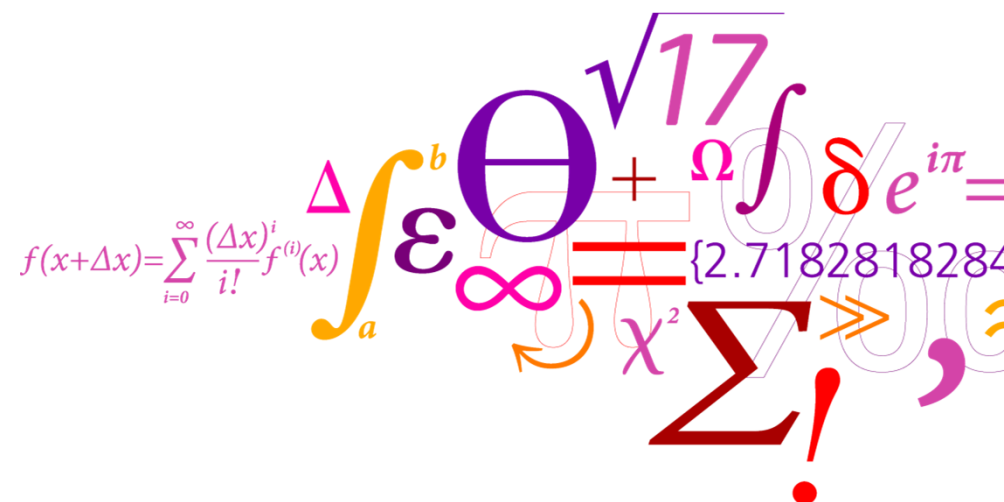




Challenges with Temocillin and tigecycline

Lina M. Cavaco

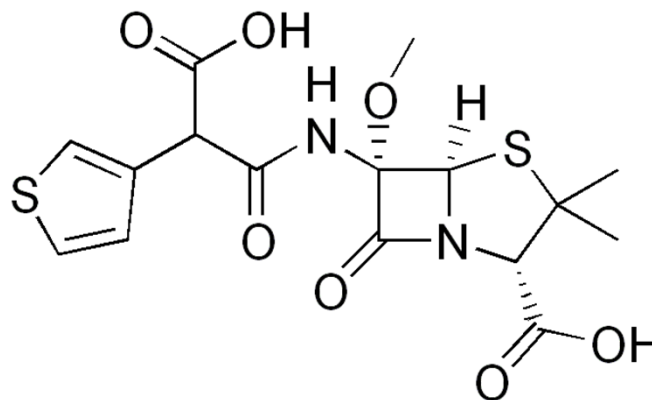
DTU Food
National Food Institute



Background

- Temocillin – lack of ECOFF for both E. coli and Salmonella - data for EUCAST ECOFF setting acc to requirements
- Tigecycline – deviations and issues tested/ observed within EQAS ST, ENT EC 2015

Temocillin project preliminary results



- Temocillin (Negaban) is a Betalactamase stable penicillin drug of the carboxypenicillin group used for treatment of MDR Gram negative bacteria in some countries
- In the monitoring it was introduced as indicator for the detection of OXA-48 as the MIC this drug is normally found at high levels when this gene is present



