Microbiology and Infectious Disease

The effect of combining antibiotics on resistance: A systematic review and meta-analysis

Reviewed Preprint

Published from the original preprint after peer review and assessment by eLife.

About eLife's process

Reviewed preprint version 1
January 22, 2024 (this version)

Sent for peer review
November 6, 2023

Posted to preprint server
October 19, 2023

Berit Siedentop, Viacheslav N. Kachalov, Christopher Witzany, Matthias Egger, Roger D. Kouyos, Sebastian Bonhoeffer

Institute of Integrative Biology, Department of Environmental Systems Science, ETH Zürich, Zurich, Switzerland • Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zürich, University of Zürich, Zürich, Switzerland • Institute of Medical Virology, University of Zurich, Zurich, Switzerland • Institute of Social and Preventive Medicine (ISPM), University of Bern, Bern, Switzerland • Population Health Sciences, University of Bristol, Bristol, UK • Centre for Infectious Disease Epidemiology and Research, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

Abstract

When and under which conditions antibiotic combination therapy decelerates rather than accelerates resistance evolution is not well understood. We examined the effect of combining antibiotics on within-patient resistance development across various bacterial pathogens and antibiotics.

We searched CENTRAL, EMBASE and PubMed for (quasi)-randomised controlled trials (RCTs) published from database inception to November 24th, 2022. Trials comparing antibiotic treatments with different numbers of antibiotics were included. A patient was considered to have acquired resistance if, at the follow-up culture, a resistant bacterium was detected that had not been present in the baseline culture. We combined results using a random effects model and performed meta-regression and stratified analyses. The trials' risk of bias was assessed with the Cochrane tool.

42 trials were eligible and 29, including 5054 patients, were qualified for statistical analysis. In most trials, resistance development was not the primary outcome and studies lacked power. The combined odds ratio (OR) for the acquisition of resistance comparing the group with the higher number of antibiotics with the comparison group was 1.23 (95% CI 0.68-2.25), with substantial between-study heterogeneity (I^2 =77%). We identified tentative evidence for potential beneficial or detrimental effects of antibiotic combination therapy for specific pathogens or medical conditions.

The evidence for combining a higher number of antibiotics compared to fewer from RCTs is scarce and overall, is compatible with both benefit or harm. Trials powered to detect differences in resistance development or well-designed observational studies are required to clarify the impact of combination therapy on resistance.

eLife assessment

This is a methodologically state-of-the-art systematic review and meta-analysis of studies that addressed the question of whether the administration of multiple antibiotics simultaneously prevents antibiotic resistance development in individuals. The findings are **solid**. Rather than providing a precise answer, the synthesis of studies eligible for analysis leads to the conclusion that "our analysis could not identify any benefit or harm of using a higher or a lower number of antibiotics regarding within-patient resistance development." This article is **important** as it articulates the existing knowledge gap, but also serves as an example to careful future use of the meta-analysis methodology, when existing data just don't allow conclusions.

Introduction

Antibiotics are one of the most significant advances in modern medicine, prescribed to treat various bacterial infections in both humans and animals and prevent infections, such as surgical site infections or opportunistic infections in immunocompromised individuals(1). However, this medical breakthrough is at risk due to the rising prevalence of antibiotic resistance and an inadequate pipeline of new antibiotics. This disturbing trend threatens to undermine the effectiveness of antibiotics and poses a severe challenge to public health worldwide.(2, 3) Hence, we need a more prudent use of antibiotics, and where antibiotics are needed, we need treatment strategies that reduce the risk that resistance emerges or spreads. Different strategies for the optimal use of antibiotics have been investigated theoretically and empirically.(4-7) Antibiotic combination therapy, i.e., the simultaneous administration of several antibiotics, is frequently discussed as a promising strategy for avoiding resistance evolution.(6-10) Importantly, it is the standard of care for some bacterial pathogens, such as *H. pylori*, *Mycobacterium tuberculosis* (Mtb), or *Mycobacterium leprae*.(11-13). However, it is unclear whether the effect of combination therapy on resistance is consistent for different pathogens.

There are several motivations for the use of antibiotic combination therapy, including to broaden the antibiotic spectrum in empirical treatment and reducing antibiotic resistance development.(14, 15) The simultaneous occurrence of resistance mutations to multiple drugs is less likely than resistance to single drugs. Combination therapy should, therefore, reduce the development of resistance.(10) This expectation is supported by viral infections such as HIV, where multiple point mutations are required for resistance to combination antiviral therapy. However, it is less clear to what extent this reasoning extends to antibiotic therapy, where the same mechanism can facilitate bacterial survival against multiple antibiotics,(16, 17) and where horizontal transfer of resistance may occur. Indeed, the benefit of combining antibiotics for reducing resistance is debated for bacterial infections.(18) Using more antibiotics overall could lead to more resistance, as overall antibiotic consumption correlates with resistance.(19)

Two meta-analyses of randomised controlled trials (RCTs) comparing beta-lactam monotherapy to beta-lactam and aminoglycoside combination therapy found no differences in resistance development.(4, 5) However, the effect of combining antibiotics on within-patient resistance development across many bacterial pathogens and various antibiotic combinations has not been addressed. Even though within-patient antibiotic resistance development is rare, it may contribute to the emergence and spread of resistance. We performed a systematic review and meta-analysis

to (i) test the effect of antibiotic combination therapy on within-patient resistance development and (ii) evaluate which factors affect the performance of combination therapy, as e.g. pathogen identity, treatment design and resistance assessment.

Results

The search identified 3082 articles, which decreased to 1837 after deduplication. A total of 488 studies were eligible for full-text review, of which 41 studies qualified for inclusion. The screening of the citations of the 41 studies identified one additional eligible study, for a total of 42 studies, 40 RCTs and two quasi-RCTs (**figure 1**, **table 1**).(20-61) Twenty-nine studies could be included in the meta-analysis; 13 were excluded due to zero events in both treatment arms.

The included studies were published between 1977 and 2021, with a median publication year of 1995 and few recent studies (**figure 2 A**). The development of antibiotic resistance was typically not the main outcome: only nine studies (21%) explicitly defined a resistance outcome (SI p 6). Consequently, most studies did not have the statistical power to detect a large effect on within-patient resistance development (**figure 2 B**, SI p 14). Twenty-two (52%) focused on a specific pathogen species (resistant *Acinetobacter baumannii*, *Escherichia coli*, *H. pylori*, Mtb, methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Staphylococcus aureus*) or pathogen group (MAC, *Salmonella enterica* subsp. *enterica* serotype Thyphi, or *Salmonella enterica* subsp. *enterica* serotype Parthypi A).

