

Highlights

- Co-trimoxazole use in UK is dramatically lower than in the late 1980s
- In older studies, decline in use did not result in decline in resistance
- Mathematical models showed that it may take decades before resistance declines
- Analysed more recently collected *Escherichia coli* blood isolates
- Co-trimoxazole resistance did not decline over time between 2002 and 2014

Will co-trimoxazole resistance rates ever go down? Resistance rates remain high despite decades of reduced co-trimoxazole consumption.

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Abstract

Objective: Several studies showed that a substantial decline in the use of co-trimoxazole did not result in a decline in resistance rates among *Escherichia coli* isolates. Since mathematical models have shown that it may take decades before resistance rates start to decline to relevant levels, we performed a new analysis using more recently collected data.

Methods: Data were extracted from Guy's and St Thomas' Hospitals Transmission and Antimicrobial Record database which contains microbiological test results from all specimens tested between 2002 and 2014. We selected all blood samples positive for *E. coli* which were tested for resistance against co-trimoxazole. Prevalence of co-trimoxazole resistance among the tested samples by year was modelled by a Poisson model.

Results: Almost all (96%) of *E. coli* blood isolates were tested for co-trimoxazole resistance. In total, 2,070 *E. coli* isolates were available for analyses. Resistance to co-trimoxazole fluctuated over the years, but there was no clear increasing or decreasing trend; the annual percentage change in the prevalence of co-trimoxazole resistance was 0.52 (95% confidence interval -0.75% to 1.81%). Including co-trimoxazole or trimethoprim use in the year before the sample was taken did not improve the model.

Conclusion: The prevalence of co-trimoxazole resistance among *E. coli* blood isolates remained high, almost three decades after a substantial decline in co-trimoxazole use. Our results further emphasize the importance of prudent antibiotics use, as antibiotic resistance may not always be easily reversible.

Keywords: *Escherichia coli*; antimicrobial resistance; bacterial infections

1. Introduction

There is a clear link between antimicrobial consumption within a population and the resistance rates of bacteria in that same population. Countries with relatively high antibiotic prescribing rates have relatively high antibiotic resistance rates [1], geographical variation within countries can be explained by variation in antibiotic use [2], and individuals recently exposed to antibiotics have a higher likelihood of carrying resistant bacteria [3].

Although there are no doubts about the causal link between antibiotic consumption and antibiotic resistance, there is debate about the reversibility of antibiotic resistance [4]. In light of the lack of new antibiotic development, lowering resistance by more prudent use of antibiotics could be an important strategy to help tackling the growing threat of antibiotic resistance. If many resistances were not reversible, the prudent use of antibiotics to prevent a further resistance development and rise in resistance levels would be even more important.

Theoretically, when selective pressure brought about by antibiotic prescribing is reduced, resistant bacteria would be outcompeted by susceptible bacteria, because carrying resistance genes is generally assumed to be associated with a fitness cost [5]. In line with this theory, reducing antimicrobial use appears to have been fairly successful in reducing the prevalence of resistance in confined environments, such as hospital wards or in intensive farming [5]. However, a major part of the apparent successes in hospital settings may be explained by a dilution effect caused by continuous influx of patients who are infected or colonized with susceptible bacteria [5]. In essence, susceptible bacteria from these patients replace the resistant bacteria [5].

Reducing resistance in bacteria considered as commensal flora, such as *Escherichia coli*, has been proven to be much harder [6]. There are several reasons why resistance may not be easily reversible:

- i) some resistances are associated with relatively low or non-existent fitness costs; ii) compensatory

evolution may reduce fitness costs; and iii) resistance genes are frequently collocated with other resistance genes (to other antimicrobial classes) on the same plasmid causing co-selection [4,6]. In addition, mathematical models have shown that, even with complete cessation of antibiotic use, it may simply take years or even decades before resistance in the community will decline to clinically relevant levels [5].

Previously, a study from the Royal London Hospital showed that a huge decline in the use of co-trimoxazole (trimethoprim/sulfamethoxazole) did not result in a decline in sulfonamide resistance rates among *E. coli* isolates within 9 years [6]. The combination of low fitness costs or even increased fitness in one strain and co-selection by other antimicrobials are thought to be the main reasons behind the lack of a decline in sulphonamide resistance [7]. In a study from Sweden, a 2-year drastic reduction in trimethoprim use had almost no effect on the frequency of trimethoprim and co-trimoxazole resistance rates [8]. Similarly, rates of co-trimoxazole resistance among *E. coli* samples remained stable despite significant decreases in co-trimoxazole use in the community in studies from Switzerland [9], Sweden [10, 11], and Spain [12].

