



Impact of ceftriaxone and temocillin on fecal abundance of extended-spectrum β -lactamase producing *Escherichia coli* in a mouse model

Rachel Chenouard, Rafael Mahieu, David Luque Paz, Estelle Marion,
Matthieu Eveillard, Vincent Dubée

► To cite this version:

Rachel Chenouard, Rafael Mahieu, David Luque Paz, Estelle Marion, Matthieu Eveillard, et al.. Impact of ceftriaxone and temocillin on fecal abundance of extended-spectrum β -lactamase producing *Escherichia coli* in a mouse model. PLoS ONE, 2021, 16 (3), pp.e0248177. 10.1371/journal.pone.0248177 . inserm-03340100

HAL Id: inserm-03340100

<https://inserm.hal.science/inserm-03340100>

Submitted on 10 Sep 2021

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.

RESEARCH ARTICLE

Impact of ceftriaxone and temocillin on fecal abundance of extended-spectrum β -lactamase producing *Escherichia coli* in a mouse model

Rachel Chenouard^{1,2}, Rafael Mahieu^{2,3}, David Luque Paz², Estelle Marion²,
Matthieu Eveillard^{1,2}, Vincent Dubée^{2,3*}

1 Microbiology Laboratory, Angers University Hospital, Angers, France, **2** Center for Research in Cancerology and Immunology Nantes-Angers, UMRS1232, Institut National de la Santé et de la Recherche Médicale, Université de Nantes, Université d'Angers, Angers, France, **3** Infectious Diseases Department, Angers University Hospital, Angers, France

* vincent.dubee@chu-angers.fr



Abstract

Background

Gut colonization by ESBL-producing *Enterobacteriaceae* (ESBL-PE) is widespread and is promoted by antibiotic exposure. Higher fecal abundance of ESBL-PE promotes the dissemination of the bacteria in the environment and is associated with increased risk of infection. Ceftriaxone and temocillin are commonly used antibiotics with a different activity on gut flora. Their impact on fecal abundance of ESBL-producing *Enterobacteriaceae* has not been studied. The objective of this study was to compare the propensity of ceftriaxone and temocillin to modify the abundance of ESBL-producing *Escherichia coli* in feces of colonized mice.

Methods

Mice received broad-spectrum antibiotics in order to disrupt their normal gut flora. A CTX-M-type ESBL-producing *E. coli* clinical isolate was then administered orally, leading to durable colonization. Thirty days later, mice received either temocillin or ceftriaxone with drinking water at a concentration simulating human intestinal exposure. Third-generation-cephalosporin resistant (3GCR) *E. coli* were enumerated in feces on selective medium before, 2 days and 10 days after the end of antibiotic exposure. The experiment was performed with two *E. coli* isolates with different temocillin minimum inhibitory concentrations.

Results

Exposure to ceftriaxone induced an increase in the fecal abundance of 3GCR *E. coli*. In contrast, temocillin had no effect or transiently decreased the number of 3GCR *E. coli*. Results obtained with the two strains were similar.

OPEN ACCESS

Citation: Chenouard R, Mahieu R, Luque Paz D, Marion E, Eveillard M, Dubée V (2021) Impact of ceftriaxone and temocillin on fecal abundance of extended-spectrum β -lactamase producing *Escherichia coli* in a mouse model. PLoS ONE 16(3): e0248177. <https://doi.org/10.1371/journal.pone.0248177>

Editor: Monica Cartelle Gestal, University of Georgia, UNITED STATES

Received: July 3, 2020

Accepted: February 19, 2021

Published: March 10, 2021

Copyright: © 2021 Chenouard et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All data are provided in the paper and [supporting information](#) files.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Conclusion

Contrary to ceftriaxone, temocillin does not promote expansion of ESBL-producing *E. coli* in feces of colonized mice. Thus temocillin may be a therapeutic of choice when a temocillin-susceptible strain infection is suspected or proven to prevent the expansion of ESBL-PE in a previously colonized patient.

