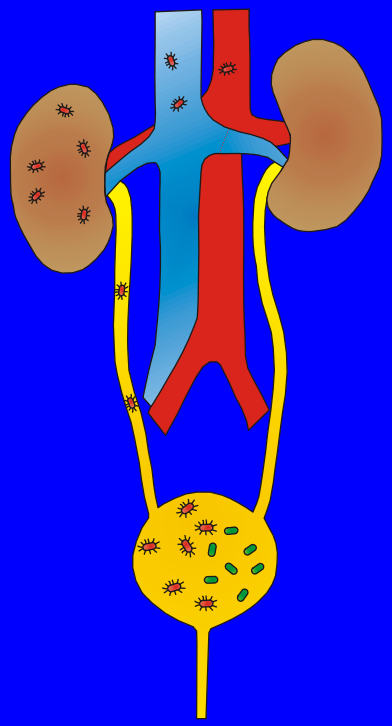
UTI, diarrhea, IBD, sepsis, meningitis

*E. coli* strains kill about 3 M/year

# Urinary Tract Infection (UTI)

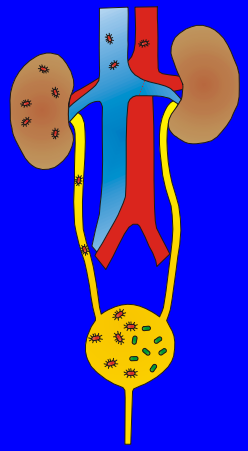
- 53% of all women will have a UTI
- 14 times more common in females than males
- 150 million cases/year worldwide, 180.000 in DK
- >80% caused by *E. coli*

Is biofilm formation involved in colonisation of the human urinary tract?

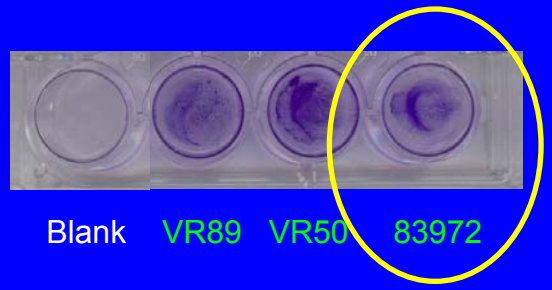


# Global gene expression of UTI strain 83972

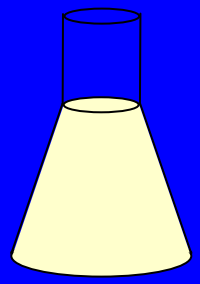
Microarray study on 3 patients carrying *E. coli* 83972



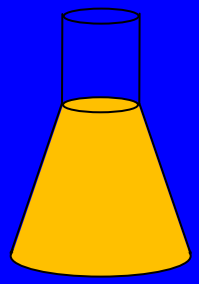
Pat1 Pat2 Pat3




Biofilm in urine



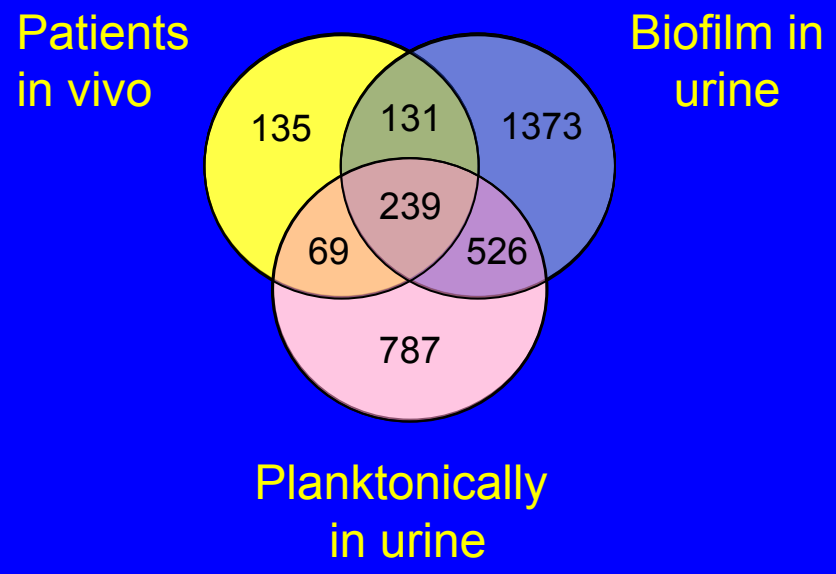
Minimal media MOPS



Human urine

Global Gene Expression Profiling of the Asymptomatic Bacteriuria *Escherichia coli* strain 83972 in the Human Urinary Tract
*Viktoria Roos and Per Klemm* Infection and Immunity (2006) 74:3565-75.