Background information

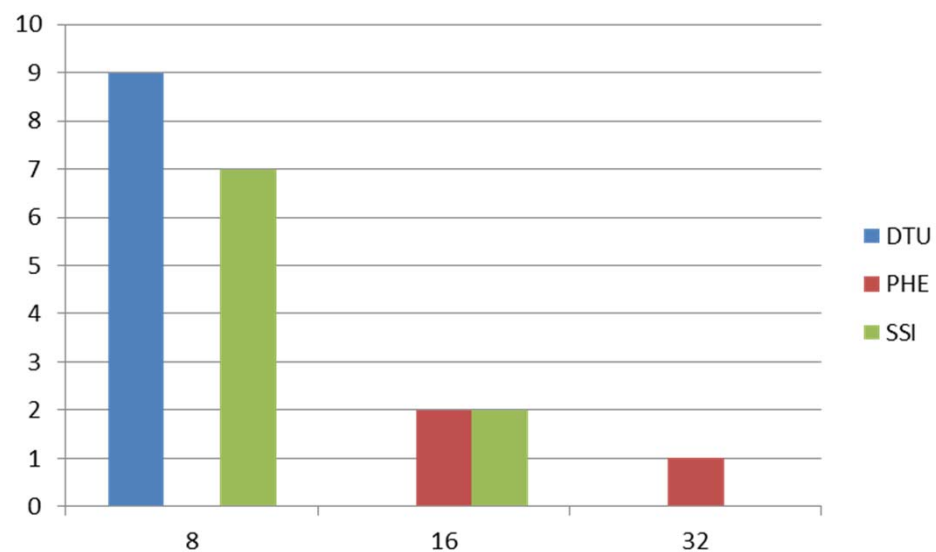
- Previous studies have been performed in the UK looking at temocillin MIC distribution in Klebsiella , E. coli and Enterobacter with Carbapenemase genes (Woodford et al, 2014), and ESBL and AmpC producing Enterobacteriaceae species incl E. coli (Livermoore et al, 2006)
- However, these studies did not include Salmonella or WT strains
- For EFSA analysis a cut off at >32mg/L was used
- The present studies are ongoing and the data is not definitive

Material and Methods

- Strains
 - 112 *E. coli* strains including strains with carbapenem resistance, ESBL, AmpC and wildtype
 - 104 Salmonella strains including strains with carbapenem resistance, ESBL, AmpC (ESC) and wildtype
- Laboratories:
 - DTU FOOD (EURL)
 - SSI
 - PHE
- Tests
 - Agar dilution test with Temocillin (Eumedica)
 - 0.5-512 mg/L range of test
 - MH agar and standard CLSI procedures
 - Controls included in all rounds:
 - K1- ATCC 25922
 - K2- *E. coli* with OXA-48
 - K3- *E. coli* CTX-M-1
 - K4- KPC-2

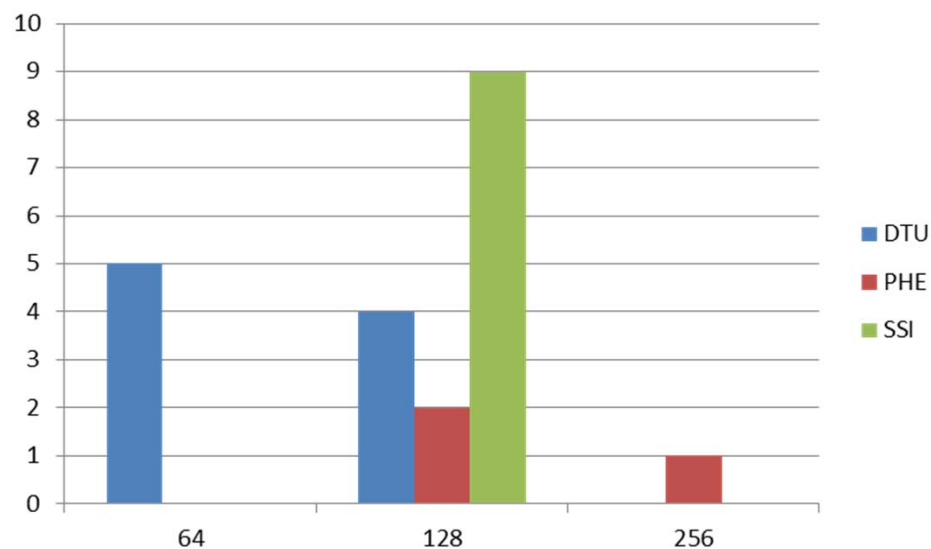
Controls Check

- K1 = *E. coli* ATCC 25922
 - Temocillin expected range unknown
 - Values tested ranged from 8 to 32mg/L
 - Different distribution of results per lab
 - Agrees with published e-test limits established at 3-24 mg/L, (Maurissen et al, 2015)