Introduction

The global prevalence of extended-spectrum β -lactamase-producing *Enterobacteriaceae* (ESBL-PE) gut colonization is estimated to have increased by 5% every year between 1990 and 2015 [1]. Colonization is the first step leading to ESBL-PE infection, which carries a higher risk of treatment failure and mortality than infections due to non-ESBL producing bacteria [2,3]. The relative abundance (RA) of ESBL-PE is a predictor of ESBL infection [4], and could be positively correlated with the risk of dissemination to the environment [5]. One of the most important factors associated with a high ESBL-PE RA is a recent antibiotic exposure [6]. Few studies have evaluated the differential effect of various antibiotic exposure on the RA of ESBL-PE [7]. Antibiotics with an activity against anaerobes could have a greater effect on the expansion of antibiotic-resistant enterococci or Gram-negative bacteria [5]. In parallel, the intrinsic activity of the antibiotic may also influence the RA of ESBL-PE in feces provided that their intestinal concentration exceeds their minimum inhibitory concentrations (MIC) against these bacteria. Third-generation cephalosporins (3GC) such as ceftriaxone are recommended as first-line treatment for numerous infections such as severe pneumonia and urinary tract infections [8,9]. Their activity against anaerobes may promote the emergence of ESBL-PE in the gut of treated patients. On the contrary, temocillin is a ticarcillin analog with increased stability toward ESBL but devoid of activity against anaerobes and Gram-positive bacteria. It is marketed in Europe where it is frequently used for treatment of ESBL-PE complicated urinary tract infections [10]. This particular antimicrobial spectrum may confer to temocillin a low propensity to select ESBL-PE in colonized patients. Here, we used an animal model to compare the propensity of ceftriaxone and temocillin to modify the RA of ESBL-producing *Escherichia coli* in the feces of colonized mice. Mice received the antibiotics orally at a concentration reproducing the one observed in treated humans.

Materials and methods

Microbiological assay to determine human and murine fecal concentration of ceftriaxone and temocillin

To reproduce in our murine model the conditions leading to expansion of antibiotic-resistant bacteria within the intestinal microbiota of patients treated with antibiotics, we determined the concentration of antibiotics in feces of treated patients by using the microbiological method described below [11]. With this experiment, we determined the optimal ceftriaxone and temocillin concentrations to be used in our mouse model to mimic concentrations in human feces in mice feces. The same assay was performed on human and murine fecal samples. For mice, feces were collected after 2, 3, and 4 days of antibiotic administration in drinking water. Fresh feces were weighed, resuspended in a saline solution, and centrifuged at 10,000 g for 10 min. The supernatant was filter sterilized using 4 mm disposable filter units with a cut-off of 0.2 μ m (Millipore). Twenty, 10 and 5 μ L of the supernatant were loaded onto

paper disks which were deposited on Mueller-Hinton agar plates inoculated with testing strains highly susceptible to the antibiotics dosed. The testing strains were clinical isolates of *Moraxella catarrhalis* for temocillin and *E. coli* for ceftriaxone, with antibiotic MICs of 0.03 mg/L. Disks loaded with known amounts of temocillin or ceftriaxone were deposited on the same plates. Plates were incubated at 37°C for 24 hours and the concentration of each antibiotic in the supernatant was deduced from the inhibition zone diameter around the paper disks [12].