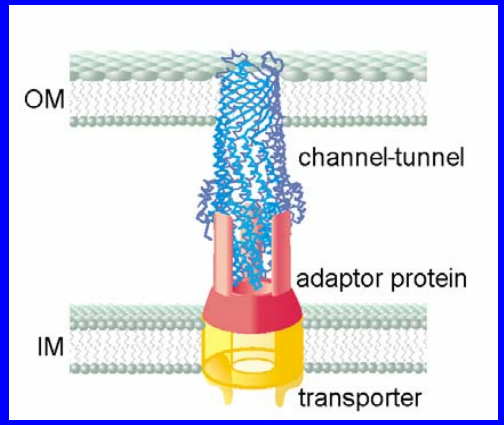
# Bacterial gene expression in human UTIs resembles that in biofilms




64% of all genes changed in patients were regulated in the same way in biofilms

# Bacterial efflux pumps are UP regulated in biofilms

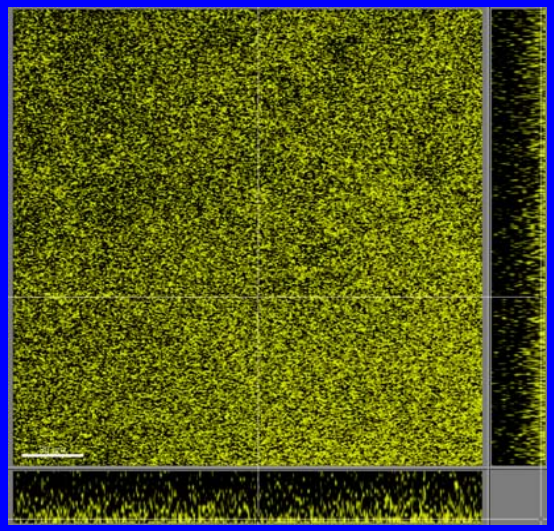
The *aaeAB* genes encode an aromatic carboxylic acid efflux pump whose physiological role may be as a metabolic relief valve to alleviate toxic effects of imbalanced metabolism. Some of the most up-regulated genes in biofilm cells (6-31-fold).




=> **Drowning in their own waste!?!**

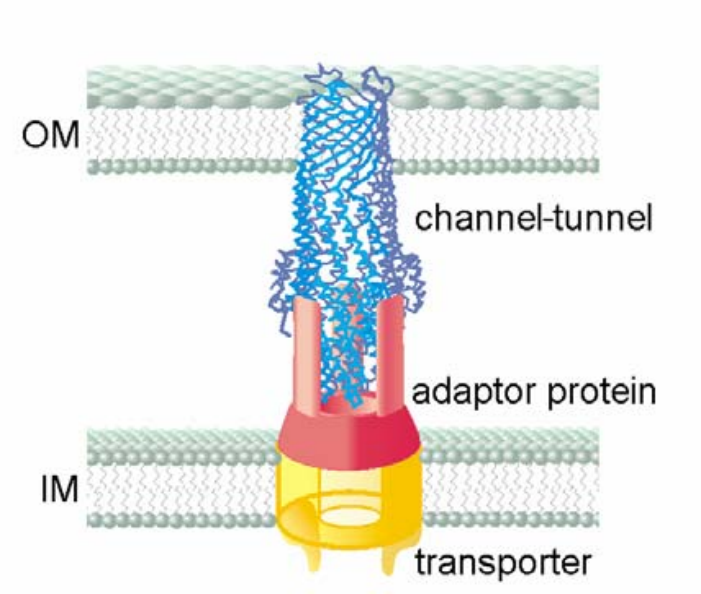
61 genes encoding transport proteins were up-regulated in biofilms (11% of all up-regulated genes)

8 of these were encoding multidrug transport proteins, up-regulated up to 15 times