The five most frequent reasons for antibiotic administration were treatment or prophylaxis of urinary tract infections (UTIs) (6 studies, 14%), MRSA (5 studies, 12%), *H.* pylori, MAC, and prophylaxis for blood cancer patients with four studies (10%) respectively. Twenty-three of the included studies (55%) compared treatment arms that with at least one administered antibiotic in common; the remaining studies compared treatment arms with no overlap in administered antibiotics (**table 1**). For the outcome acquisition of resistance, only two of all 42 studies had a low overall risk of bias according to the risk of bias assessment. Twelve (29%) were at high risk of bias, 28 (67%) at moderate risk of bias (SI p 3).

The overall pooled OR for acquisition of resistance comparing a lower number of antibiotics versus a higher one was 1.23 (95% CI 0.68 – 2.25), with substantial heterogeneity between studies (I^2 =77.4%). The latter OR was compatible with the OR for *de novo* emergence of resistance (pooled OR 0.74, 95% CI 0.34 – 1.59; I^2=77%). Since the results for the two resistance outcomes are comparable, our focus in the following is on the acquisition of resistance (details on emergence of resistance can be found in the SI p 2, p 4, p 8).

Stratified analyses revealed that a higher number of antibiotics performed better than a lower number in case of *H. pylori*, (pooled OR 0.14, 95% CI 0.03 – 0.55; I^2 =41.7%, **figure 2A**), and *MAC* (pooled OR 0.18, 95% CI 0.06 – 0.52; I^2 =26.8%, **figure 2A**), but worse in case of *P. aeruginosa* (pooled OR 3.42, 95% CI 1.03 – 11.43; I^2=1.54%, **figure 2A**). Furthermore, a lower number of antibiotics performed better than a higher number if the compared treatment arms had no antibiotics in common (pooled OR 4.73, 95% CI 2.14 – 10.42; I^2=37%, SI p 7). In contrast, when restricting the analysis to studies with at least one common antibiotic in the treatment arms are pooled there was little evidence of a difference (pooled OR 0.55, 95% CI 0.28 – 1.07; I^2 =74%, **figure 3B**). When considering only resistance measurements of antibiotics common to both treatment arms instead of all resistance measurements, the arm with a higher number of antibiotics shows a benefit in comparison to the one with fewer (pooled OR 0.39, 95% CI 0.18 – 0.81; I^2=75%, SI p 6). If the study measured the acquisition of resistance of both gram negative and positive bacteria, fewer antibiotics performed better (pooled OR 3.38, 95% CI 1.08 – 10.58; I^2=38.35%, SI p 5). Other sub-group analyses did not show any harm or benefit of using a higher number of antibiotics. The results for all subgroup analyses are presented in the supplement (SI p 4). The multi-model

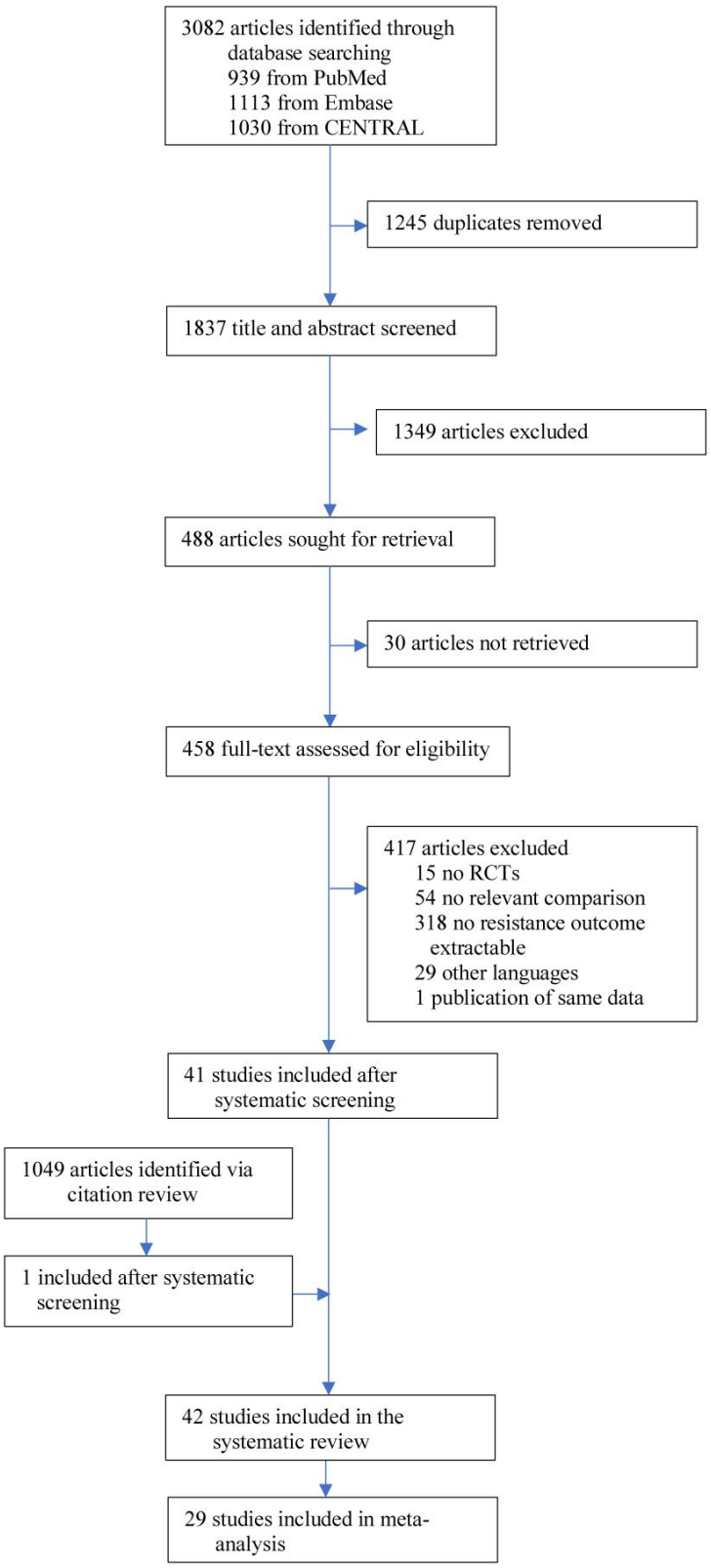

Figure 1.