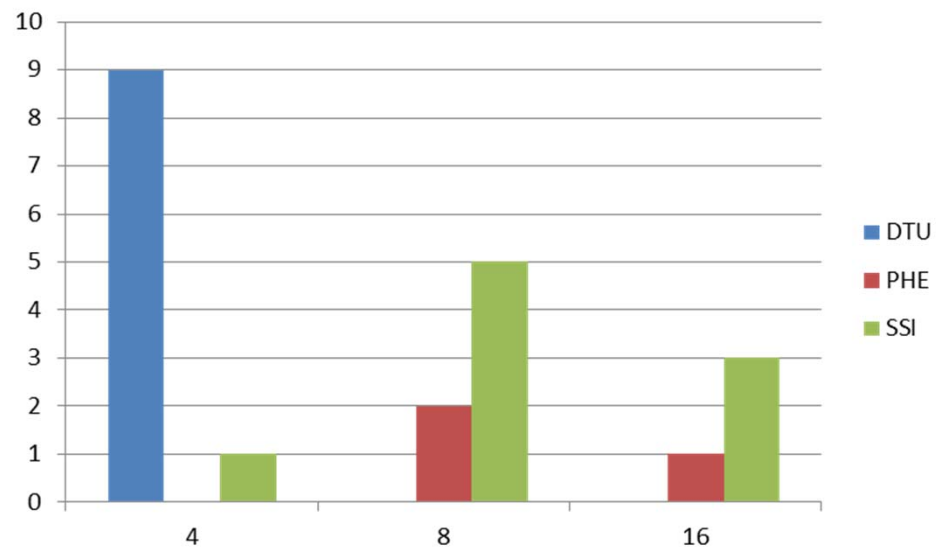
Controls Check

- K2 E. coli OXA-48
 - Temocillin expected range high
 - Values tested ranged from 64 to 256mg/L
 - Different distribution of results per lab



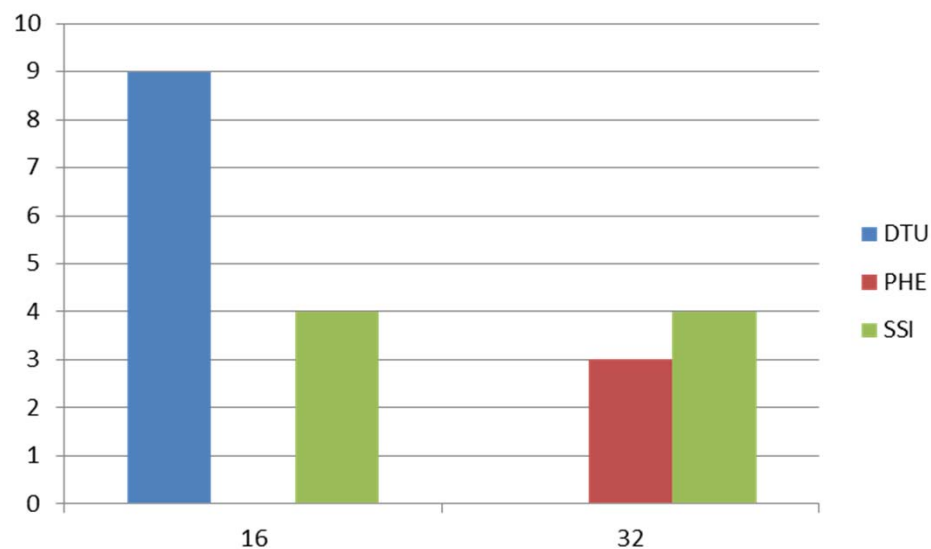
Controls Check

- K3 *E. coli* CTX-M-1
 - Temocillin expected range medium
 - Values tested ranged from 4 to 16 mg/L
 - Different distribution of results per lab



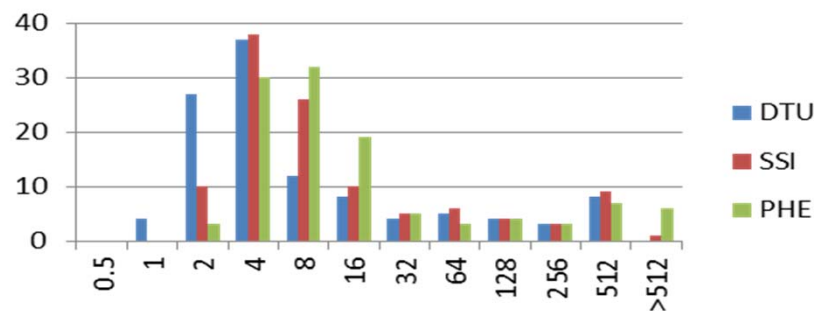
Controls Check

- K4 *E. coli* KPC-2
 - Temocillin expected range medium
 - Values tested ranged from 16 to 32 mg/L
 - Different distribution of results per lab