Colonization procedure

In a preceding study [13], we developed a model of stabilized gut colonization with ESBL-producing *E. coli* in six-week old female Swiss mice (Janvier Labs). Briefly, the normal flora was disrupted by oral administration of antibiotics and then, mice received a high inoculum of the strain of interest by oral gavage as described below. A period of thirty days was considered mandatory to stabilize the level of ESBL-producing *E. coli* as observed in human and in previous model [14]. This model was used in the present study with two *E. coli* clinical isolates: one isolate with a temocillin MIC of 1 mg/L (TEMO-S) and one isolate with a temocillin MIC of 16 mg/L (TEMO-R). We used clinical isolates from our lab collection instead of reference isolates to be able to more easily choose a temocillin-susceptible and a temocillin-resistant *E. coli*. As currently recommended in animal experimentation, we aimed limiting the number of mice as much as possible. Therefore, we used 33 mice (5 mice per experimental group in the temocillin resistant group and 6 in the temocillin susceptible group because of unexpected death in the first experimentation). A trial profile is provided in Fig 1. Normal gut flora was disrupted following administration of ceftriaxone (25 mg/kg/day), metronidazole (25 mg/kg/day) and vancomycin (50 mg/kg/day) by oral gavage once daily from day 1 to day 5 (the morning) [13]. Mice were randomly divided into two groups, and each group received one of the two ESBL-producing *E. coli* clinical isolates (5×10^9 CFU/mL) by oral gavage once daily on days 4, 5 and 8 (the afternoon). Thereafter, mice were left untreated for thirty days. Colonization by 3GCR *E. coli* was assessed regularly by culture of serial dilutions of feces resuspended in saline on a commercial selective chromogenic medium for isolation of 3GCR Gram-negative bacilli and species identification (CHROMID ESBL, bioMérieux, France). Additionally, total abundance of *E. coli* was evaluated by culture on a chromogenic medium allowing species identification (Brilliance UTI, Oxoid, UK). Plates were incubated for 48 h at 37°C under aerobic atmosphere.

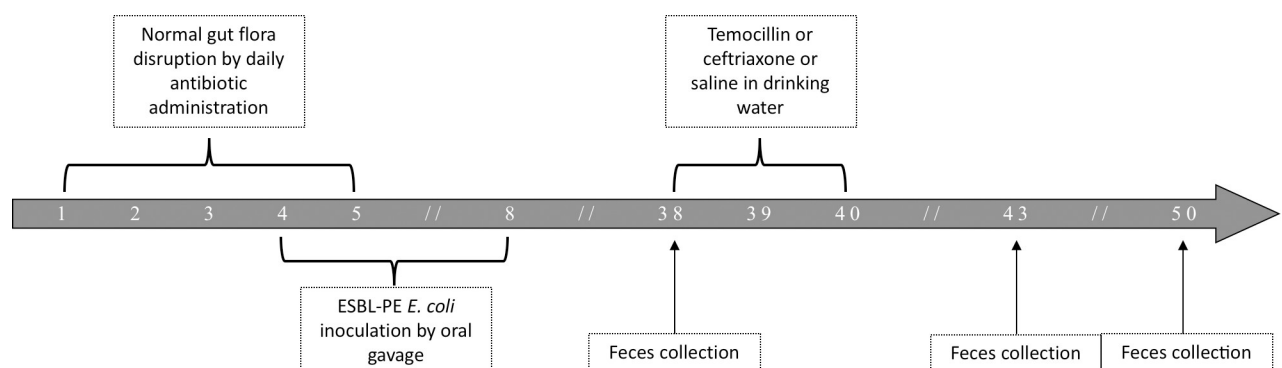


Fig 1. Trial profile. After gut flora disruption, mice are inoculated with the strain of 3GCR *E. coli* and left untreated for 30 days before exposure to temocillin, ceftriaxone or saline. Numbers represent days.

<https://doi.org/10.1371/journal.pone.0248177.g001>

Thirty days after the end of the colonization procedure, mice were randomly divided into three groups of five mice. One group received temocillin and the other group received ceftriaxone for three days in drinking water. The remaining mice were left unexposed to antibiotic (control group with saline). During this period, drinking water with or without antibiotics was changed every day. As mice drank this water for their usual need, we used tap water and not sterile water. The mice included in the different groups had similar characteristics (Swiss type, 6-week old, 25 ± 3 g) and had been provided by the same company (Janvier Labs, feeding by industrial granules). Fresh feces were collected (the morning) two and ten days following the end of antibiotic exposure to enumerate 3GCR *E. coli* as previously described. Abundance of 3GCR *E. coli* was expressed as CFU/g of feces. The lower limit of detection was dependent on feces weight.

