ECCMID 2021 | Poster #02235

High Prevalence of Tetracycline Resistance Genes among *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* Clinical Isolates Causing Infections in Europe (2019)

Lalitagauri Deshpande, Timothy Doyle, Andrew Davis, Mariana Castanheira, Rodrigo E. Mendes
JMI Laboratories, North Liberty, IA, USA

Introduction

- Tetracycline antibiotics have been in clinical use since the discovery of chlortetracycline in 1948.
- Over the next two decades, other natural and semisynthetic derivatives of this drug class (e.g., demethylchlortetracycline, doxycycline, lymecycline, methacycline, minocycline, and rolitetracycline) were introduced with improved antibacterial potency, oral bioavailability, resistance coverage, solubility, and spectrum.
- Tetracyclines exhibit broad-spectrum of activity against gram-positive and -negative bacteria as well as obligate intracellular bacteria, protozoan, parasites, and spirochetes.
- Resistance to the older generation of tetracycline agents is due to long and widespread use of tetracycline compounds to treat infections as well as their addition to livestock feed to promote growth and improve productivity.
- This study updates the literature on the distribution of tetracycline resistance (*tet*) genes among *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* recovered from patients in European medical centres that met the CLSI MIC criteria for the screening of β-lactamase genes.
- All isolates were submitted to the SENTRY Antimicrobial Surveillance Program in 2019.

Materials and Methods

- The SENTRY Antimicrobial Surveillance Program collected 2,627 *Enterobacterales* isolates in 2019 from Europe, including 1,809 *E. coli*, 638 *K. pneumoniae*, and 180 *P. mirabilis*.
  - Isolates were recovered from bloodstream (BSI) and urinary tract (UTI) infections.
  - Isolates were from patients in 37 medical centres located in 19 European countries.
- Isolates were tested for susceptibility using CLSI broth microdilution methods.
- A total of 592 isolates (22.5% overall), including 283 *E. coli* (15.6% of the isolates), 282 *K. pneumoniae* (44.2% of the isolates), and 27 *P. mirabilis* (15.0% of the isolates), met the criteria for β-lactamase screening and were subjected to Whole Genome Sequencing (WGS).
- WGS was performed using MiSeq (Illumina, San Diego, CA, USA).
  - High quality genomic DNA was extracted using KingFisher Cell and Tissue DNA kit (Thermo Scientific, Waltham, MA USA) in a robotic workstation KingFisher™ Flex Magnetic Particle Processor (Thermo Scientific).
  - Total genomic DNA was used as input material for library construction utilizing the Nextera XT™ library construction protocol and index kit (Illumina) and sequenced on a MiSeq Sequencer using MiSeq Reagent Kit v3 (600 cycle) with a target depth of coverage >30X.
  - Each raw data set was quality assured, error corrected, and *de novo* assembled using SPAdes v. 3.11.1.
- Sequencing data was screened *in silico* for β-lactamase and *tet* genes using an in-house proprietary bioinformatic pipeline.