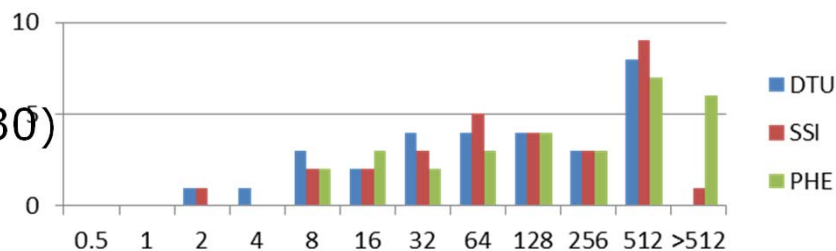


Results *E. coli*

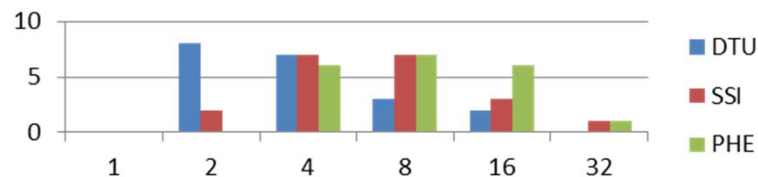
- General distribution (n=112)



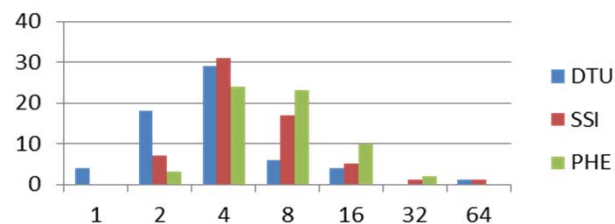
- All CPE (incl OXA) (n=30)



- ESC (n=20)



- WT (n=62)

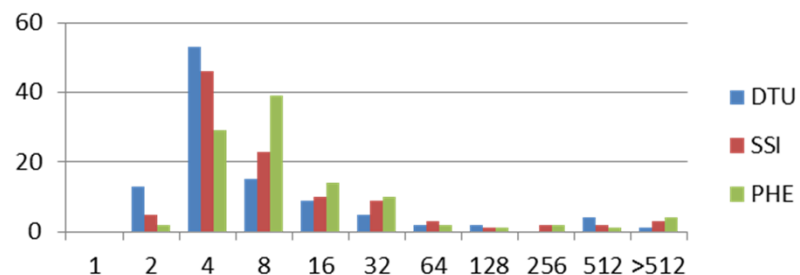


Results *E. coli* CPE, divided by genes

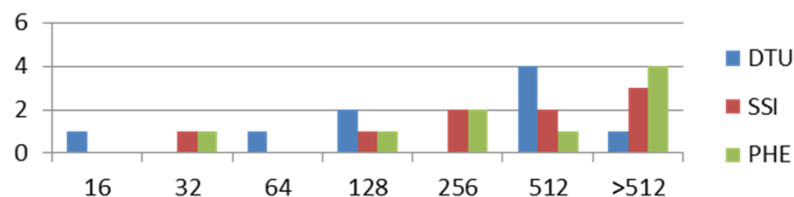
- OXA
 - OXA genes (OXA-48, -181, -232 and -244) (n=10) range MIC 64- >512 mg/L most strains, one strain tested 8-16 and is being checked
- Other CPE high MIC
 - VIM genes (VIM-1 and -4) (n=4) range between 128 and 512 mg/L
 - NDM-4, -5-7 and -8 (n=4) range between 129 and >512 mg/L
- Middle level
 - NDM-1 (n=4) range between 16 and 128 mg/L
- Low level
 - Other (GES-5, IMP-1, KPC-2 and -3) (n=5) range between 2 and 32 mg/L

Results *Salmonella* spp

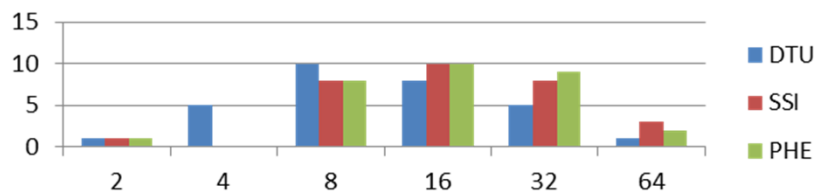
- General distribution (n=104)