Study selection

Study	Type Of Study	Focused pathogen/ Reason for antibiotic treatment	Objective(s)	Antibiotics used in Study arms		Explicit Definition Of Resistance Outcome	Secondary Outcomes extracted
				Less antibiotics	More Antibiotics		
Bender et al. (1979)(20)	RCT	Infection prophylaxis for patients with acute leukemia or malignant lymphomas receiving remission induction chemotherapy	Tolerance, suppression of microbial flora, protection against colonisation and infection	Gentamicin	Gentamicin, and vancomycin	no	All-cause mortality
Black et al. (1982)(21)	RCT	enterotoxigenic *Escherichia coli* (ETEC)	Compare two treatments options against ECET.	Trimethoprim	Trimethoprim, and sulfamethoxazole	no	-
Chaisson et al. (1997)(22)	RCT	MAC	Safety and activity	Clarithromycin, and ethambutol	Clarithromycin, ethambutol, and clofazimine	no	All-cause mortality
Cometta et al. (1997)(23)	RCT	Nosocomial pneumonia, nosocomial	Clinical efficacy and tolerance, emergence of	Imipenem	Imipenem, and netilmicin	no	Mortality attributable to infection,
		sepsis, or severe diffuse peritonitis	resistance and risk of superinfection				treatment failure, treatment failure due to a change of resistance against the study drugs
Dawson et al. (2015)(24)	RCT	Mtb	Efficacy, and safety	Moxifloxacin, pretomanid, and pyrazinamide	Isoniazid, rifampicin, pyrazinamide, and ethambutol	no	Proportion of patients with alterations of the prescribed treatment due to adverse events
Dekker et al. (1987)(25)	RCT	Prophylaxis for acute nonlymphocytic or lymphocytic leukemia	Efficacy of protecting against infections	Ciprofloxacin	Trimethoprim, and sulfamethoxazole	no	All-cause mortality, mortality attributable to infection, acquisition of resistance against non-administered antibiotics
Dickstein et al. (2019)(26)	RCT	Carbapenem resistant, colistin-susceptible, and gram-negative infections	Development of colistin resistance (secondary outcome of a clinical trial)	Colistin	Colistin, and meropenem	yes	All-cause mortality, proportion of patients with alterations of the prescribed treatment due to adverse events
Dubé et al. (1997)(27)	RCT	MAC	Risk of recrudescent MAC bacteraemia, emergence of resistance to clarithromycin.	Clarithromycin, and clofazimine	Clarithromycin, clofazimine, and ethambutol	no	All-cause mortality, proportion of patients with alterations of the prescribed treatment due to adverse events
Durante-Mangoni et al. (2013)(28)	RCT	Extensively drug resistant *Acinetobacter baumannii*	Mortality	Colistin	Colistin, and rifampicin	yes	All-cause mortality, mortality attributable to infection, treatment failure
Fournier et al. (1999)(29)	RCT	MAC	Efficacy and tolerance.	Clarithromycin, ethambutol	Clarithromycin, ethambutol, and clofazimine	no	All-cause mortality, proportion of patients with alterations of the prescribed treatment due to adverse events
Gerecht et al. (1989)(30)	RCT	Cholangitis	Compare a single drug treatment to a two-drug treatment.	Mezlocillin	Ampicillin, and gentamicin	yes	All-cause mortality, treatment failure as reported in each study, treatment failure due to a change of resistance against the study drugs

Table 1.

Overview of the 42 RCTs or quasi-RCTs included in the systematic review and meta-analysis. The underlined antibiotics indicate that resistance measurements were made for this antibiotic, reported and extractable from the studies. Resistance outcome definitions marked with (*) indicate that the definition was not specifically provided by the study itself but states our justification for extraction.

Gibson et al. (1989)(31)	RCT	Febrile neutropenia	Efficacy and side effects	Ceftazidime	Azlocillin, and amikacin	no	All-cause mortality, mortality attributable to infection, proportion of patients with alterations of the prescribed treatment due to adverse events, acquisition of resistance against non-administered antibiotics, emergence of resistance against non-administered antibiotics
Haase et al. (1984)(32)	RCT	UTI	Efficacy, tolerance, and safety	Norfloxacin	Trimethoprim, and sulfamethoxazole	no	Treatment failure, acquisition of resistance against non-administered antibiotics
Hartbarth et al. (2015)(33)	RCT	MRSA	Assess the non-inferiority of a multiple drug treatment in comparison of a single drug treatment.	Linezolid	Trimethoprim, sulfamethoxazole, and rifampicin	no	All-cause mortality, mortality attributable to infection, treatment failure
Hodson (1987)(34)	RCT	Cystic fibrosis patients with *P. aeruginosa*	Compare an oral one drug treatment to an intravenous two drug treatment.	Ciprofloxacin	Azlocillin, and gentamicin	no	-
Hoepelman et al. (1988)(35)	RCT	Serious bacterial infections	Emergence of resistance of fecal flora	Ceftriaxone	Cefuroxime, and gentamicin	no	Proportion of patients with alterations of the prescribed treatment due to adverse events
Hultén et al. (1997)(36)	RCT	*H. pylori*	Antibacterial efficacy, emergence of clarithromycin resistance.	Clarithromycin	Clarithromycin, and lymecycline	no	-
Iravani et al. (1981)(37)	RCT	Acute UTI	Efficacy, treatment effects on fecal flora, resistance emergence in the infecting pathogen	Nalidixic acid	Trimethoprim, and sulfamethoxazole	no	-
Jacobs et al. (1993)(38)	RCT	Bacterial infections in neutropenic children	Efficacy and safety, tolerance, emergence of resistance and risk of superinfection	Ceftazidime	Ceftazidime, and tobramycin	yes	All-cause mortality, treatment failure, treatment failure due to a change of resistance against the study drugs
Jo et al. (2021)(39)	RCT	Determine impact of antibiotics on healthy skin microbiota	Investigate short and long term of the skin microbiome	Doxcycycline	Trimethoprim, and sulfamethoxazole	no	-
Macnab et al. (1994)(40)	Quasi-RCT	Mtb	Efficacy, primary drug resistance, bacteriological conversion rates, compliance, and side effects	Isoniazid, and rifampicin	Isoniazid, rifampicin, and ethambutol	no	Proportion of patients with alterations of the prescribed treatment due to adverse event
Markowitz et al. (1992)(41)	RCT	*S. aureus*	Efficacy and safety	Vancomycin	Trimethoprim, and sulfamethoxazole	no	All-cause mortality, treatment failure, proportion of patients with alterations of the prescribed treatment due to adverse events
Mavromanolakis et al. (1997)(42)	RCT	Recurrent UTIs	Effect on the aerobic bowel flora, frequency of resistant strains in the fecal flora during and after treatment.	Norfloxacin, or Nitrofurantoin	Trimethoprim, and sulfamethoxazole	no	-
May et al. (1997)(43)	RCT	MAC	Clinical and bacteriological efficacy, safety, tolerability	Clarithromycin, and clofazimine	Clarithromycin, rifabutin, and ethambutol	no	All-cause mortality, treatment failure, proportion of patients with alterations of the prescribed treatment due to adverse events
Mc Carty et al. (1988)(44)	RCT	Cystic fibrosis patients with *P. aeruginosa*	Safety, pharmacokinetics of a high-dose singe drug treatment, the effectiveness	Piperacillin	Piperacillin, and tobramycin	no	All-cause mortality, mortality attributable to infection
Menon et al. (1986)(45)	RCT	Acute UTI	Efficacy, selection of resistance in Enterobacteriaceae	Trimethoprim	Trimethoprim, and sulfamethoxazole	no	Acquisition of resistance against non-administered antibiotics
Miehlke et al. (1998)(46)	RCT	*H. pylori*	Effectiveness, tolerability	Amoxicillin	Clarithromycin, and metronidazole	no	Proportion of patients with alterations of the prescribed treatment due to adverse events

