ECA 2021

sciforum

*Conference Proceedings Paper*

# Differentiation of Trimethoprim Resistance Genes among Eschericha coli Strains from an Environment with Intensive Supply of Antibiotics †

**Justyna Mazurek-Popczyk [1,*] and Katarzyna Baldy-Chudzik [2]**

[1] Department of Microbiology and Molecular Biology, Institute of Health Sciences, Collegium Medicum, University of Zielona Góra ; j.mazurek-popczyk@cm.uz.zgora.pl
[2] Department of Microbiology and Molecular Biology, Institute of Health Sciences, Collegium Medicum, University of Zielona Góra; k.baldy-chudzik@cm.uz.zgora.pl
* Correspondence: j.mazurek-popczyk@cm.uz.zgora.pl; Tel.: (+48683287333; J.M-P)
† Presented at: The 1st International Electronic Conference on Antibiotics—The Equal Power of Antibiotics And Antimicrobial Resistance, https://eca2021.sciforum.net/, 08/05/2021–17/05/2021.

Received: date; Accepted: date; Published: date

**Abstract:** Trimethoprim (TMP) is chemotherapeutic agent, which is mostly used to treat community-acquired urinary tract infections in medicine, but TMP is also commonly used in veterinary and during livestock production. There are multiple mechanisms by which strains become resistant to trimethoprim like bypass mechanism encoding by *dfr* genes. Aim of the research was to assess the differentiation of *dfrA* genes in commensal *Escherichia coli* strains isolated from pigs from a breeding farm with intense supplementation of antibiotics in metaphylaxis program. A total of one hundred and sixty-four *E.coli* strains were isolated from feces of fifty animals. Trimethoprim resistance was tested by microdilution method and revealed that 92% of tested strains were resistant with high MIC values (>32 mg/L). Resistance genes were tested by PCR and PCR-RFLP methods and six different genes were detected: *dfrA1*, *dfrA5*, *dfrA7*, *dfrA12*, *dfrA14*, *dfrA21*. Among 37% of the strains two to four different *dfrA* genes were detected in different combinations. In only three cases, all the strains derived from one individual had the same pattern of resistance genes among the others there was significant variability. *DfrA* genes were also detected in strains sensitive to TMP. Genes sequence analysis revealed nucleotide changes within some genes, which highlight the potential for alterations leading to the emergence of new resistance gene variants. The study shows the great diversity of the trimethoprim resistance genes, both within the tested animal population and in the individual host.

**Keywords:** antibiotic resistance; trimethoprim; *Escherichia coli*

## 1. Introduction

Trimethoprim (TMP) is a synthetic, broad-spectrum chemotherapeutic agent. TMP inhibits dihydrofolate reductase (DHFR)- an enzyme, that catalyzes the reduction of dihydrofolate to tetrahydrofolate in microbial cells . The main role of DHFR is related to biosynthesis pathways of the thymidylate, purines and several amino acids. TMP is mostly used to treat community-acquired urinary tract infections in medicine [1,2]. In veterinary medicine and during livestock production, trimethoprim is commonly used in combination with sulfonamides and can be administered orally, as a bolus, medicated fodder or in drinking water [3]. An intense use of antibiotics in these sectors leads to the rapid selection of resistant strains which, through the environment, can pose a serious health risk. Resistance to trimethoprim persists over the years [4].

There are multiple mechanisms by which strains become resistant to trimethoprim; to these mechanisms belongs impermeability of cell membranes, activity of the efflux pump, overproduction

chromosomal of DHFR and one of the most frequently identified- bypass mechanism. The last mentioned mechanism is associated with the presence of the acquired *dfr* genes encoding a non-allelic variants of dihydrofolate reductase (DHFR) and is responsible for high levels of trimethoprim resistance [1,5]. Two main families of *dfr* genes which are *dfrA* and *dfrB* among Gram negative bacteria have been distinguish. The *dfrA* family is larger, includes subfamilies *dfrA1*-group and *dfrA12*-group, consists of genes that are at least 474 nucleotides long and share even less than 25% amino acid sequence identity [6]. Currently thirty eight *dfrA* genes identified in different species are already described [7]. *Dfr* genes are found mostly as gene cassettes within variable parts of class 1 and class 2 integrons but also with common regions IS*CR [*8]. Association with the mobile genetic elements contributes to the rapid spread of resistance to trimethoprim among bacteria. Trimethoprim (TMP) was first used chemotherapeutic to treat infection in humans in 1962 and in 1974 Sköld described first plasmid-mediating enzyme responsible for resistance to high levels of trimethoprim in *E. coli* strains [9]. In addition to horizontal gene transfer, selection of resistant mutants in an environment of high antibiotic pressure is the second driving force for the emergence and spread of antibiotic resistance.