The protocol was ethically reviewed and approved by the Ethical Committee in Animal Testing, Pays de la Loire, France (AFAPIS 11747-201710111159930v2). Mice were bred in cage by four or five. All animals in the same cage received the same antibiotic.

Statistical analysis

Results of bacterial counts are given as medians. Variations in bacterial counts are expressed as $\Delta \log_{10}$ CFU/g of feces. Whenever no 3GCR bacteria could be detected, the lower limit of detection was used for all calculations. Statistical paired differences between treatment groups were assessed by 2-way ANOVA for repeated measures. We used the False Discovery Rate method to take into account the multiple comparisons.

Results

Determination of optimal ceftriaxone and temocillin concentration in mice drinking water

Temocillin concentrations in feces of three patients treated for complicated urinary tract infection were 0.25, 0.7, and 1 mg/g of feces 3, 3, and 5 days after antibiotic initiation, respectively. It has been shown previously that mean concentration of ceftriaxone in feces of treated patients ranges between 0.15 and 0.26 mg/g [15].

Using the same microbiological method, we assessed the proportion of each antibiotic excreted in feces after oral ingestion in drinking water (bottles). Fecal concentration of the active form of both antibiotics was approximately 70% (median result of 20 measurements) of the concentration ingested with drinking water. This proportion remained stable over time (no difference between day 2, 3, and 4), with limited interindividual variations (interquartile range of the proportion 60–120%). Therefore, mice received ceftriaxone and temocillin in drinking water at a concentration of 250 and 650 mg/L, respectively, in subsequent experiments.

Impact of ceftriaxone and temocillin on the abundance of ESBL-producing *E. coli* in feces of colonized mice

During the first thirty days, the fecal abundance of 3GC-resistant (3GCR) bacteria decreased, and then stabilized at a low level similar to the level observed in colonized humans. After the end of the colonization procedure, all mice colonized with TEMO-S and TEMO-R strains had detectable 3GCR *E. coli* in feces. For the TEMO-S strain, thirty days after the end of colonization procedure, the median density of 3GCR *E. coli* was $3.5 \log_{10}$ CFU/g of feces (Fig 2A). After a three-day oral antibiotic challenge, no change in the density of 3GCR bacteria was observed in the control group (P-adjusted = 0.24 and 0.49 at day 2 and 10, respectively). In