Table 1. (continued)

Parras et al. (1995)(47)	RCT	MRSA	Efficacy to eradicate, safety	Mupirocin	Sodium fusidate, trimethoprim, and sulfamethoxazole	no	All-cause mortality, proportion of patients with alterations of the prescribed treatment due to adverse events
Parry et al. (1977)(48)	Quasi-RCT	*Pulmonary infection*	Effectiveness, treatment failure, treatment success, frequency of ticarcillin resistant organisms, influence of resistance on disease development	Ticarcillin	Ticarcillin, and gentamicin	no	-
Parry et al. (2007)(49)	RCT	Multidrug resistant typhoid fever	Efficacy	Ofloxacin, or Azithromycin	Ofloxacin, and Azithromycin	no	Treatment failure
Paul et al. (2015)(50)	RCT	MRSA	Test whether a two-drug treatment is non-inferior to a two-drug treatment.	Vancomycin	Trimethoprim, and sulfamethoxazole	yes	All-cause mortality, treatment failure, acquisition of resistance against non-administered antibiotics, emergence of resistance against non-administered antibiotics
Pogue et al. (2021)(51)	RCT	Gram negative resistant bloodstream infections or pneumonia	Assess superiority of a combination of colistin to monotherapy.	Colistin	Colistin, and meropenem	yes	All-cause mortality, treatment failure
Pujol et al. (2021)(52)	RCT	MRSA	Assess treatment success.	Daptomycin	Daptomycin, and fosfomycin		All-cause mortality, treatment failure, proportion of patients with alterations of the prescribed treatment due to adverse events
Rubinstein et al. (1995)(53)	RCT	Gram-negative hospital acquired infections	Efficacy, safety	Ceftazidime	Ceftriaxone, and Tobramycin	yes	All-cause mortality, mortality attributable to infection, treatment failure, proportion of patients with alterations of the prescribed treatment due to adverse events
Schaeffer et al. (1981)(54)	RCT	UTI	Effectiveness, safety, incidence of resistance in faecal and vaginal flora before, and after treatment.	Cinoxacin	Trimethoprim, and sulfamethoxazole	no	Proportion of patients with alterations of the prescribed treatment due to adverse events
Schaeffer et al. (1985)(55)	RCT	UTI	Effectiveness, safety, incidence of resistance in fecal and vaginal flora before, and after treatment.	Norfloxacin	Trimethoprim, and sulfamethoxazole	no	-
Smith et al. (1999)(56)	RCT	P. aeruginosa	Efficacy	Azlocillin	Azlocillin, and tobramycin	no	Acquisition of resistance against non-administered antibiotics, emergence of resistance against non-administered antibiotics
Stack et al. (1998)(57)	RCT	*H. pylori*	Efficacy, safety	Clarithromycin	Clarithromycin, and metronidazole, or amoxycillin	no	-
Walsh et al. (1993)(58)	RCT	MRSA	Efficacy of eradication, emergence of resistance, safety	Novobiocin, and rifampicin	Rifampicin, trimethoprim, and sulfamethoxazole	yes	Treatment failure, acquisition of resistance against non-administered antibiotics, emergence of resistance against non-administered antibiotics
Winston et al. (1986)(59)	RCT	Prophylaxis for blood cancer patients	Efficacy and safety	Norfloxacin	Vancomycin, and polymyxin	no	Mortality attributable to infection
Winston et al. (1990)(60)	RCT	Prophylaxis for blood cancer patients	Efficacy and safety	Ofloxacin	Vancomycin, and polymyxin	no	-
Wurzer et al. (1997)(61)	RCT	*H. pylori*	Effectiveness, emergence of resistance.	Clarithromycin	Clarithromycin, and amoxycillin	no	-

Table 1. (continued)