The aim of the study was to assess the differentiation of *dfrA* genes determining resistance to trimethoprim in commensal *Escherichia coli* strains isolated from pigs from a breeding farm with an intensive supply of antibiotics in metaphylaxis program.

## 2. Materials and Methods

### *2.1. Material Collection and Bacterial Isolation*

*E.coli* were isolated from fecal samples of 50 pigs, piglets and sows form one farm in Lubuskie province in Poland. The material was from swine farming, where piglets in the period after weaning were subject to the medical metaphylaxis program. The animals were treated with medicated fodder contained amoxicillin, trimethoprim, sulfamethoxazole for four weeks.

Fresh feces were collected and transported under refrigeration to the laboratory, and inoculated on McConkey agar. After cultivation ten typical, pink colonies per sample were further identified by biochemical testing and finally frozen in -80˚C. *E. coli* isolates were differentiated based on BOX-PCR fingerprint analysis [10,11].

### *2.2. Antibiotic Susceptibility Testing*

Trimethoprim resistance was tested by microdilution method. Colonies of *E. coli* cultures (18h) were suspended and diluted to a value of 0.5 McFarland, then inoculated with Mueller Hinton Broth (Merck) and spread into the wells of the 96-well plate with TMP solutions. The range of tested TMP values were 0.25-32 mg/l. After 18h incubation at 36˚C the MIC values were calculate. The results were interpreted according to EFSA [12].

### *2.3. Detection of Trimethoprim Resistance dfrA Genes*

Thermal lysates were the source of template DNA. PCR method was used to identify *dfrA5, dfrA7*, *dfrA14*, *dfrA24*, *dfrA26* genes [13]. PCR-RFLP method was used to identify genes from two *dfr* gene groups: group A1 (*dfrA1, dfrA15, dfrA15b, dfrA16, dfrA16b, dfrA28*) and A12 (*dfrA12, dfrA13, dfrA21* and *dfrA22*). In this method primers covering the conservative regions of *dfr* gene groups is used and the resulting amplicons are digested with restriction enzymes PvuI, AluI, TruI, MspI, TasI. The obtained products of a certain length indicate the type of the *dfr* gene [14].

### *2.4. Sequence Analysis*

Selected PCR products from amplification of groups A1 genes, due to non-specific restriction digest products were sequence (Genomed, Poland). The sequence has been assembled in Nucleotide BLAST (https://blast.ncbi.nlm.nih.gov), the description of an exemplary alignment has been carried out using T-Coffee (http://tcoffee.crg.cat) and BOXSHADE (https://embnet.vital-it.ch/cgi-bin/BOX_form_parser) online tools.

## 3. Results

### 3.1. Resistance to TMP

A total of one hundred and sixty-four *E.coli* strains were isolated from feces of fifty animals, from two to six non-identical isolates per animal samples. In this set one hundred and fifty one (92%) isolates were resistant to TMP and they derived from samples of forty-nine individuals. In samples from ten pigs, *E. coli* both sensitive both resistant to TMP were detected. Studies revealed that 92% of tested strains were resistant with high MIC values (>32 mg/L).

### 3.2. Prevalence of dfrA Genes

In the tested set of *E.coli* (164), six different genes were detected: *dfrA1, dfrA5, dfrA7, dfrA12, dfrA14* and *dfrA21*. The *dfrA1* gene was the most common (n= 105/64%), and was not detected in only one strains from one animal. *Dfr12* gene was detected in 60 *E.coli* (37%), *dfr7* gene in 28 strains (17%) and *dfr5*, *dfrA14* and *dfr21* genes were detected respectively in 18 (11%), 15 (9%) and 14 (9%) of tested strains (Table 1.).