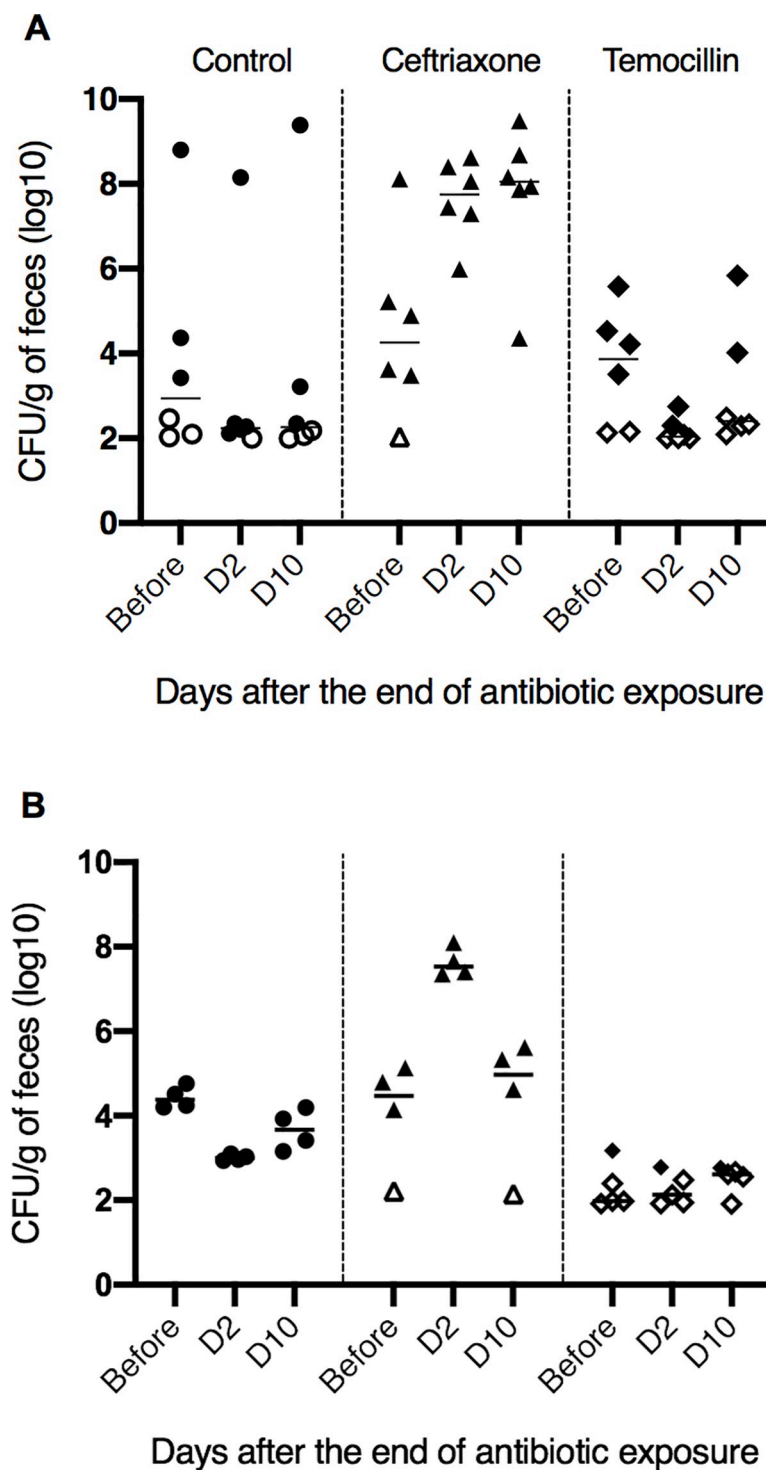


Fig 2. Effect of ceftriaxone and temocillin on the fecal abundance of 3GCR *E. coli* in a mouse model. Mice were colonized either with a temocillin-susceptible (A) or temocillin-resistant (B) CTX-M type ESBL-producing *E. coli*. Black horizontal bars represent medians of bacterial counts. Empty symbols represent the lower limit of detection in mice without detectable 3GCR *E. coli*.

<https://doi.org/10.1371/journal.pone.0248177.g002>

contrast, the density of 3GCR *E. coli* in feces of mice exposed to ceftriaxone increased by a median of 3.5 and 4.6 log₁₀ CFU/g at day two and ten, respectively, in comparison with bacterial density observed at baseline (P-adjusted = 0.03 and 0.03). Exposure to temocillin led to a non-significant decrease in the density of 3GCR *E. coli* bacteria at day 2, in comparison to baseline (median $\Delta\log_{10} = -1.50 \log_{10}$ CFU/g, P-adjusted = 0.12). At day ten, no difference was observed in comparison with baseline (median $\Delta\log_{10} = 0.51 \log_{10}$ CFU/g, P = 0.55). Ceftriaxone increased the number of 3GCR *E. coli* in feces of exposed mice significantly more than temocillin at day 2 (P-adjusted < 0.001 and < 0.01 for day 2 and day 10, respectively).