Because mathematical models have shown that it may take several years or even decades before resistance rates start to decline to clinically relevant levels [5], and several years have passed since the previous studies assessed co-trimoxazole resistance levels, we performed a new analysis using more recently collected data.

2. Methods

2.1 Antibiotic prescribing

Data on national annual primary care antibiotic prescriptions between 1998 and 2013 were obtained from NHS Business services authority (NHSbsa). The data includes all antibiotics prescribed in England which were dispensed in the community, except items prescribed by dentists or by

hospitals. UK Veterinary sales of trimethoprim/sulfonamides were obtained from the UK Veterinary Antibiotic Resistance and Sales Surveillance reports of the Veterinary Medicines Directorate [13].

2.2 Bacterial isolates

Data were extracted from Guy's and St Thomas' Hospitals (London, UK) Transmission and Antimicrobial Record database which contains microbiological test results from all specimens tested between 2002 and 2014. This collection contains specimens from inpatients, outpatients, accident and emergency departments, and general practice patients within the hospitals' catchment area. National Ethical approval for research databases is not required under the Research Governance Framework as directed by the Health Research Authority, UK. We selected all blood samples positive for *E. coli* which were tested for resistance against co-trimoxazole. We only included the first isolate of a specific species for each patient in each year [6]. During the entire period, zone diameter breakpoints were ≤ 15 (resistant) and ≥ 16 (susceptible) [14].

2.3 Statistical analyses

Prevalence of co-trimoxazole resistance among the tested samples by year was modelled by taking the count of co-trimoxazole resistant samples as the response variable in a Poisson model with robust standard errors [15]. To take into account that the number of tests may differ per year, the natural logarithm of the number of samples tested for co-trimoxazole resistance each year was included as an offset variable. Initially, a model with only time (calendar year – 2002) was considered as a predictor variable. Potential non-linear effect of time was taken into account by including an additional quadratic function for time into the model. Subsequently, we evaluated whether including co-trimoxazole items dispensed per 100,000 inhabitants, trimethoprim items dispensed per 100,000 inhabitants, or veterinary sales of trimethoprim/sulfonamides (tonnes active ingredient) in the year before improved the model. Finally, it was evaluated whether there was a delayed (or lagged) association between co-trimoxazole or trimethoprim use and co-trimoxazole resistance. Because

127 antibiotic dispensing data was available up to 3 years before the first resistant measurements, we
128 evaluated whether the model fit improved by including 1, 2 and 3 year lagged measurements for co-
129 trimoxazole use , trimethoprim use, and veterinary trimethoprim/sulphonamide sales
130 simultaneously using an unconstrained distributed lag model [15]. The best-fitting model was
131 obtained using backward stepwise regression based on the Akaike Information Criterion (AIC).
132 Partial autocorrelation function plots were used to check for residual autocorrelation in the Poisson
133 models. The percentage annual change in prevalence of co-trimoxazole resistance was estimated
134 using the formula $(\exp(\beta)-1)*100$, where β refers to the sum of the relevant parameter estimates
135 from the Poisson model. We used R 3.2.1 for all our statistical analyses.

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3. Results

3.1 Antibiotic prescribing

A substantial decline in sulfonamide use, largely driven by a switch from co-trimoxazole to trimethoprim only, has been observed in the UK. While there were over 4 million annual prescriptions in the late 1980s, the use declined to 320,000 in 1991, and remained below 70,000 between 2000 and 2004 [6,7]. The data for England between April 1998 and March 2013 indicate that annual co-trimoxazole prescriptions initially declined to 47,000 in 2002, but thereafter increased again to 99,000 prescriptions in 2013 (Figure 1). Nevertheless, the total number of co-trimoxazole prescriptions is still substantially lower than in the 1980s. There was also an increase in annual trimethoprim prescriptions, from 2.7 million in 2002 to 3.8 million in 2013 (Figure 1). Veterinary sales of trimethoprim/sulfonamides decreased between 1999 and 2013 (Figure 2).