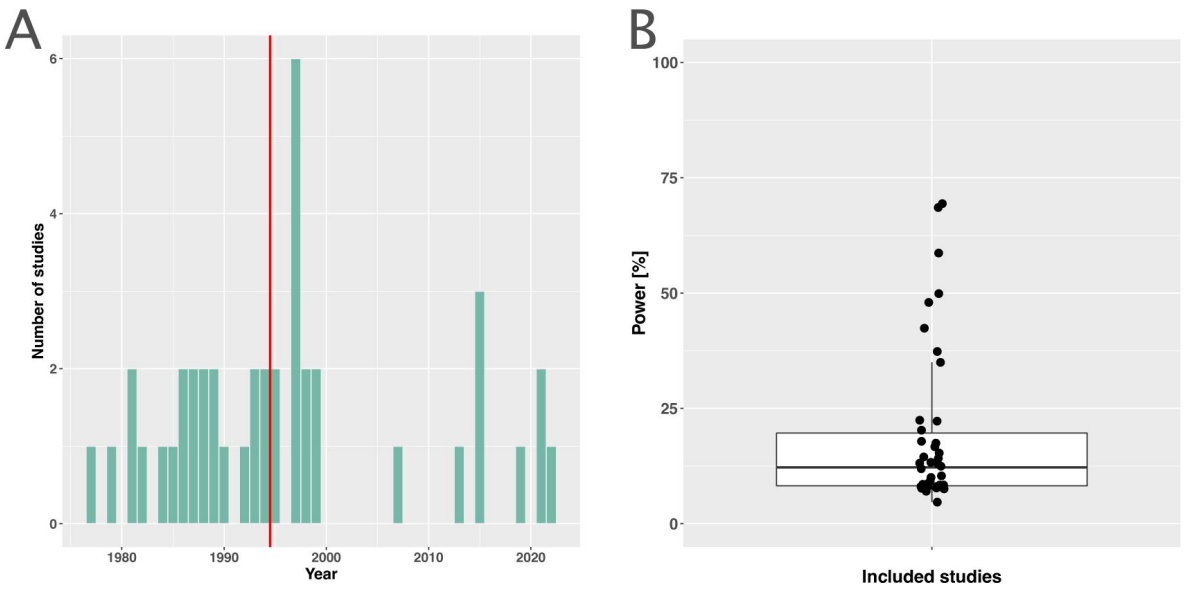

Figure 2.

Measuring antibiotic resistance is not a current main objective of RCTs. A) Distribution of the publishing year of included studies, where n indicates the number of studies, and the red vertical line the median of the distribution. B) Calculated power of included studies to detect an odds ratio of 0.5. The power calculations were based on equal treatment arm sizes. For the calculations the treatment arm with the higher number of patients of the respective studies was used.

inference for our meta-regression showed that the only significant factor influencing the outcome acquisition of resistance is whether at least one common antibiotic was used in the comparator arms (for details see SI p 8).

The inspection of the funnel plot and the modified Egger's test showed no indication of a publication bias (SI p 4). The results were largely robust to the choice of the random effects model (SI p 8). The probability of the secondary outcome "alterations of the prescribed treatment due to adverse events", was higher using more antibiotics in comparison to fewer (pooled OR 1.61, 95% CI 1.12 – 2.31; I^2=5%; SI p 10). In 15 studies (36%), the proportion of patients with alterations of the prescribed treatment due to adverse events was reported, with three studies (20%) reporting zero cases in both treatment arms. All other analyses of secondary outcomes showed no indication of harm or benefit of treating with a higher number of antibiotics (SI p 9).

Discussion

We performed a meta-analysis of RCTs and quasi-RCTs not limited to a particular bacterial species, specific condition, or antibiotic combinations to assess the effect of antibiotic combination therapy on within-patient resistance development. Our analysis could not identify any benefit or harm of using a higher or a lower number of antibiotics regarding within-patient resistance development. However, we found some evidence that combining antibiotics may be beneficial or harmful for specific pathogens or infection types. Acquisition of resistance was rarely a primary objective of the included RCTs. Hence, they were typically not designed to detect differences in resistance development between treatment arms and underpowered for this endpoint. Therefore, the absence of evidence does not mean that there is convincing evidence for the lack of an effect of using more or fewer antibiotics on resistance development but rather highlights a knowledge gap. This is remarkable given that the general rise of resistance is an increasing concern(18, 62) and a priority area for health policy and public health.(63)

Our analysis showed that combining antibiotics reduced resistance development for *H. pylori* or MAC, in line with the current standard of care.(11, 64) Surprisingly, we found only two studies that satisfied our inclusion criteria for Mtb(24, 40), which may be considered the prime example of effective antibiotic combination therapy. The limited number of Mtb studies may be because antibiotic administration commonly varies during Mtb treatment, which conflicted with our inclusion criteria that necessitated a consistent treatment regimen for susceptibility measurements (see SI, page 2). Both eligible Mtb studies were excluded from the analysis due to the absence of any events in either treatment arm.

Our main result, the absence of a general effect of combining antibiotics on resistance development, aligns with the two previous meta-analyses.(4, 5) With 42 trials in our systematic review and 29 in the meta-analysis, our study provided a comprehensive assessment of the effect of antibiotic combination therapy on within-patient resistance. Whereas previous meta-analyses focused on a combination of specific antibiotic classes and included fewer than ten studies each, our study aimed to assess the general effect of combining antibiotics on resistance evolution across different bacterial pathogens. By including trials with different antibiotic combinations and bacterial pathogens, we increased clinical and statistical heterogeneity. We accounted for many sources of heterogeneity using stratification and meta-regression, but analyses were limited by missing information and sparse data.

Our findings have implications for the design of future studies of resistance development. Generally, the development of resistance within a patient is a rare event. However, even small differences could be relevant at the population level. To obtain reliable estimates of such differences and to better understand the factors influencing them, very large RCTs would be needed, which systematically investigate the development of antibiotic resistance and include