The colonization procedure with TEMO-R led to a non-different fecal abundance of 3GCR *E. coli* to that observed with TEMO-S. One mouse in the ceftriaxone and one in the control group died during the colonization procedure. No difference in the number of 3GCR bacteria was observed in mice exposed to temocillin at two and ten days (Fig 2B). In contrast, ceftriaxone significantly increased the abundance of 3GCR *E. coli* at day two (median increase 2.97 log₁₀ CFU/g of feces, P-adjusted < 0.01). No significant difference was observed at day ten (median variation +0.52 log₁₀ CFU/g, P-adjusted = 0.08).

Discussion and conclusion

In this study, we assessed the impact of temocillin and ceftriaxone on the fecal abundance of 3GCR *E. coli* in a mouse model of ESBL-producing *E. coli* gut colonization. For this purpose, antibiotics were given orally in order to reproduce antibiotic concentrations observed in treated humans. Our results show that ceftriaxone leads to a major increase in the excretion of 3GCR *E. coli* after the end of antibiotic exposure, whereas temocillin does not. This may arise from two major differences between these two antibiotics. Contrary to ceftriaxone, temocillin lacks activity against anaerobes, and is active against most ESBL-producing *E. coli*. A vast literature supports the hypothesis that activity against anaerobes is a major determinant of the ability of an antibiotic to promote gut colonization by antibiotic-resistant bacteria [16,17]. The impact of antibiotic intrinsic activity on excretion of resistant bacteria has been less studied. In our study, a non-significant decrease in the number of the temocillin susceptible strain of 3GCR *E. coli* was observed during temocillin exposure ($\Delta\log_{10} = -1.50 \log_{10}$ CFU/g) suggesting that temocillin may have a direct effect on the resistant bacteria. Moreover, several reports show that carbapenems, despite their activity against anaerobes, decrease the fecal density of ESBL-producing bacteria or do not promote gut colonization [7,18], suggesting that antibiotics with activity against resistant bacteria could have a favorable profile. The differences between human and mouse gut microbiota may query the conclusions of our study. However, most conclusions of animal models assessing the impact of antibiotics on selection of resistant bacteria have been confirmed in human clinical studies [19]. Another limitation is the number of mice included in the protocol, but this choice was justified by a balance between the minimum number needed to provide significative results and the respect of ethic in animal experimentation.

Third-generation cephalosporins are recommended as first-line therapy for treatment of complicated urinary tract infections [20]. Our results, in line with others, suggest that prescription of these antibiotics promotes ESBL-selection in the gut of treated patients, which is the first step to infection and may be associated with an increased risk of transmission of resistant clones. Temocillin is active against most *E. coli* isolates responsible for urinary tract infections [21] and our study shows that it has minimal impact on the fecal abundance of ESBL-producing *E. coli*. Thus, in a context of growing ESBL burden, temocillin is an interesting option for empirical therapy of complicated urinary tract infections.

Supporting information

S1 File. Temocillin susceptible *E. coli*.
(PZFX)

S2 File. Temocillin resistant *E. coli*.
(PZFX)

Author Contributions

Conceptualization: Rachel Chenouard, Rafael Mahieu, Estelle Marion, Matthieu Eveillard, Vincent Dubée.

Data curation: Rachel Chenouard, Vincent Dubée.

Formal analysis: Rafael Mahieu.

Investigation: Rachel Chenouard, Rafael Mahieu, Vincent Dubée.

Methodology: Rachel Chenouard, Rafael Mahieu, David Luque Paz, Estelle Marion, Matthieu Eveillard, Vincent Dubée.

Project administration: Rachel Chenouard.

Supervision: Rafael Mahieu, Estelle Marion.

Validation: Rachel Chenouard, Rafael Mahieu, David Luque Paz, Estelle Marion, Matthieu Eveillard, Vincent Dubée.

Writing – original draft: Rachel Chenouard, Rafael Mahieu, David Luque Paz, Estelle Marion, Matthieu Eveillard, Vincent Dubée.

Writing – review & editing: Rachel Chenouard, Rafael Mahieu, David Luque Paz, Estelle Marion, Matthieu Eveillard, Vincent Dubée.