3.2 Co-trimoxazole resistance rates

Almost all (96%) of *E. coli* blood isolates were tested for co-trimoxazole resistance. After removing repeated blood samples retrieved from patients within one year (i.e. 2nd, 3rd, ...ⁿth sample), 2,070 *E. coli* isolates were available for analyses. Resistance to co-trimoxazole fluctuated over the years, but there was no clear increasing or decreasing trend (Figure 3). The absence of a clear trend was also reflected by the fact that the best fitting model only included an intercept. Including co-trimoxazole or trimethoprim use, or veterinary trimethoprim/sulfonamides sales in the 1-3 years before the sample was taken did not improve the model fit, as evidenced by higher AIC values being obtained when adding these variables to the model. When time in calendar years was forced into the model, the annual percentage change in the prevalence of co-trimoxazole resistance was 0.52% (95% confidence interval -0.75% to 1.81%). Partial autocorrelation function plots indicated that there was no significant residual autocorrelation.

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4. Discussion

Although co-trimoxazole prescribing increased compared to the previous decade in England, prescribing levels are still dramatically lower than those observed in the late 1980s. Nevertheless, similarly to previous studies from England that did not observe an effect on sulfonamide resistance [6,7], we did not observe a decrease in co-trimoxazole resistance among *E. coli* blood isolates. However, it is of note that the recent slight increase in consumption of co-trimoxazole did not result in an increase in co-trimoxazole resistance among *E. coli* blood isolates.

Reasons explaining why co-trimoxazole and sulfonamide resistance remained high in several countries despite a dramatic decrease in co-trimoxazole use have been discussed extensively [4,6,7,10-12]. One suggested explanation was that there may be a considerable time delay (even decades) before reductions in use are mirrored by reductions in resistance. Our results, together with other older studies [4,6,7,9-11], suggest that time since the decline in co-trimoxazole use is not a major factor determining the prevalence of co-trimoxazole resistance among *E. coli* isolates. Likely explanations for rather stable levels of resistance are co-selection by continued prescribing of other antibiotics and relatively low or non-existent fitness costs [6,16]. For example, trimethoprim is still widely used in the community and may partly explain continued high co-trimoxazole, a combination of trimethoprim and sulfamethoxazole, resistance rates. Although common resistance genes against trimethoprim do not confer resistance against sulfonamides [17], the genes conferring the resistance against trimethoprim, *dfr*, and sulphonamides, *sul*, are often linked in the widely distributed class 1 integrons [17, 18].

An important strength of this study is that the MCS database allowed us to perform analyses based on more than 2,000 blood isolates obtained from consecutive years, reducing the likelihood that findings are due to random variation in some selected years.

The current study has some important limitations however. In the database, the actual minimum inhibitory concentrations (MICs) or zone diameters are not stored. Hence, it was not possible to evaluate whether there were changes in the average MIC. Similarly, it was not possible to estimate the prevalence of co-trimoxazole resistance among urinary samples as those types of samples were not regularly tested for co-trimoxazole resistance.

In addition, we only had information about community antibiotic prescriptions on the national level, rather than at a regional or more granular level (which would have enabled us to determine prescribing within the catchment population of the hospitals from which resistance rates were determined). However, although there may be some geographical variation, given the magnitude of the reduction in national prescribing rates, it is almost certain that the conclusion that co-trimoxazole prescriptions are substantially lower than in the 1980's also holds for the catchment area of Guy's and St Thomas' Hospitals.

We did not have information about co-trimoxazole dispensed in the hospitals. However, the majority of antibiotics are dispensed in primary care [19] and co-trimoxazole is not commonly used in English hospitals [20], thereby limiting the potential impact of changes in hospital prescribing. We cannot exclude the possibility that local co-trimoxazole resistance rates were partly influenced by intercontinental travel to areas with high co-trimoxazole use and resistance rates. Nevertheless, given the fact that similar observations have been made using older data in the UK and other countries [4,6,7,9-11], results from this local hospital are likely generalizable to the national level.

In conclusion, the prevalence of co-trimoxazole resistance among *E. coli* blood isolates remained high almost three decades after a substantial decline in co-trimoxazole use. Our results further emphasize the importance of prudent antibiotics use, as antibiotic resistance may not always be easily reversible.