Results

- The isolates selected for β-lactamase, and *tet* gene screening by WGS, were highly resistant, showing >90% susceptibility only to ceftazidime-avibactam and tigecycline (Table 1).
  - Meropenem (82.8%/85.0% susceptible per CLSI/EUCAST), amikacin (87.0%/78.4% susceptible per CLSI/EUCAST), and colistin (89.5% susceptible per EUCAST) showed moderate activity.
  - Nitrofurantoin (47.3% susceptible per CLSI) and trimethoprim-sulfamethoxazole (28.1% susceptible) showed modest activity.
  - Only 36.5% of isolates were susceptible to tetracycline according to CLSI criteria.
- Among the 592 isolates sequenced, 294 (49.7%) carried 1 or more *tet* genes, including (Figure 1):
  - 149 (52.6% tested isolates) *E. coli*,
  - 140 (49.6%) *K. pneumoniae*, and
  - 5 (18.5%) *P. mirabilis*.
- *tet*(A) was observed in 40.2% of all sequenced isolates, followed by *tet*(D) (5.1%), *tet*(B) (4.7%), and *tet*(G) (0.2%).
  - *tet*(A) was similarly prevalent in *E. coli* (42.8% of tested isolates) and *K. pneumoniae* (39.7%), whereas *tet*(B) was only detected in *E. coli* (9.9%; Figure 1).
  - *tet*(D) was more prevalent in *K. pneumoniae* (9.9% of tested isolates) than in *E. coli* (0.7%).
- The distribution of *tet* and β-lactamase genes among species is shown in Figure 2.
  - The majority (270/294; 91.8%) of isolates positive for *tet* genes also carried bla_{CTX-M}.
  - 209 (83.6%) of the bla_{CTX-M} alleles were $bla_{CTX-M-15}$.
- Carbapenemase genes were noted in (Figure 2):
  - 111/282 sequenced *K. pneumoniae* (39.4%): 38 bla_{KPC}, 15 bla_{NDM}, 1 bla_{VIM-1}, and 58 bla_{OXA-48}-like.
  - 6/283 sequenced *E. coli* (2.1%): 3 bla_{KPC-3}, 1 bla_{VIM-1}, and 2 bla_{OXA-48}-like.
  - 46/117 carbapenemase producing isolates also carried a *tet* gene (39.3%).

Table 1. Antimicrobial susceptibility profiles of 592 *Enterobacterales* isolates evaluated for presence of β-lactamase and *tet* genes

Antimicrobial Agent	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)	CLSI[a] %S	CLSI[a] %I	CLSI[a] %R	EUCAST[a] %S	EUCAST[a] %I	EUCAST[a] %R
Ampicillin-sulbactam	64	>64	4 to >64	9.3	10.5	80.2	9.3		90.7[c]
Aztreonam	>16	>16	0.12 to >16	11.3	8.8	79.9	3.5	7.8	88.7
Ceftriaxone	>8	>8	0.12 to >8	2.2	0.8	97.0	2.2		97.8[e]
							2.2	0.8	97.0[f]
Cefepime	>32	>32	0.03 to >32	8.6	9.3	82.1[g]	5.7	7.4	86.8
Ceftazidime	32	>32	0.12 to >32	19.1	7.8	73.1	4.9	14.2	80.9
Ceftazidime-avibactam	0.25	2	≤0.015 to >32	97.3		2.7	97.3		2.7
Piperacillin-tazobactam	8	>128	0.25 to >128	62.2	9.0	28.9	53.0		47.0
Meropenem	0.03	16	≤0.015 to >32	82.8	2.2	15.0	85.0		15.0
							85.0	3.2	11.8[f]
Gentamicin	1	>16	≤0.12 to >16	57.9	2.2	39.9	56.6		43.4[h]
Amikacin	4	>32	0.5 to >32	87.0	2.7	10.3	78.4		21.6[h]
Tobramycin	8	>16	≤0.12 to >16	42.2	10.8	47.0	38.5		61.5[h]
Levofloxacin	8	>32	≤0.015 to >32	23.0	5.1	72.0	23.0	5.1	72.0
Tetracycline	>16	>16	≤0.5 to >16	36.5	3.0	60.5			
Minocycline	2	32	0.25 to >32	71.1	10.0	18.9			
Doxycycline	8	>8	0.5 to >8	42.9	15.2	41.9			
Tigecycline	0.25	2	≤0.06 to 8	94.8	4.9	0.3[g]			
Nitrofurantoin	64	>64	≤4 to >64	47.3	10.8	41.9			
Trimethoprim-sulfamethoxazole	>16	>16	≤0.12 to >16	28.1		71.9	28.1	1.0	70.9
Colistin	0.25	4	≤0.06 to >8		89.5	10.5	89.5		10.5

[a] Criteria as published by CLSI (2021) and EUCAST (2021).
[b] For infections originating from the urinary tract. For systemic infections, aminoglycosides must be used in combination with other active therapy.
[c] These breakpoints for oral administration are relevant for uncomplicated urinary tract infections only.
[d] Intermediate is interpreted as susceptible-dose dependent.
[e] Using meningitis breakpoints.
[f] Using non-meningitis breakpoints.
[g] US FDA breakpoints were applied.