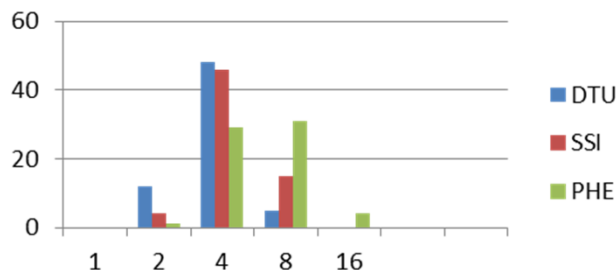
- All CPE (incl OXA-48)(n=9)



- ESC (n=30)



- WT (n=65)



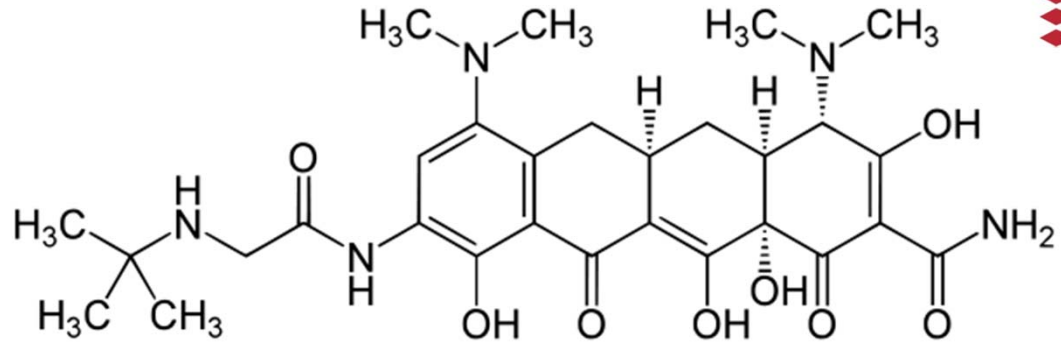
Results *Samonella* CPE, divided by genes

- OXA CPE (OXA-48)
 - OXA genes (n=3) range 512->512mg/L
- Other CPE high MIC
 - VIM-2 genes (n=2) range 512->512mg/L
- Middle level
 - NDM (NDM-1 and NDM) (n=3) range 64-256 mg/L
- Low level
 - One isolate with unknown carba and CTX-M-1- MIC at 16-32 mg/L

Discussion /Conclusions

- Some differences between labs observed
- Temocillin can be used as an indicator
- Higher MIC values observed in Salmonella collection
- Data will be sent to EUCAST to set ECOFFs

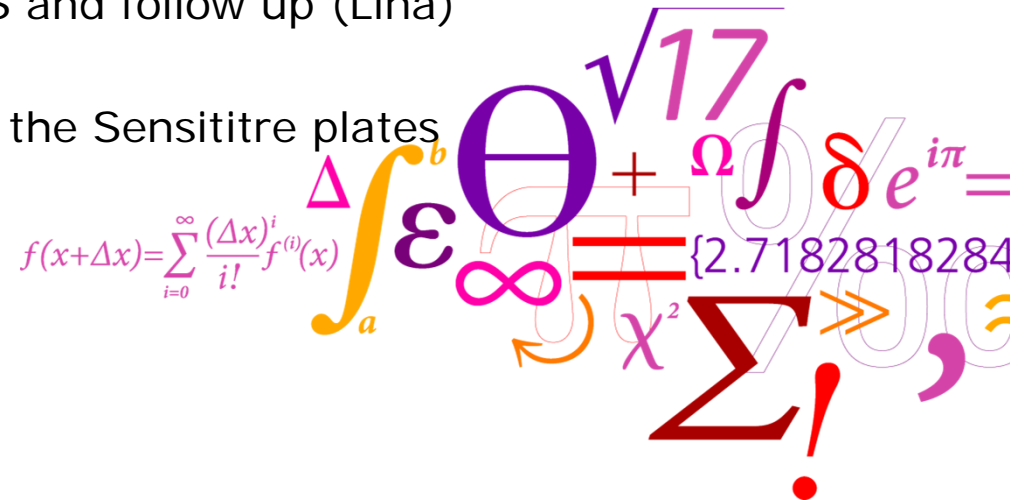
Tigecycline



<https://en.wikipedia.org/wiki/Tigecycline>

Part 1- Challenges observed in EQAS and follow up (Lina)