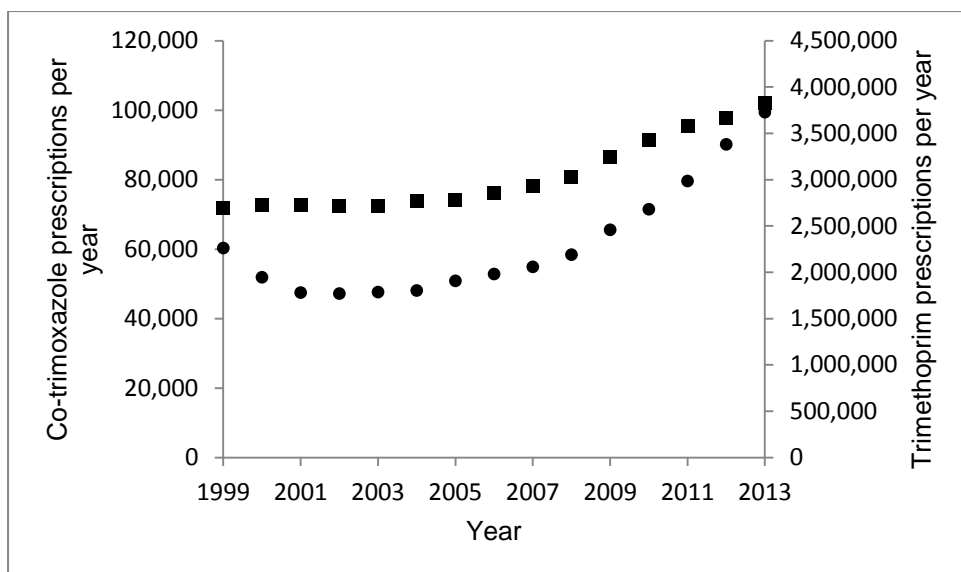


Figure 1. Annual co-trimoxazole (dots) and trimethoprim (squares) prescriptions in England.

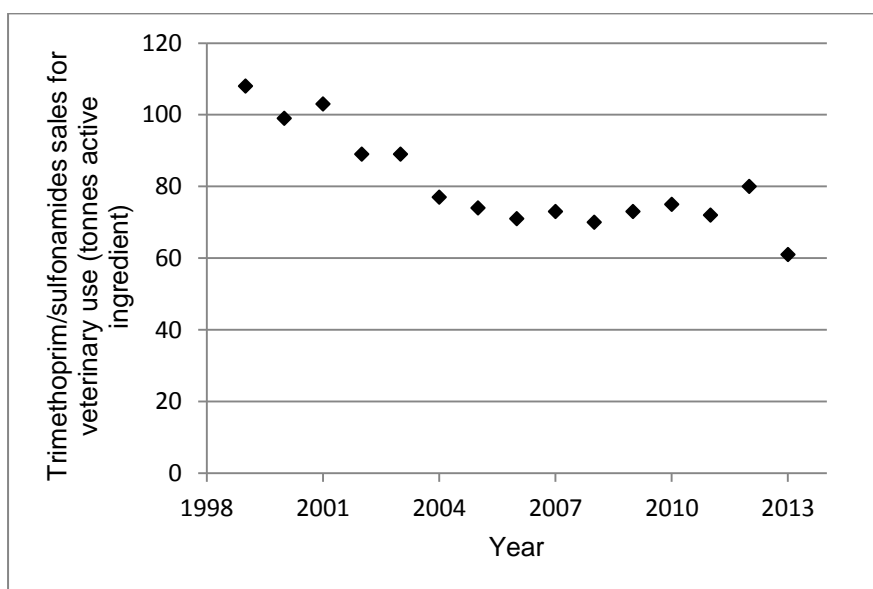


Figure 2. Annual trimethoprim/sulfonamides sales for veterinary use in the UK.