**Table 1.** Prevalence of trimethoprim resistance *dfrA* genes among *E. coli* isolates from pigs.

| ***dfrA1*** | ***dfr12*** | ***dfr7*** | ***dfr5*** | ***dfrA14*** | ***dfr21*** |
|---|---|---|---|---|---|
| 105 (65%) | 60 (36.6%) | 28 (17.1%) | 18 (11%) | 15 (9.1%) | 14 (8.5%) |

36.6% of the strains (n=59) carried two to four different *dfrA* genes in different combinations. In 32 strains (20.1%) two *dfrA* genes were detected, in 19 strains (11.6%) three genes were detected, in 6 (3.7%) four genes were detected and in one (0.6%) strain five TMP resistance genes were detected (Table 2.). *DfrA* genes were also detected in three strains sensitive to TMP.

**Table 2.** Distribution of trimethoprim resistance genes among *E. coli* isolates from pigs.

| **Number of Strains with Multiple *dfrA* Genes** | | | | | |
|---|---|---|---|---|---|
| 18 | *dfrA1* | *dfrA12* | | | |
| 7 | *dfrA1* | *dfrA7* | | | |
| 3 | *dfrA1* | *dfrA5* | | | |
| 1 | *dfrA1* | *dfrA21* | | | |
| 1 | *dfrA5* | *dfrA7* | | | |
| 1 | *dfrA5* | *dfrA14* | | | |
| 1 | *dfrA7* | *dfrA12* | | | |
| 1 | *dfrA12* | *dfrA21* | | | |
| 7 | *dfrA1* | *dfrA12* | *dfrA21* | | |
| 5 | *dfrA1* | *dfrA5* | *dfrA14* | | |
| 1 | *dfrA1* | *dfrA5* | *dfrA7* | | |
| 2 | *dfrA1* | *dfrA7* | *dfrA12* | | |
| 1 | *dfrA1* | *dfrA12* | *dfrA14* | | |
| 2 | *dfrA5* | *dfrA7* | *dfrA14* | | |
| 1 | *dfrA5* | *dfrA12* | *dfrA14* | | |
| 2 | *dfrA1* | *dfrA7* | *dfrA12* | *dfrA21* | |
| 1 | *dfrA1* | *dfrA7* | *dfrA12* | *dfrA14* | |
| 2 | *dfrA1* | *dfrA12* | *dfrA14* | *dfrA21* | |
| 1 | *dfrA1* | *dfrA5* | *dfrA7* | *dfrA14* | |
| 1 | *dfrA1* | *dfrA5* | *dfrA7* | *dfrA12* | *dfrA21* |
| Total number 59 | | | | | |

In only four cases all of the strains derived from one individual had the same pattern of TMP resistance genes, among the others there was significant variability was shown. Taking into account the number of genes in the strains from a given animal, strains from 46 individuals had from two to

six different genes. In *E. coli* from samples from 17 animals, two different genes were detected, from 19 - three genes, from 6 - four genes, from 3 - five genes, and in *E. coli* strains from 2 animals, 6 different genes were detected (Table 3).

**Table 3.** Number of animals in which *E.coli* strains with different *dfrA* genes detected.

| 1 Gene | 2 Genes | 3 Genes | 4 Gene | 5 Gene | 6 Genes |
|---|---|---|---|---|---|
| | | per animal sample | | | |
| 3 (6%) | 17 (34%) | 19 (38%) | 6 (12%) | 3 (6%) | 1 (2%) |

### 3.3. Sequence Analysis of *dfrA1* Genes

During restriction enzyme analysis of PCR products of *dfrA1* genes group, in five cases digestion with PvuI and TasI enzymes indicated the presence of the *dfrA1* gene but non-specific products after TruI and AluI digesting were revealed. PCR products were sequenced and nucleotide sequences were analyzed in NCBI Nucleotide BLAST. Sequence alignment revealed the highest identity score with *dfrA1*at 89.27% (GB: NG_047685.1). Numerous single nucleotide changes and several deletions were detected (Figure 1.). Comparing translated amino acid sequence to the NCBI data base, the highest protein identity score was 83.02% (GB:EFY9453405.1).