References

1. Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E. Fecal Colonization With Extended-spectrum Beta-lactamase-Producing Enterobacteriaceae and Risk Factors Among Healthy Individuals: A Systematic Review and Metaanalysis. *Clin Infect Dis*. 2016; 63: 310–318. <https://doi.org/10.1093/cid/ciw283> PMID: 27143671
2. Anesi JA, Lautenbach E, Nachamkin I, Garrigan C, Bilker WB, Omorogbe J, et al. Poor clinical outcomes associated with community-onset urinary tract infections due to extended-spectrum cephalosporin-resistant Enterobacteriaceae. *Infect Control Hosp Epidemiol*. 2018; 39: 1431–1435. <https://doi.org/10.1017/ice.2018.254> PMID: 30375298
3. Melzer M, Petersen I. Mortality following bacteraemic infection caused by extended spectrum beta-lactamase (ESBL) producing *E. coli* compared to non-ESBL producing *E. coli*. *J Infect*. 2007; 55: 254–259. <https://doi.org/10.1016/j.jinf.2007.04.007> PMID: 17574678
4. Ruppé E, Lixandru B, Cojocaru R, Büke C, Paramythiotou E, Angebault C, et al. Relative fecal abundance of extended-spectrum- β -lactamase-producing *Escherichia coli* strains and their occurrence in urinary tract infections in women. *Antimicrob Agents Chemother*. 2013; 57: 4512–4517. <https://doi.org/10.1128/AAC.00238-13> PMID: 23836184
5. Ruppé E, Andremont A. Causes, consequences, and perspectives in the variations of intestinal density of colonization of multidrug-resistant enterobacteria. *Front Microbiol*. 2013; 4: 129. <https://doi.org/10.3389/fmicb.2013.00129> PMID: 23755045
6. de Lastours V, Chopin D, Jacquier H, d'Humières C, Burdet C, Chau F, et al. Prospective Cohort Study of the Relative Abundance of Extended-Spectrum-Beta-Lactamase-Producing *Escherichia coli* in the Gut of Patients Admitted to Hospitals. *Antimicrob Agents Chemother*. 2016; 60: 6941–6944. <https://doi.org/10.1128/AAC.01328-16> PMID: 27600033
7. Pultz MJ, Donskey CJ. Effects of imipenem-cilastatin, ertapenem, piperacillin-tazobactam, and ceftriaxone treatments on persistence of intestinal colonization by extended-spectrum-beta-lactamase-