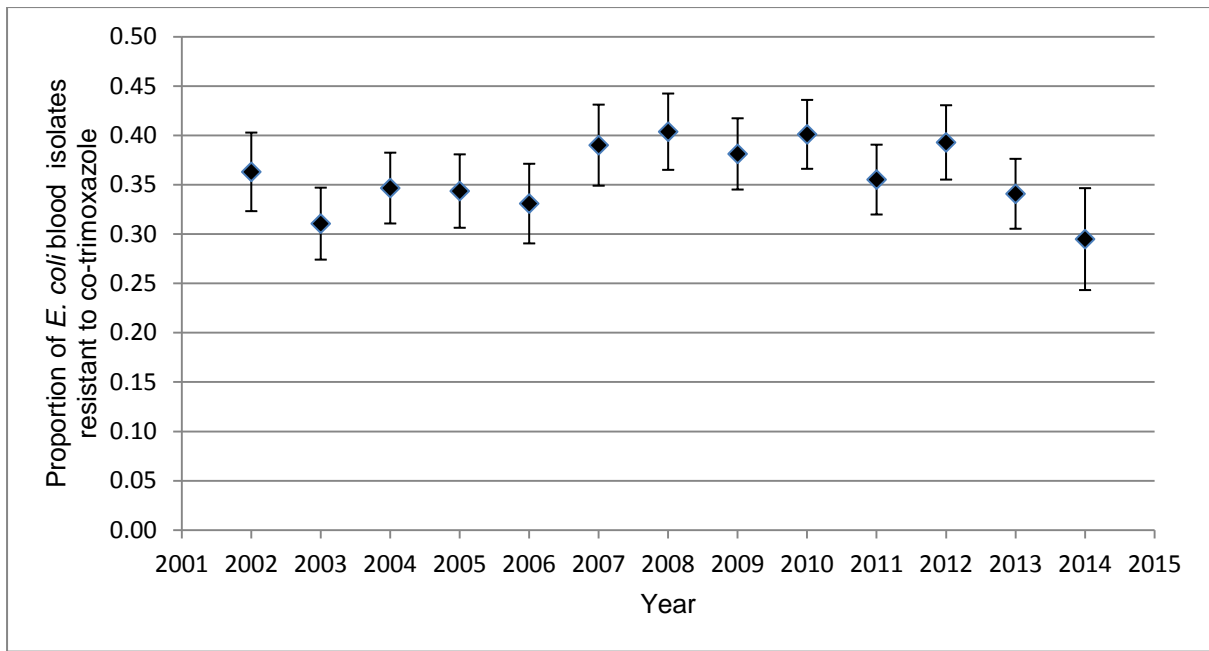


Figure 3. Annual proportion of *E. coli* blood isolates resistant to co-trimoxazole.

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243 **References**

- 244 1. Goossens H, Ferech M, Vander Stichele R, Elseviers M, ESAC Project Group. Outpatient antibiotic
245 use in Europe and association with resistance: a cross-national database study. *Lancet*. 2005 Feb 12-
246 18;365(9459):579-87.
- 247 2. Garcia-Rey C, Fenoll A, Aguilar L, Casal J. Effect of social and climatological factors on antimicrobial
248 use and *Streptococcus pneumoniae* resistance in different provinces in Spain. *J Antimicrob*
249 *Chemother*. 2004 Aug;54(2):465-71.
- 250 3. Costelloe C, Metcalfe C, Lovering A, Mant D, Hay AD. Effect of antibiotic prescribing in primary
251 care on antimicrobial resistance in individual patients: systematic review and meta-analysis. *BMJ*.
252 2010 May 18;340:c2096.
- 253 4. Andersson DI, Hughes D. Antibiotic resistance and its cost: is it possible to reverse resistance? *Nat*
254 *Rev Microbiol*. 2010 Apr;8(4):260-71.
- 255 5. Levin BR. Minimizing potential resistance: a population dynamics view. *Clin Infect Dis*. 2001 Sep
256 15;33 Suppl 3:S161-9.
- 257 6. Enne VI, Livermore DM, Stephens P, Hall LM. Persistence of sulphonamide resistance in
258 *Escherichia coli* in the UK despite national prescribing restriction. *Lancet*. 2001 Apr
259 28;357(9265):1325-8.
- 260 7. Bean DC, Livermore DM, Papa I, Hall LM. Resistance among *Escherichia coli* to sulphonamides and
261 other antimicrobials now little used in man. *J Antimicrob Chemother*. 2005 Nov;56(5):962-4.
- 262 8. Sundqvist M. Reversibility of antibiotic resistance. *Ups J Med Sci*. 2014 May;119(2):142-8.
- 263 9. Vernaz N, Huttner B, Musciconico D, Salomon JL, Bonnabry P, Lopez-Lozano JM, et al. Modelling
264 the impact of antibiotic use on antibiotic-resistant *Escherichia coli* using population-based data from
265 a large hospital and its surrounding community. *J Antimicrob Chemother*. 2011 Apr;66(4):928-35.
- 266 10. Farra A, Skoog G, Wallen L, Kahlmeter G, Kronvall G, Sorberg M, et al. Antibiotic use and
267 *Escherichia coli* resistance trends for quinolones and cotrimoxazole in Sweden. *Scand J Infect Dis*.
268 2002;34(6):449-55.
- 269 11. Sorberg M, Farra A, Ransjo U, Gardlund B, Rylander M, Wallen L, et al. Long-term antibiotic
270 resistance surveillance of gram-negative pathogens suggests that temporal trends can be used as a
271 resistance warning system. *Scand J Infect Dis*. 2002;34(5):372-8.
- 272 12. Oteo J, Lazaro E, de Abajo FJ, Baquero F, Campos J, Spanish members of EARSS. Antimicrobial-
273 resistant invasive *Escherichia coli*, Spain. *Emerg Infect Dis*. 2005 Apr;11(4):546-53.
- 274 13. Veterinary Medicines Directorate. UK Veterinary Antibiotic Resistance and Sales Surveillance
275 (UK-VARSS). Reports 2006-2014. [https://www.gov.uk/government/publications/veterinary-](https://www.gov.uk/government/publications/veterinary-antimicrobial-resistance-and-sales-surveillance-2014)
276 [antimicrobial-resistance-and-sales-surveillance-2014](https://www.gov.uk/government/publications/veterinary-antimicrobial-resistance-and-sales-surveillance-2014)