# Bacterial efflux pumps

An efflux pump is a mechanism used by bacteria to pump out harmful agents



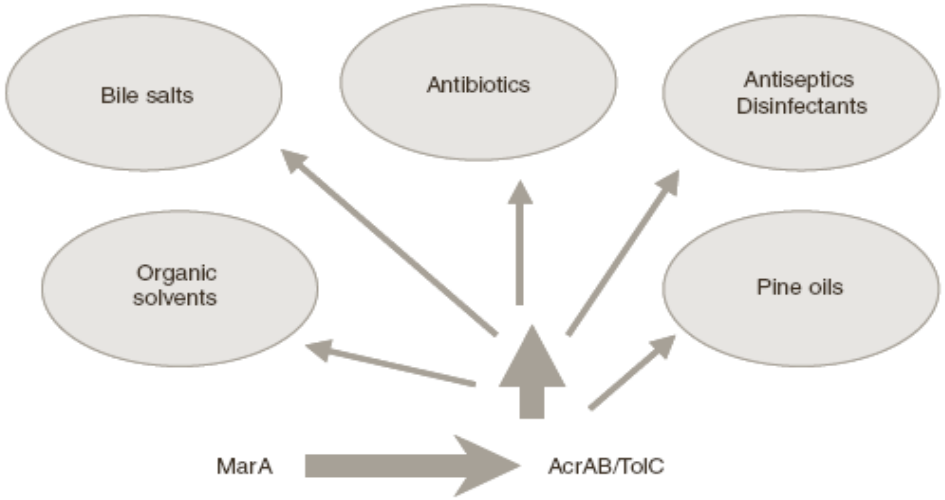
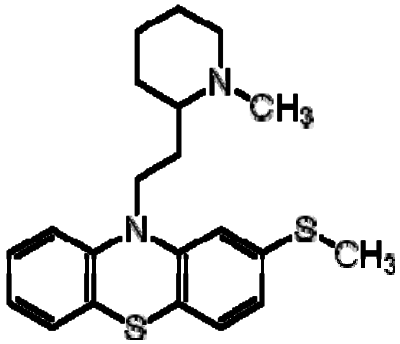
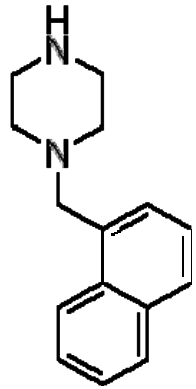




**Fig. 3** MarA from the *mar* locus in *Escherichia coli* causes upregulation of the AcrAB/TolC complex leading to the efflux of antibiotics, organic solvents, antiseptics, disinfectants and other diverse structural compounds

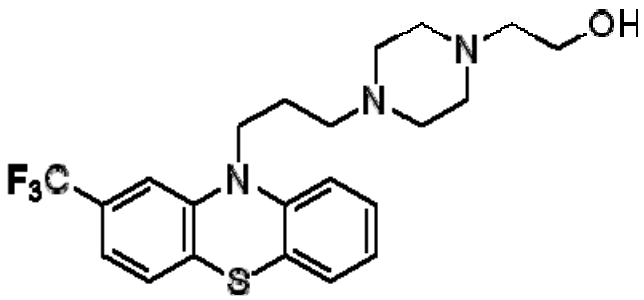
# Bacterial efflux pump inhibitors, EPIs



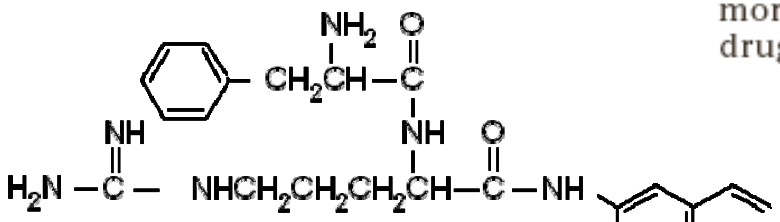

Thioridazine (T)



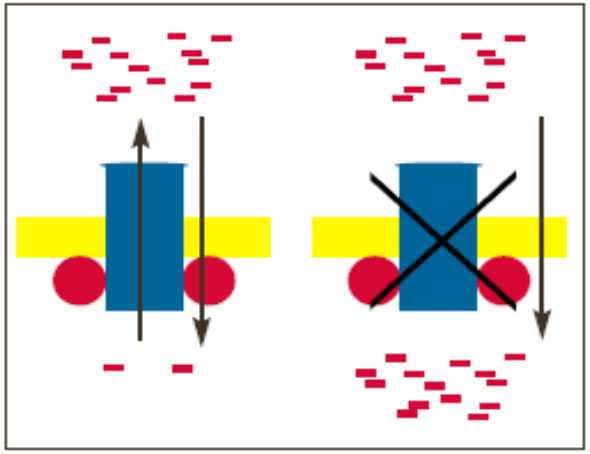

1-(1-naphtylmethyl)piperazine (NMP)




Fluphenazine (F)