- producing *Klebsiella pneumoniae* strains in mice. *Antimicrob Agents Chemother*. 2007; 51: 3044–3045. <https://doi.org/10.1128/AAC.00194-07> PMID: 17562802
8. Malmros K, Huttner BD, McNulty C, Rodríguez-Baño J, Pulcini C, Tängdén T, et al. Comparison of antibiotic treatment guidelines for urinary tract infections in 15 European countries: Results of an online survey. *Int J Antimicrob Agents*. 2019; 54: 478–486. <https://doi.org/10.1016/j.ijantimicag.2019.06.015> PMID: 31229671
 9. Ferreira-Coimbra J, Tejada S, Campogiani L, Rello J. Levels of evidence supporting European and American community-acquired pneumonia guidelines. *Eur J Clin Microbiol Infect Dis*. 2020. <https://doi.org/10.1007/s10096-020-03833-8> PMID: 32030566
 10. Soubirou JF, Rossi B, Couffignal C, Ruppé E, Chau F, Massias L, et al. Activity of temocillin in a murine model of urinary tract infection due to *Escherichia coli* producing or not producing the ESBL CTX-M-15. *J Antimicrob Chemother*. 2015; 70: 1466–1472. <https://doi.org/10.1093/jac/dku542> PMID: 25564564
 11. Kager L, Brismar B, Malmborg AS, Nord CE. Imipenem concentrations in colorectal surgery and impact on the colonic microflora. *Antimicrob Agents Chemother*. 1989; 33: 204–208. <https://doi.org/10.1128/aac.33.2.204> PMID: 2719464
 12. Fuchs PC, Barry AL, Thornsberry C, Jones RN. Interpretive criteria for temocillin disk diffusion susceptibility testing. *Eur J Clin Microbiol*. 1985; 4: 30–33. <https://doi.org/10.1007/BF02148656> PMID: 3872793
 13. Mahieu R, Cassisa V, Hilliquin D, Coron N, Pailhoriès H, Kempf M, et al. Impact of faecal microbiota transplantation on mouse digestive colonization with two extensively resistant bacteria. *J Infect*. 2017; 75: 75–77. <https://doi.org/10.1016/j.jinf.2017.04.008> PMID: 28461230
 14. Mahieu R, Cassisa V, Sanderink D, Chenouard R, Pailhoriès H, Kempf M, et al. Iterative Fecal Microbiota Transplantations for Eradicating Digestive Colonization With Carbapenemase-Producing Enterobacteriaceae: Is It Worth It? *Infect Control Hosp Epidemiol*. 2017; 1–2. <https://doi.org/10.1017/ice.2017.173> PMID: 28807067
 15. Pletz MWR, Rau M, Bulitta J, De Roux A, Burkhardt O, Kruse G, et al. Ertapenem pharmacokinetics and impact on intestinal microflora, in comparison to those of ceftriaxone, after multiple dosing in male and female volunteers. *Antimicrob Agents Chemother*. 2004; 48: 3765–3772. <https://doi.org/10.1128/AAC.48.10.3765-3772.2004> PMID: 15388432
 16. Høyen CK, Pultz NJ, Paterson DL, Aron DC, Donskey CJ. Effect of parenteral antibiotic administration on establishment of intestinal colonization in mice by *Klebsiella pneumoniae* strains producing extended-spectrum beta-lactamases. *Antimicrob Agents Chemother*. 2003; 47: 3610–3612. <https://doi.org/10.1128/aac.47.11.3610-3612.2003> PMID: 14576127
 17. Pultz NJ, Stiefel U, Subramanyan S, Helfand MS, Donskey CJ. Mechanisms by which anaerobic microbiota inhibit the establishment in mice of intestinal colonization by vancomycin-resistant *Enterococcus*. *J Infect Dis*. 2005; 191: 949–956. <https://doi.org/10.1086/428090> PMID: 15717271
 18. Dinubile MJ, Friedland I, Chan CY, Motyl MR, Giezek H, Shivaprakash M, et al. Bowel colonization with resistant gram-negative bacilli after antimicrobial therapy of intra-abdominal infections: observations from two randomized comparative clinical trials of ertapenem therapy. *Eur J Clin Microbiol Infect Dis*. 2005; 24: 443–449. <https://doi.org/10.1007/s10096-005-1356-0> PMID: 15990986
 19. Donskey CJ, Chowdhry TK, Hecker MT, Høyen CK, Hanrahan JA, Hujer AM, et al. Effect of antibiotic therapy on the density of vancomycin-resistant enterococci in the stool of colonized patients. *N Engl J Med*. 2000; 343: 1925–1932. <https://doi.org/10.1056/NEJM200012283432604> PMID: 11136263
 20. Caron F, Galperine T, Flateau C, Azria R, Bonacorsi S, Bruyère F, et al. Practice guidelines for the management of adult community-acquired urinary tract infections. *Médecine et Maladies Infectieuses*. 2018; 48: 327–358. <https://doi.org/10.1016/j.medmal.2018.03.005> PMID: 29759852
 21. Alexandre K, Réveillon-Istin M, Fabre R, Delbos V, Etienne M, Pestel-Caron M, et al. Temocillin against Enterobacteriaceae isolates from community-acquired urinary tract infections: low rate of resistance and good accuracy of routine susceptibility testing methods. *J Antimicrob Chemother*. 2018; 73: 1848–1853. <https://doi.org/10.1093/jac/dky101> PMID: 29635629