Figure 1. Prevalence of *tet* genes among *Enterobacterales* isolates from Europe during the 2019 SENTRY Program

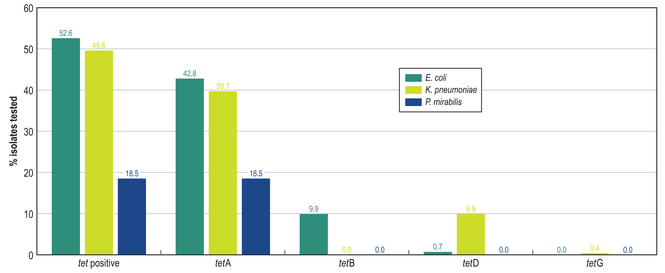
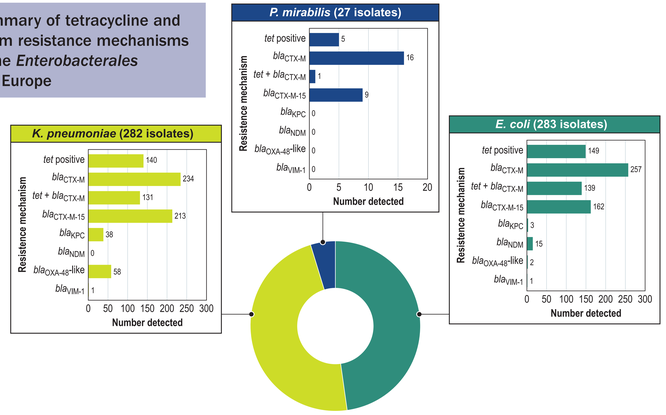


Figure 2. Summary of tetracycline and select β-lactam resistance mechanisms detected in the *Enterobacterales* isolates from Europe



Conclusions

- *tet* genes were commonly encountered among *E. coli*, *K. pneumoniae*, and *P. mirabilis* isolates from BSIs and UTIs that met the MIC criteria for the screening of β-lactamases.
- Only genes encoding for tetracycline efflux-pump proteins were detected.
  - *tet*(A) was the most prevalent gene in both *E. coli* and *K. pneumoniae*.
  - *tet* genes were commonly associated with bla_{CTX-M}, especially $bla_{CTX-M-15}$.
- These data suggest that infections caused by these organisms would likely require new generation β-lactam-β-lactamase inhibitor combinations and/or tetracycline agents not affected by these resistance mechanisms.

Acknowledgements

This poster has been funded by JMI Laboratories. The authors acknowledge excellent support from microbiology and molecular biology teams at JMI Laboratories.

References

CLSI. *M07 Eleventh edition. Methods for Dilution Antimicrobial Susceptibility Testing for Bacteria That Grow Aerobically.* Clinical and Laboratory Standards Institute, Wayne, PA, 2018.

Clinical and Laboratory Standards Institute (2021). M100Ed31. Performance standards for antimicrobial susceptibility testing: 31st informational supplement. Wayne, PA: CLSI.

EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, v 11.0, January 1, 2021. http://www.eucast.org (Access Date Jan 31, 2021).

Speer BS, Shoemaker NB, Salyers AA. Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. Clin Microbiol Rev. 1992 Oct;5(4):387-99. doi: 10.1128/cmr.5.4.387. PMID: 1423217; PMCID: PMC358256.

Roberts MC. Tetracycline therapy: update. Clin Infect Dis. 2003 Feb 15;36(4):462-7. doi: 10.1086/367622. Epub 2003 Jan 28. PMID: 12567304.

Contact

Rodrigo E. Mendes
JMI Laboratories
345 Beaver Kreek Centre, Suite A
North Liberty, Iowa 52317
Phone: 319-665-3370
Email: Rodrigo-mendes@jmilabs.com

To obtain a PDF of this poster:
Scan the QR code or visit https://www.jmilabs.com/data/posters/ECCMID2021_TetraGenesInEuro.pdf

Charges may apply. No personal information is stored.