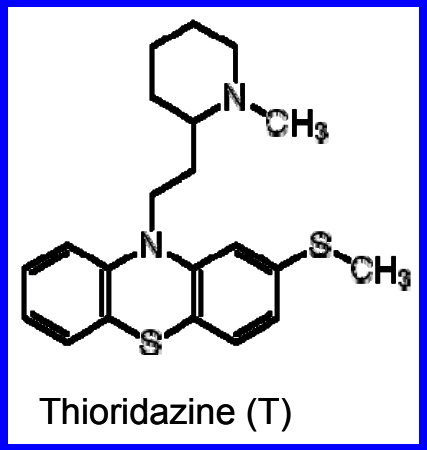


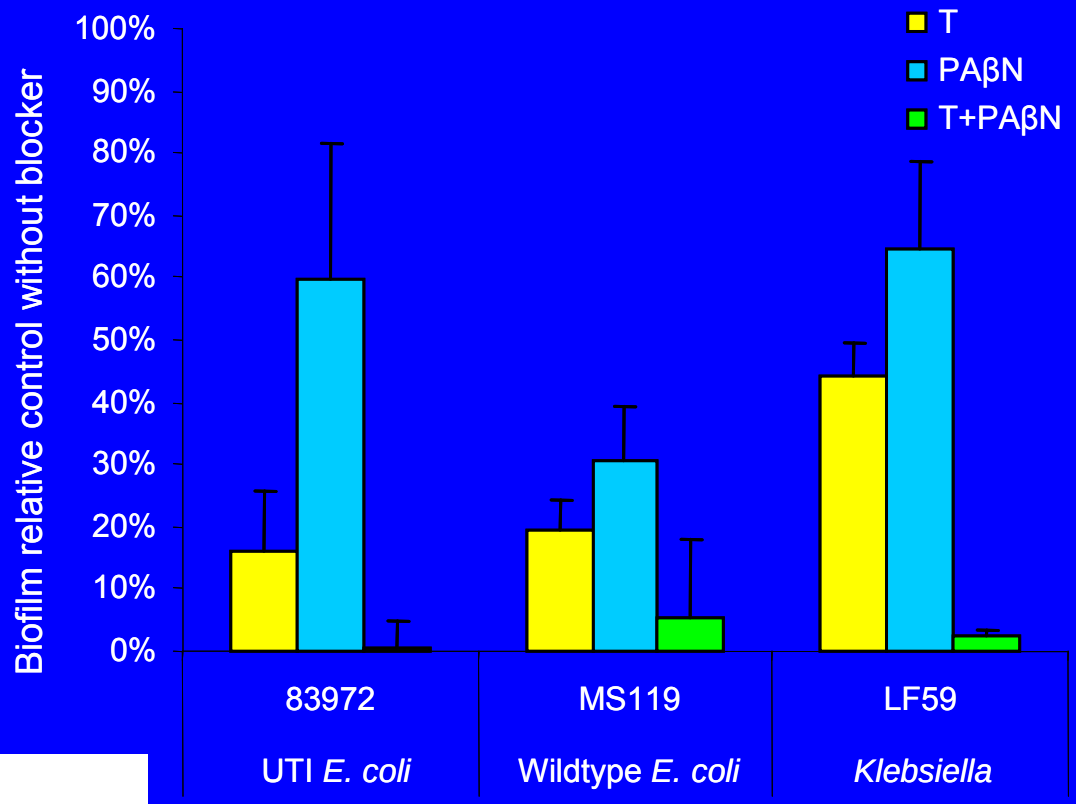

Phe-Arg β-naphtylamide (PAβN)

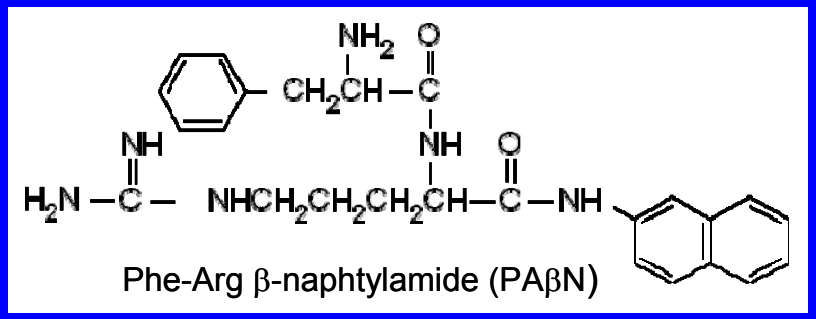


When a microbe's efflux pumps spew out drugs (red rectangles) across the cell's membrane (yellow), the potentially deadly compounds are unable to accumulate inside (left). If the pumps are blocked, the microbe is more likely to succumb to the drugs (right).