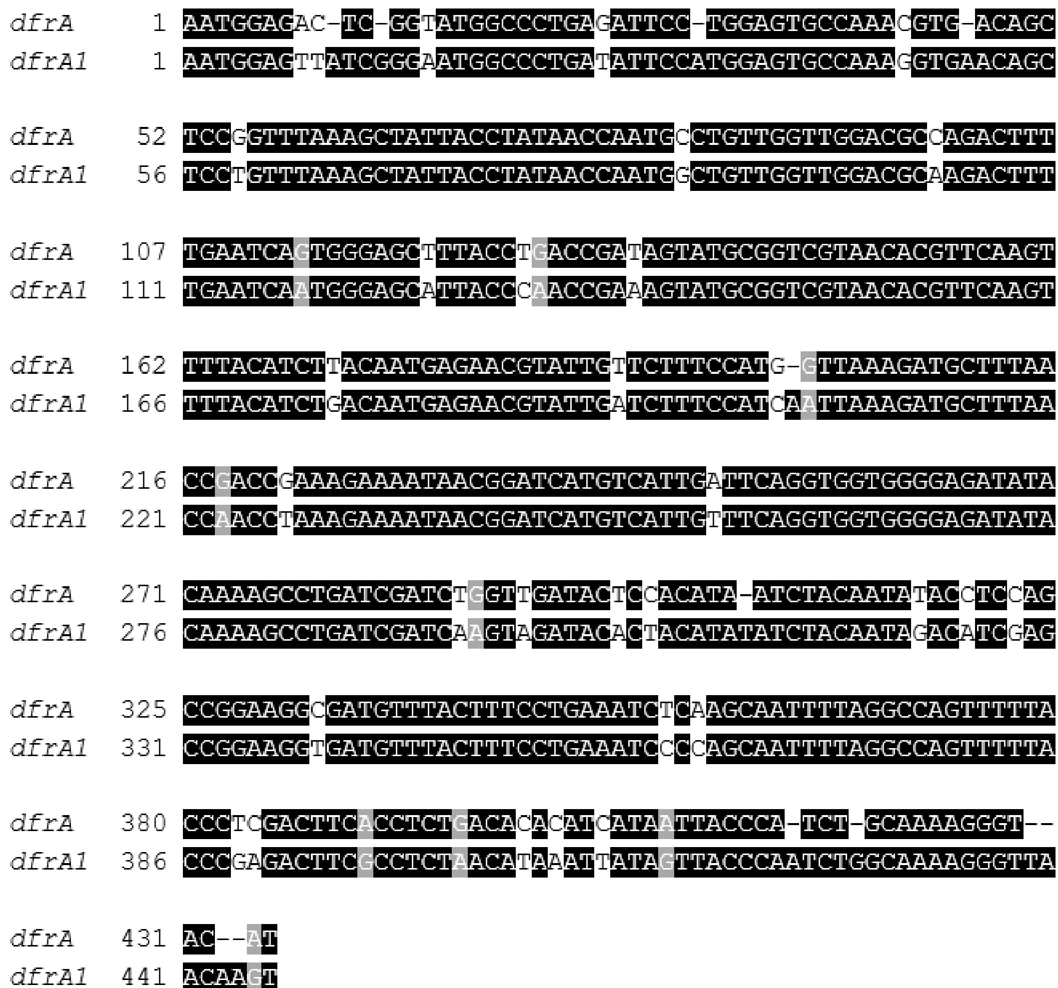


**Figure 1.** Alignment result with *dfrA1* sequence GB: NG_047685.1.

## 4. Discussion

The prevalence of the resistant strains in the sample collection from pigs was very high, reaching 92%. Only the strains from one animal showed a sensitivity to TMP. This results clearly show the

enormous power of selection pressure. An animal husbandry where antibiotics are intensively administered selected resistant strains.

In addition to the high prevalence, the high level of resistance has also been demonstrated among most of the strains. For all resistant *E.coli* the minimum growth inhibitory concentration exceeded 32mg/L. It is worth mentioning that the cut-off values for *E.coli* resistance both for zoonotic and human strains are significantly lower (R>2 and R>4mg/L respectively) [12,15].

The research showed a large diversity of *dfrA* genes in the studied population. Literature reports the detection of multiple TMP resistance genes but mostly in strains that are isolated from a larger collection or from different environments [13,16–18]. It is also rare to identify six different genes corresponding to resistance to the same antibiotic in one strain.