Part 2- The potency of tigecycline in the Sensititre plates
(Michel Rapallini)



A collection of mathematical symbols and a formula. The formula $f(x+\Delta x) = \sum_{i=0}^{\infty} \frac{(\Delta x)^i}{i!} f^{(i)}(x)$ is shown in pink. Other symbols include a yellow integral \int_a^b , a purple Θ , a purple $\sqrt{17}$, a red Ω , a red \int , a red δ , a red $e^{i\pi} =$, a red ∞ , a purple χ^2 , a red Σ , a red $!$, a red $>>$, and a red \approx .

Tigecycline -Challenges observed in EQAS

- No ENT strains were expected resistant
- 23 deviations observed in test strains all with higher MIC value and observed resistance
- Deviations in control strain ATCC 29212 above range (3 participants)
- Deviations numerous and concentrated for six laboratories (1-8 deviations per lab), all deviations pointing in same direction

First thoughts- batch of plates or media issue??

- Collection of data batches/media
 - Labs with deviation used 3 different batches of plates
 - Quite different media combinations used
- Collect at EURL plates from three batches and different labs
- Test at EURL with one batch of media
- Collect information about similar observations
 - Light/air sensitivity observed in the Netherlands
 - Issues with media observed in other NRL

Results tests at EURL

MIC determinations

Lab	Strain nr	batch	TGC
EURL	ATCC	Exp result	0,03-0,12
1	ATCC	B4423A	0,06
2	ATCC	B4255	0,12
3	ATCC	B4255	0,06
4	ATCC	B4423A	0,12
EURL	EURL ENT 9.1	Exp result	0,25
1	EURL ENT 9.1	B4423A	0,25
2	EURL ENT 9.1	B4255	0,12
3	EURL ENT 9.1	B4255	0,12
EURL	EURL ENT 9.2	Exp result	0,25
1	EURL ENT 9.2	B4423A	0,12
2	EURL ENT 9.2	B4255	0,12
3	EURL ENT 9.2	B4255	0,25
EURL	EURL ENT 9.3	Exp result	0,25
1	EURL ENT 9.3	B4423A	0,12
2	EURL ENT 9.3	B4255	0,25
3	EURL ENT 9.3	B4255	0,12
EURL	EURL ENT 9.4	Exp result	0,25
1	EURL ENT 9.4	B4423A	0,12
2	EURL ENT 9.4	B4255	0,12
3	EURL ENT 9.4	B4255	0,12

Lab	Strain nr	batch	TGC
EURL	EURL ENT 9.5	Exp result	0,12
1	EURL ENT 9.5	B4423A	0,12
2	EURL ENT 9.5	B4255	0,06
3	EURL ENT 9.5	B4255	0,12
EURL	EURL ENT 9.6	Exp result	0,25
1	EURL ENT 9.6	B4423A	0,12
2	EURL ENT 9.6	B4255	0,12
3	EURL ENT 9.6	B4255	0,12
EURL	EURL ENT 9.7	Exp result	0,25
1	EURL ENT 9.7	B4423A	0,12
2	EURL ENT 9.7	B4255	0,25
3	EURL ENT 9.7	B4255	0,12
EURL	EURL ENT 9.8	Exp result	0,25
1	EURL ENT 9.8	B4423A	0,12
2	EURL ENT 9.8	B4255	0,12
3	EURL ENT 9.8	B4255	0,12

Issues not exclusively to batch of plates

Need to look for other reasons

Other possible sources of error (referred by NRLs)

- Light sensitivity of Tigecycline
 - Oxidation of drug (contact with air)
 - Long incubation time (observed in one of the labs with most deviations)
 - Media batches or older medium (one NRL observed improvement with use of fresh made medium)
 - Pipetting error (one occasional deviation observed reg the QC strain)
-
- Need to be careful about methods and handling plates and media, as this drug is very sensitive
 - Resistance of Enterococci to tigecycline should be investigated and confirmed if it is found as it is very rare!