277 14. BSAC methods for antimicrobial susceptibility testing (<http://www.bsac.org.uk/previous-versions>).
278 Accessed 17 November 2016.

279 15. Bhaskaran K, Gasparrini A, Hajat S, Smeeth L, Armstrong B. Time series regression studies in
280 environmental epidemiology. *Int J Epidemiol*. 2013;42(4):1187-1195.

281 16. Sundqvist M, Geli P, Andersson DI, Sjolund-Karlsson M, Runeheggen A, Cars H, et al. Little
282 evidence for reversibility of trimethoprim resistance after a drastic reduction in trimethoprim use. *J*
283 *Antimicrob Chemother*. 2010 Feb;65(2):350-60.

284 17. Huovinen P. Resistance to trimethoprim-sulfamethoxazole. *Clin Infect Dis*. 2001;32:1608-14.

285 18. Grape M, Farra A, Kronvall G, Sundstrom L. Integrons and gene cassettes in clinical isolates of co-
286 trimoxazole-resistant Gram-negative bacteria. *Clin Microbiol Infect*. 2005;11(3):185-92.

287

288 19. Public Health England. English surveillance programme for antimicrobial utilisation and
289 resistance (ESPAUR).
290 [http://www.gov.uk/government/uploads/system/uploads/attachment_data/file/575626/ESPAUR_Report_201](http://www.gov.uk/government/uploads/system/uploads/attachment_data/file/575626/ESPAUR_Report_2016.pdf)
291 [6.pdf](http://www.gov.uk/government/uploads/system/uploads/attachment_data/file/575626/ESPAUR_Report_2016.pdf).

292

293 20. Cooke J, Stephens P, Ashiru-Oredope D, Johnson AP, Livermore DM, Sharland M. Antibacterial
294 usage in English NHS hospitals as part of a national antimicrobial stewardship programme. *Public*
295 *Health* 2014;128:693-7.