It is known that the relationship between *dfr* genes and mobile elements is mostly responsible for this genetic diversity. The *dfr* genes are found as gene cassettes within variable parts of class 1 and class 2 integrons and common regions like IS*CR2 [*8]. Trimetoprim resistance genes detected in this study: *dfrA1, dfrA5, dfrA7, dfrA12, dfrA14, dfrA17, dfrA21* have been found as gene cassettes, present within variable regions of class 1 and 2 integrons [16–20]. It was shown that among *E. coli* form one pig farm, large variety of plasmids carrying multiple resistance genes can exist [20].

One of the theories that explain great diversity and global distribution of *dfr* genes is that they originate from a wide variety of bacteria and are disseminated through horizontal gene transfer [5,21]. The *dfrA21* gene is a good illustration of this assumption. This gene was first detected in *Salmonella enterica* Livingstone producing beta-lactamase CTX-M-27 in the neonatal unit in 2005 in Tunisia [22]. Currently, a large number of records with *dfrA21* gene are available in the NCBI database, like in *Klebsiella pneumoniae* from Chilean hospital (GenBank accession no. KY286108), or from United Kingdom (NZ_FLGL01000034.1), *Pseudomonas aeruginosa* from Brazilian states (NZ_JADPHL010000124.1), *Citrobacter freundii* from the Unites States (NZ_JADVDT010000023.1). Recently described new *dfr* genes- *dhfr*37 has been detected in gram-negative *Rheinheimera* spp. from Yellow Sea, China [23] and *dfrA38* was found in *Acinetobacter baumannii* isolates [7]. It is a matter of a short time when these genes will be identified in our environment.

The second mechanism contributing to the emergence of resistance are mutations within the genes. An example are the changes in the sequence of the *dfrA1* gene observed in the presented studies, which were caught by changing the restriction site. Nucleotide changes could lead to a new variant of the gene, encoding a trimethoprim-insensitive dihydrofolate reductase. Selection of resistant mutants is accelerated in the presence of antibiotic pressure like in the animal breeding environment with an intensive supply of antibiotics. However mutations and rearrangements of horizontal gene transfer can lead to switching off the expression of these genes [24]. It is often assumed that detected resistance gene are expressed, but it has been shown that *dfrA* genes present in the cells may not be expressed, particularly gene cassettes of class 1 integrons [24]. After all, resistance genes are detected in sensitive strains.

Various approaches to the effects of antibiotics and selection pressure on the development of resistance are discussed. It has been shown that exposure to very low antibiotic concentrations can select low-level resistant mutants and use of an antibiotic at a concentration above the minimal inhibitory, would limit the emergence of resistant mutants [25–27]. The use of antibiotics in the livestock sector has been a great concern. Governmental and international agencies have been pointing to the rational use of antibiotics. Fortunately, the latest data of European Medicines Agency in European surveillance of veterinary antimicrobial consumption, shows a downward trend over several years and overall decline in antibiotic sales [28]. Unfortunately whenever antibiotics are used improperly, resistance will be generated. The current research adds voice to the appeal and the rational management of antibiotics.

## 4. Conclusions

The study shows the great diversity of the trimethoprim resistance genes, both within the tested animal population and in the individual host. In addition, genes sequence analysis revealed nucleotide changes within some genes, which highlight the potential for alterations leading to the

emergence of new resistance gene variants. Research shows how the intensive use of antibiotics in the population contributes significantly to the development of antibiotic resistance.

**Author Contributions:** Conceptualization, J.M-P. and K.B.-Ch; methodology, J.M.-P.; investigation, J.M.-P.; resources, J.M-P., K.B.-Ch; data curation, J.M.-P., K.B.-Ch; writing—original draft preparation, J.M.-P..; writing—review and editing, J.M.-P., K.B.-Ch;. supervision, K.B.-Ch.; project administration, J.M-P., K.B.-Ch.; funding acquisition, K.B.-Ch. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Ethical review and approval were waived for this study, due to fact, that feces not rectal swabs so studies not involved and harm animals.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References