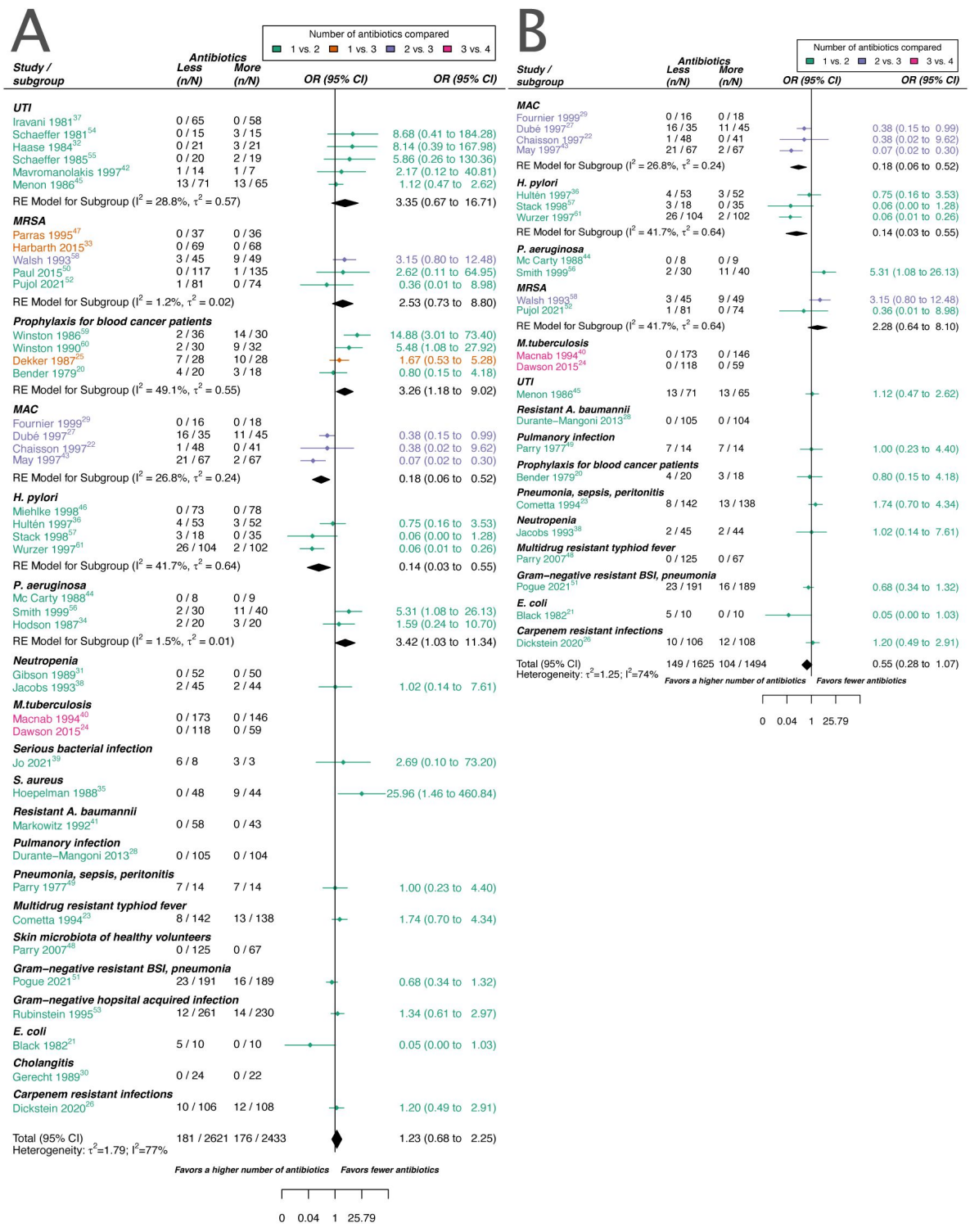

Figure 3.

Forest plot of acquisition of bacterial resistance stratified by the reason antibiotics were administered. The coloring indicates the number of antibiotics that were compared in each study. A) The overall pooled LOR of all included studies. B) The pooled LOR of studies with at least one antibiotic in common in the treatment arms. UTI stands for urinary tract infection, MRSA for methicillin-resistant Staphylococcus aureus, MAC for Mycobacterium avium complex, and BSI for blood stream infection.

resistance testing of each administered antibiotic. To better evaluate the effect of combination therapy, especially more RCTs would be needed where the basic antibiotic treatment is consistent across both treatment arms, i.e. the antibiotics used in both treatment arms should be identical, except for the additional antibiotic added in the comparator arm (**figure 3**). As such RCTs are costly and associated with high hurdles, the analysis of cohort studies could be an alternative approach. Over 25 years ago, Fish et al. published a systematic summary of prospective observational studies reporting data on resistance development, including antibiotic combination therapy.(65) Similarly, today, relevant cohort studies could be analysed collaboratively using various modern statistical methods to address confounding by indication and other biases(66, 67).

The main strength of this study is its comprehensive and systematic approach. For one, it allowed identifying a knowledge gap regarding the effect of antibiotic combination therapy on resistance development. Further, our study highlights several issues in the evidence base evaluating antibiotic combination therapy and resistance development. The included trials did not always test for susceptibility against all administered antibiotics (**table 1**). Some antibiotics might have had reduced potency or were ineffective due to pre-existing resistance mutations. Furthermore, in studies where treatment was not targeted against a specific pathogen, some antibiotics may have been inactive against the causative pathogen due to intrinsic resistance. Indeed, one of the reasons for using combination therapy is to broaden the bacterial spectrum for empirical therapy(15), which could contribute to an increased risk of antibiotic resistance spread.

Our study had several limitations. First, despite our systematic search, we might have missed relevant studies. Since resistance development is typically not a primary endpoint and often not reported systematically, relevant trials are challenging to identify. Our search strategy aimed to identify a broad range of trials considering resistance development. However, as a trade-off, our search strategy might have missed trials addressing a specific medical condition or drug combination. Second, our systematic review and meta-analysis included many older studies that did not follow the relevant reporting guidelines(68), thereby hampering data extraction and potentially introducing bias. Third, it is often challenging to discern the specific mechanisms by which resistance develops based on the data from clinical trials. This includes distinguishing whether resistance arises *de novo*, if the pathogen acquires resistance through horizontal gene transfer, if the patient becomes newly infected with a resistant pathogen, or if the pathogen was present but undetected at the beginning of treatment. These scenarios can impact the effectiveness of combination therapy. For example, combination therapy may be more likely to select any pre-existing resistant pathogens compared to monotherapy due to the use of multiple antibiotics. We addressed some of this heterogeneity by employing two different measures of resistance (see SI, page 2). Furthermore, the variation in standards that classify bacteria as susceptible or resistant adds another layer of heterogeneity alongside the technical limitations in detecting resistance development.

In conclusion, combination therapy offers potential advantages and disadvantages regarding resistance evolution and spread. On the one hand, combination therapy typically increases the genetic barrier to resistance, and it has become the standard therapy for pathogens notorious for resistance evolution. Therefore, combination therapy remains a plausible candidate strategy to slow down resistance evolution. On the other hand, combination therapy generates selection pressure for resistance to multiple antibiotics simultaneously and could, therefore, accelerate resistance evolution – especially in the microbiome. Given the critical nature of this context, it is profoundly disconcerting that there is a lack of evidence elucidating the impact of combining antibiotics on the development of resistance

Materials and Methods

Inclusion criteria and search strategy

We did a systematic review and meta-analysis to summarise the evidence on the effect of antibiotic combination therapy on resistance development. We included RCTs and quasi-RCTs comparing treatments with a higher number of antibiotics to treatments with a lower number of antibiotics. Studies were classified as quasi-RCTs if the allocation of participants to study arms was not truly random. We did not consider antiseptics or compounds supporting the activity of antibiotics, such as beta-lactam inhibitors as antibiotics itself. Whereas the antibiotic substances administered within one treatment arm had to be the same for all patients, the antibiotics could differ between treatment arms. We required baseline and follow-up cultures with resistance measurements to determine the treatment impact on resistance. We considered only antibiotic treatment regimens fixed for the period between two resistance measurements. Hence, we excluded sequential and cycling regimens.