Figure 1

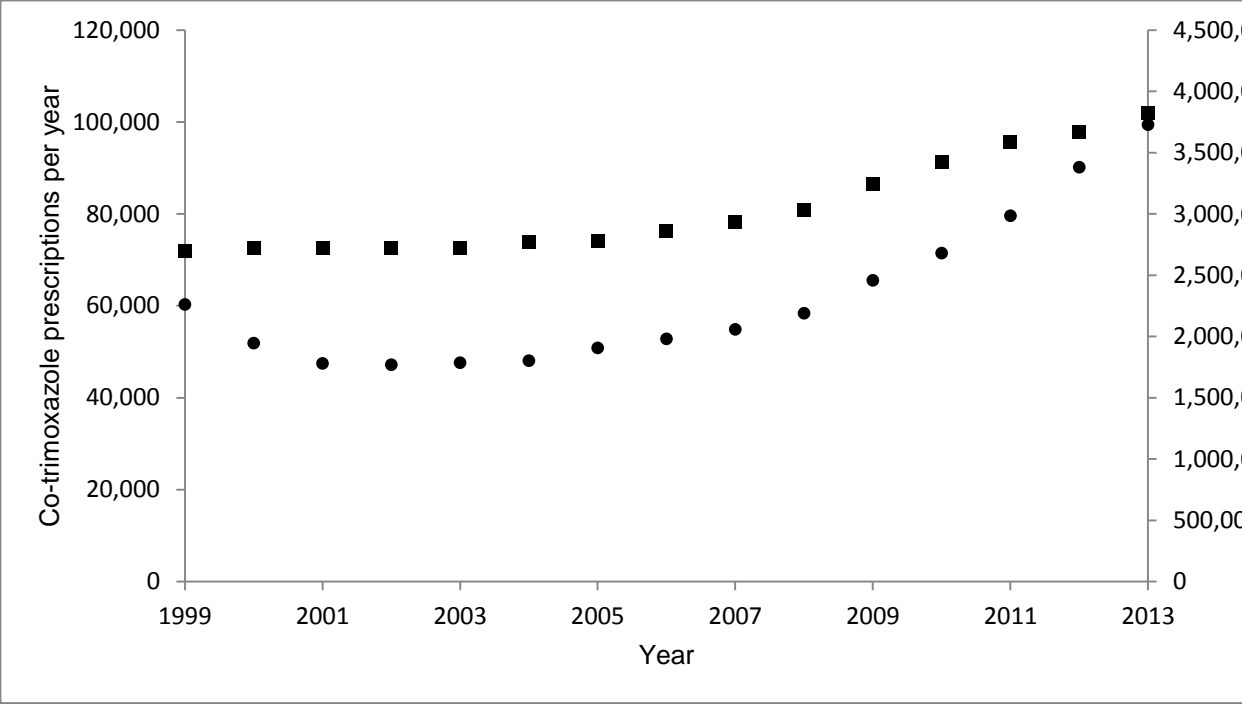


Figure 2

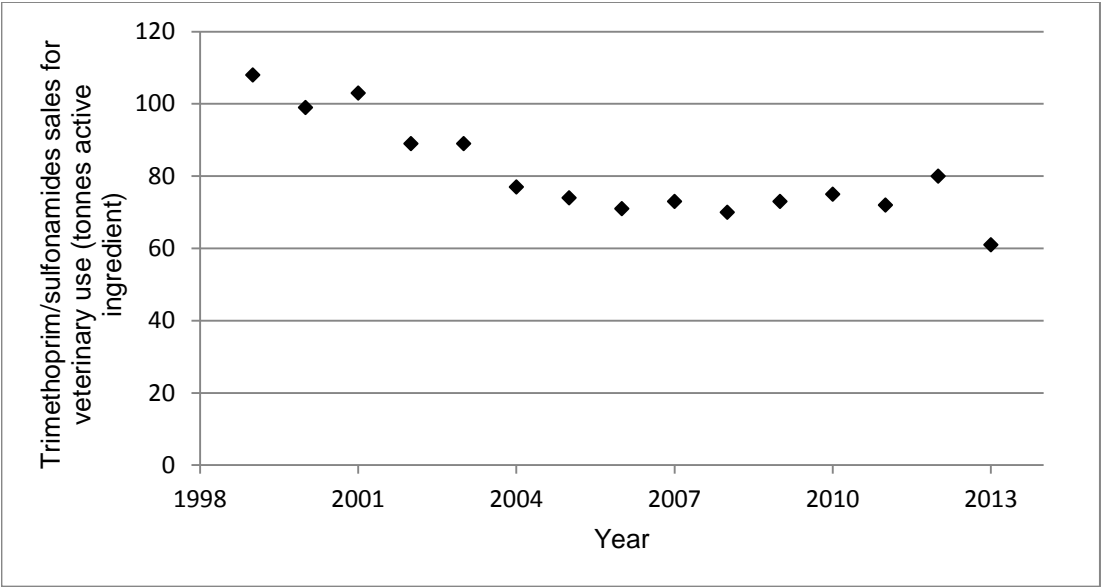


Figure 3