1. Huovinen, P.; Sundström, L.; Swedberg, G.; Sköld O. Trimethoprim and sulfonamide resistance. *Antimicrob Agents Chemother* **1995**, *39*, 279–289, doi:10.1128/AAC.39.2.279.
2. Caron, F.; Wehrle, V.; Etienne, M. The comeback of trimethoprim in France. *Med Mal Infect*. **2017**, *47*, 253–260, doi:10.1016/j.medmal.2016.12.001.
3. Moulin, G.; Cavalié, P.; Pellanne, I.; Chevance, A.; Laval, A.; Millemann, Y.; Colin, P.; Chauvin, C. Antimicrobial Resistance ad hoc Group of the French Food Safety Agency. A comparison of antimicrobial usage in human and veterinary medicine in France from 1999 to 2005. *J Antimicrob Chemother* **2008**, *62*, 617–625, doi:10.1093/jac/dkn213.
4. Pormohammad, A.; Nasiri, M.J.; Azimi, T. Prevalence of antibiotic resistance in *Escherichia coli* strains simultaneously isolated from humans, animals, food, and the environment: A systematic review and meta-analysis. *Infect. Drug Resist.* **2019**, *12*, 1181–1197. https://doi.org/10.2147/IDR.S201324.
5. Sköld, O. Resistance to trimethoprim and sulfonamides. *Vet Res* **2001**, *32*, 261–273, doi:10.1051/vetres:2001123.
6. van Hoek, A.H.; Mevius, D.; Guerra, B.; Mullany, P.; Roberts, A.P.; Aarts, H.J. Acquired antibiotic resistance genes: An overview. *Front Microbiol*. **2011**, *28*, 203, doi:10.3389/fmicb.2011.00203.
7. Ambrose, S.J.; Hall, R.M. A novel trimethoprim resistance gene, *dfrA38*, found in a sporadic *Acinetobacter baumannii* isolate. *J Antimicrob Chemother*. **2020**, *75*, 3694–3695, doi:10.1093/jac/dkaa379.
8. Wüthrich, D.; Brilhante, M.; Hausherr, A.; Becker, J.; Meylan, M.; Perreten, V. A Novel Trimethoprim Resistance Gene, *dfrA36*, Characterized from *Escherichia coli* from Calves. mSphere, **2019**, *4*, e00255–e19. doi.org/10.1128/mSphere.00255-19.
9. Sköld O.; Widh A.; A new dihydrofolate reductase with low trimethoprim sensitivity induced by an R-factor mediating high resistance to trimethoprim, *J. Biol. Chem*. **1974**, *249*, 4324–4325.
10. Versalovic, J.; Schneider, M.; de Bruijn, F.J.; Lupski, J.R. Genomic fingerprinting of bacteria using repetitive sequence-based polymerase chain reaction. *Method. Mol. Cell. Biol*. **1994**, *5*, 25–40.
11. Bok, E.; Mazurek, J.; Pusz, P.; Stosik, M.; Baldy-Chudzik, K. Age as a factor influencing diversity of commensal *E. coli* microflora in pigs. *Pol. J. Microbiol*. **2013**, *62*, 165–171.
12. EFSA (European Food Safety Authority), Amore G.; Beloeil P.-A.; Stoicescu A.-V. Manual for reporting 2020 antimicrobial resistance dat within the framework of Directive 2003/99/EC and Decision 2013/652/EU. EFSA supporting publication **2021**, *2021:EN-6442*. 26 pp, doi:10.2903/sp.efsa.2021.EN-6442.
13. Brolund, A.; Sundqvist, M.; Kahlmeter, G.; Grape, M. Molecular Characterisation of Trimethoprim Resistance in *Escherichia coli* and *Klebsiella pneumoniae* during a Two Year Intervention on Trimethoprim Use. *PLoS ONE* **2010**, *5,* e9233, doi:10.1371/journal.pone.0009233.
14. Seputiene, V.; Povilonis, J.; Ruzauskas, M.; Pavilonis, A.; Suziedeliene, E. Prevalence of trimethoprim resistance genes in *Escherichia coli* isolates of human and animal origin in Lithuania. *J. Med. Microbiol*. **2010**, *59*, 315–322.
15. European Committee on Antimicrobial Susceptibility Testing. Breakpoints tables for interpretation of MICs and zones diameters. Version 9.0 **2019**. http://www.eucast.org.
16. Blahna, M.T.; Zalewski, C.A.; Reuer, J.; Kahlmeter, G.; Foxman, B.; Marrs, C.F. The role of horizontal gene transfer in the spread of trimethoprim-sulfamethoxazole resistance among uropathogenic *Escherichia coli* in Europe and Canada. *J. Antimicrob. Chemother*. **2006**, *57*, 666–672.