We searched PubMed, EMBASE, and the Cochrane Central Register of Controlled Trials (CENTRAL) from inception up to 24.11.2022, using keywords, medical subject headings (MeSH), and EMTREE terms related to bacterial infection, antibiotics, combination therapy, resistance and RCTs. We excluded complementary and alternative medicine and bismuth. The search strategy is detailed in the SI (p 26). After a systematic deduplication process,(69) VNK (or CW) and BS independently screened the titles and abstracts, and, if potentially eligible, the full texts. Any discrepancies between VNK (or CW) and BS were discussed and resolved. At full-text screening, we excluded articles that were not accessible in English or German. We screened the references of eligible studies and the trials included in two previous meta-analyses.(4, 5) We followed the PRISMA reporting guidelines(70) and registered our protocol with PROSPERO (CRD42020187257).

Outcomes

We used two definitions for the primary outcome resistance. A broader definition, “acquisition of resistance”, and a stricter “*de novo* emergence of resistance” definition, where the latter is a subset of the former. A patient was considered to have acquired resistance if, at the follow-up culture a resistant bacterium was detected that was not present in the baseline culture. *De novo* emergence of resistance was defined as the detection of a resistant bacterium that was present at baseline but sensitive. Additional secondary outcomes included mortality from all causes and infection, treatment failure overall, treatment failure due to resistance, treatment change due to adverse effects, and acquisition/*de novo* emergence of resistance against non-administered antibiotics. The SI (p 2, p 18) provides further details.

Data extraction and analysis

VNK (or CW) and BS independently extracted all study data using a standardised form (see *https://osf.io/gwefy/?view_only=f6a4c1f4c79241038b203bd03c8e1845*). The data extracted included the proportion of patients who developed the two primary outcomes and the secondary outcomes and study characteristics such as type of trial (RCT or quasi-RCT), follow-up and treatment duration, number of antibiotics in the treatment arms, type of antibiotic, and presence of comorbidities. Any discrepancies in data extraction were discussed and resolved.

We calculated odds ratios (ORs) with 95% confidence intervals (CIs), comparing a higher with a lower number of antibiotics for each study. We combined ORs using a modified version of the Simmonds and Higgins random effects model.(71) If a study had more than two eligible treatment arms, they were merged for statistical analysis. Studies with zero events in both treatment arms were excluded from the statistical analysis. We used subgroup analyses and meta-regressions with multi-model inference to examine the influence of pre-specified variables on summary ORs. Variables included whether the antibiotic(s) used in the arm with the lower

number of antibiotics are also part of the arm(s) with the higher number of antibiotics, the number of antibiotics administered, the age of the antibiotics (time since market entry), the administration of other non-antibiotic drugs, whether participants had specific comorbidities or were in intensive care, gram-status of the tested pathogens, and the length of antibiotic treatment and follow-up. We extended our predefined analysis regarding the reason for antibiotic treatment/type of pathogen, which was initially restricted to only *H. pylori* and Mtb, as we found enough studies to stratify by other conditions/pathogens. We furthermore performed post-hoc subgroup analyses to examine the following factors: treatment of resistant pathogens, additional antibiotic administration besides the fixed treatment, and the way of antibiotic administration (SI p 14).

Between study heterogeneity was estimated with I^2, using the criteria for I^2specified in Higgins et al. for classifying the degree of heterogeneity.(72) CW and BS assessed each study's quality for the main outcomes using the Risk of Bias tool (RoB 2, SI p 7).(73) To assess publication bias, we visually inspected the funnel plot and a modified Egger's test (SI p 10). We performed sensitivity analyses on the model choice (SI p 8), and risk of bias (SI p 9), and performed a post-hoc trial sequential analysis (SI p 17). Statistical analyses and visualisations were done in R (version 4.2.1) using packages *metafor* and *MuMIn*.(74, 75)

Data Availability

All data are contained in the the manuscript, supplementary information or online at *https://osf.io/gwefy/?view_only=f6a4c1f4c79241038b203bd03c8e1845*.

https://osf.io/gwefy/?view_only=f6a4c1f4c79241038b203bd03c8e1845

Acknowledgements

Support from the Swiss National Science Foundation (grant 310030B_176401 (SB, BS, CW), grant 32FP30-174281 (ME), grant 324730_207957 (RDK)) and from the National Institute of Allergy and Infectious Diseases (NIAID, cooperative agreement AI069924 (ME)) is gratefully acknowledged. We thank Anthony Hauser, João Pires and Frédérique Lachmann for helpful discussions and Annelies Zinkernagel and Johannes Nemeth for critical reading of the manuscript. Furthermore, we are grateful for all contacted study authors that responded to our inquiries and provided further information.

References

1. Taplitz RA, Kennedy EB, Bow EJ, Crews J, Gleason C, Hawley DK, et al. (2018) **Antimicrobial Prophylaxis for Adult Patients With Cancer-Related Immunosuppression: ASCO and IDSA Clinical Practice Guideline Update** *Journal of Clinical Oncology* **36**:3043–54

2. Hutchings MI, Truman AW, Wilkinson B (2019) **Antibiotics: past, present and future** *Current Opinion in Microbiology* **51**:72–80

3. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, et al. (2022) **Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis** *The Lancet* **399**:629–55

4. Bliziotis IA, Samonis G, Vardakas KZ, Chrysanthopoulou S, Falagas ME (2005) **Effect of Aminoglycoside and β-Lactam Combination Therapy versus β-Lactam Monotherapy on the Emergence of Antimicrobial Resistance: A Meta-analysis of Randomized, Controlled Trials** *Clinical Infectious Diseases* **41**:149–58

5. Paul M, Lador A, Grozinsky-Glasberg S, Leibovici L (2014) **Beta lactam antibiotic monotherapy versus beta lactam-aminoglycoside antibiotic combination therapy for sepsis** *Cochrane Database of Systematic Reviews*

6. Angst DC, Tepekule B, Sun L, Bogos B, Bonhoeffer S (2021) **Comparing treatment strategies to reduce antibiotic resistance in an in vitro epidemiological setting** *Proceedings of the National Academy of Sciences* **118**