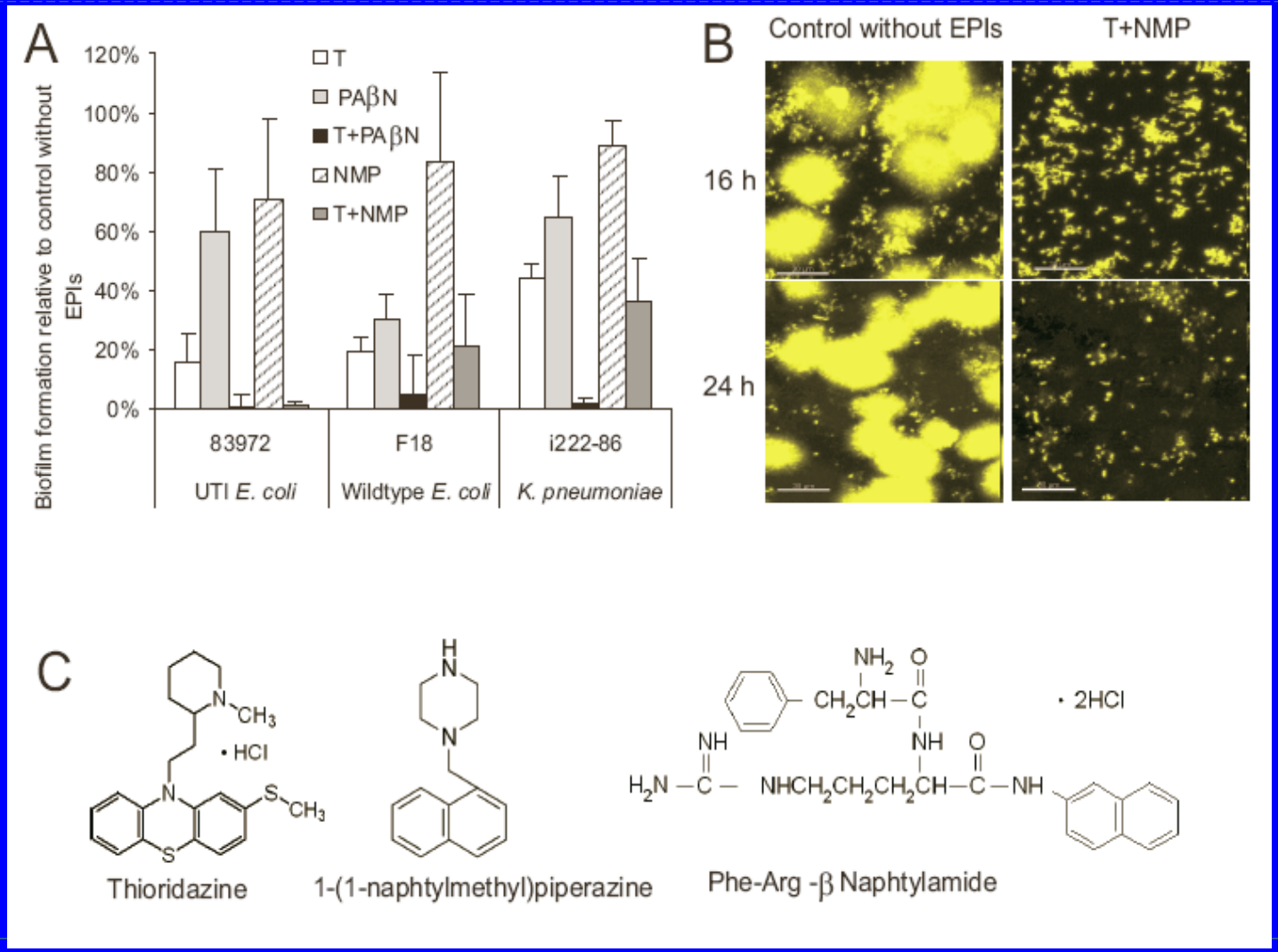
# Inhibition of biofilm by EPIs



# Efflux Pump inhibitors abolish biofilm formation



# Conclusions

Transcriptomics identified **Efflux Pumps** as being specifically upregulated during biofilm formation. EPs could be the Achilles heel of bacterial populations living as biofilms

A range of Efflux Pump Inhibitors (EPIs) are known and we have demonstrated that many of these abolish biofilm formation

In effect EPIs are anti-biofilm drugs

EPIs could supplement classic antibiotics

Some EPIs are harmless constituents of green tea and herbs and many more can be identified