17. Grape, M.; Motakefi, A.; Pavuluri, S.; Kahlmeter, G. Standard and real-time multiplex PCR methods for detection of trimethoprim resistance *dfr* genes in large collections of bacteria. *Clin Microbiol Infect*. **2007**, *13*, 1112–1118.
18. Domínguez, M.; Miranda, C.D.; Fuentes, O.; de la Fuente, M.; Godoy, F.A.; Bello-Toledo, H.; González-Rocha, G. Occurrence of Transferable Integrons and *sul* and *dfr* Genes Among Sulfonamide-and/or Trimethoprim-Resistant Bacteria Isolated From Chilean Salmonid Farms. *Front Microbiol*. **2019**, *12*, 748, doi:10.3389/fmicb.2019.00748.
19. Karczmarczyk, M.; Walsh, C.; Slowey, R.; Leonard, N.; Fanning, S. Molecular characterization of multidrug-resistant *Escherichia coli* isolates from Irish cattle farms. *Appl Environ Microbiol*. **2011**, *77*, 7121–7127, doi:10.1128/AEM.00601-11.
20. Mazurek, J.; Bok, E.; Baldy-Chudzik, K. Complexity of Antibiotic Resistance in Commensal *Escherichia coli* Derived from Pigs from an Intensive-Production Farm. *Microbes Environ*. **2018**, *33*, 242–248, doi:10.1264/jsme2.ME17041.
21. Kraupner, N.; Ebmeyer, S.; Hutinel, M.; Fick, J.; Flach, C.F.; Larsson, D.G.J. Selective concentrations for trimethoprim resistance in aquatic environments. *Environ. Int*. **2020**, *144*, 106083, doi:10.1016/j.envint.2020.106083.
22. Bouallègue-Godet, O.; Ben Salem, Y.; Fabre L. Nosocomial outbreak caused by *Salmonella enterica* serotype Livingstone producing CTX-M-27 extended-spectrum beta-lactamase in a neonatal unit in Sousse, Tunisia. *J. Clin. Microbiol*. **2005**, *43*, 1037–1044, doi:10.1128/JCM.43.3.1037-1044.2005.
23. Fu, J.; Zhong, C.; Zhang, P.; Zong, G.; Liu, M.; Cao, G. Novel Mobilizable Genomic Island GEI-D18A Mediates Conjugational Transfer of Antibiotic Resistance Genes in the Multidrug-Resistant Strain *Rheinheimera* sp. D18. *Front. Microbiol*. **2020**, *11*, 627, doi:10.3389/fmicb.2020.00627.
24. Mazurek, J.; Bok, E.; Stosik, M.; Baldy-Chudzik, K. Antimicrobial resistance in commensal *Escherichia coli* from pigs during metaphylactic trimethoprim and sulfamethoxazole treatment and in the post-exposure period. *Int J. Env. Res. Public Health.* **2015**, *12*,2150-63, doi:10.3390/ijerph120202150.
25. Baquero, F. Low-level antibacterial resistance: A gateway to clinical resistance. *Drug Resist. Updates* **2001**, *4*, 93–105, doi:10.1054/drup.2001.0196.
26. Cantón, R.; Lode, H.; Graninger, W.; Milkovich, G. Respiratory tract infections: At-risk patients, who are they? Implications for their management with levofloxacin. *Int J* Antimicrob Agents. **2006**, *28*, S115–S127, doi:10.1016/j.ijantimicag.2006.06.001.
27. Lee, D.S.; Lee, S.J.; Choe, H.S. Community-Acquired Urinary Tract Infection by *Escherichia coli* in the Era of Antibiotic Resistance. *Biomed. Res. Int*. **2018**, *2018*,7656752, doi:10.1155/2018/7656752.
28. European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption, 2020. 'Sales of veterinary antimicrobial agents in 31 European countries in 2018. **2020** (EMA/24309/2020).


Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).