7. Tepekule B, Uecker H, Derungs I, Frenoy A, Bonhoeffer S (2017) **Modeling antibiotic treatment in hospitals: A systematic approach shows benefits of combination therapy over cycling, mixing, and mono-drug therapies** *PLOS Computational Biology* **13**

8. Sullivan GJ, Delgado NN, Maharjan R, Cain AK (2020) **How antibiotics work together: molecular mechanisms behind combination therapy** *Current Opinion in Microbiology* **57**:31–40

9. Tyers M, Wright GD (2019) **Drug combinations: a strategy to extend the life of antibiotics in the 21st century** *Nature Reviews Microbiology* **17**:141–55

10. Bonhoeffer S, Lipsitch M, Levin BR (1997) **Evaluating treatment protocols to prevent antibiotic resistance** *Proceedings of the National Academy of Sciences* **94**:12106–11

11. De Francesco V, Bellesia A, Ridola L, Manta R, Zullo A (2017) **First-line therapies for Helicobacter pylori eradication: a critical reappraisal of updated guidelines** *Ann Gastroenterol* **30**:373–9

12. Singh R, Dwivedi SP, Gaharwar US, Meena R, Rajamani P, Prasad T (2020) **Recent updates on drug resistance in Mycobacterium tuberculosis** *Journal of Applied Microbiology* **128**:1547–67

13. Alemu Belachew W, Naafs B (2019) **Position statement: LEPROSY: Diagnosis, treatment and follow-up** *Journal of the European Academy of Dermatology and Venereology* **33**:1205–13

14. Pletz MW, Hagel S, Forstner C (2017) **Who benefits from antimicrobial combination therapy?** *The Lancet Infectious Diseases* **17**:677–8

15. Roemhild R, Bollenbach T, Andersson DI (2022) **The physiology and genetics of bacterial responses to antibiotic combinations** *Nature Reviews Microbiology*

16. Du D, Wang-Kan X, Neuberger A, van Veen HW, Pos KM, Piddock LJV, et al. (2018) **Multidrug efflux pumps: structure, function and regulation** *Nature Reviews Microbiology* **16**:523–39

17. Lázár V, Snitser O, Barkan D, Kishony R (2022) **Antibiotic combinations reduce Staphylococcus aureus clearance** *Nature* **610**:540–6

18. Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, et al. (2016) **Understanding the mechanisms and drivers of antimicrobial resistance** *The Lancet* **387**:176–87

19. Goossens H, Ferech M, Vander Stichele R, Elseviers M (2005) **Outpatient antibiotic use in Europe and association with resistance: a cross-national database study** *The Lancet* **365**:579–87

20. Bender JF, Schimpff SC, Young VM, Fortner CL, Brouillet MD, Love LJ, et al. (1979) **Role of vancomycin as a component of oral nonabsorbable antibiotics for microbial suppression in leukemic patients** *Antimicrobial agents and chemotherapy* **15**:455–60

21. Black RE, Levine MM, Clements ML, Cisneros L, Daya V (1982) **Treatment of experimentally induced enterotoxigenic Escherichia coli diarrhea with trimethoprim, trimethoprim-sulfamethoxazole, or placebo** *Reviews of infectious diseases* **4**:540–5

22. Chaisson RE, Keiser P, Pierce M, Fessel WJ, Ruskin J, Lahart C, et al. (1997) **Clarithromycin and ethambutol with or without clofazimine for the treatment of bacteremic Mycobacterium avium complex disease in patients with HIV infection. AIDS (London** *England* **11**:311–7

23. Cometta A, Baumgartner JD, Lew D, Zimmerli W, Pittet D, Chopart P, et al. (1994) **Prospective randomized comparison of imipenem monotherapy with imipenem plus netilmicin for treatment of severe infections in nonneutropenic patients** *Antimicrobial agents and chemotherapy* **38**:1309–13

24. Dawson R, Diacon AH, Everitt D, van Niekerk C, Donald PR, Burger DA, et al. (2015) **Efficiency and safety of the combination of moxifloxacin, pretomanid (PA-824), and pyrazinamide during the first 8 weeks of antituberculosis treatment: a phase 2b, open-label, partly randomised trial in patients with drug-susceptible or drug-resistant pulmonary tuberculosis** *Lancet (london, england)* **385**:1738–47

25. Dekker AW, Rozenberg-Arska M, Verhoef J (1987) **Infection prophylaxis in acute leukemia: a comparison of ciprofloxacin with trimethoprim-sulfamethoxazole and colistin** *Annals of internal medicine* **106**:7–11

26. Dickstein Y, Lellouche J, Schwartz D, Nutman A, Rakovitsky N, Dishon Benattar Y, et al. (2019) **Colistin Resistance Development Following Colistin-Meropenem Combination Therapy vs. Colistin Monotherapy in Patients with Infections Caused by Carbapenem-Resistant Organisms** *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*

27. Dubé MP, Sattler FR, Torriani FJ, See D, Havlir DV, Kemper CA, et al. (1997) **A randomized evaluation of ethambutol for prevention of relapse and drug resistance during treatment of Mycobacterium avium complex bacteremia with clarithromycin-based combination therapy** *Journal of Infectious Diseases* **176**:1225–32

28. Durante-Mangoni E, Signoriello G, Andini R, Mattei A, De Cristoforo M, Murino P, et al. (2013) **Colistin and rifampicin compared with colistin alone for the treatment of serious infections due to extensively drug-resistant Acinetobacter baumannii: a multicenter, randomized clinical trial** *Clinical infectious diseases* **57**:349–58

29. Fournier S, Burguière AM, Flahault A, Vincent V, Treilhou MP, Eliaszewicz M (1999) **Effect of adding clofazimine to combined clarithromycin-ethambutol therapy for Mycobacterium avium complex septicemia in AIDS patients** *European journal of clinical microbiology & infectious diseases* **18**:16–22

30. Gerecht WB, Henry NK, Hoffman WW, Muller SM, LaRusso NF, Rosenblatt JE, et al. (1989) **Prospective randomized comparison of mezlocillin therapy alone with combined ampicillin and gentamicin therapy for patients with cholangitis** *Archives of internal medicine* **149**:1279–84

31. Gibson J, Date L, Joshua DE, Young GA, Wilson A, Benn R, et al. (1989) **A randomised trial of empirical antibiotic therapy in febrile neutropenic patients with hematological disorders: ceftazidime versus azlocillin plus amikacin** *Australian and New Zealand journal of medicine* **19**:417–25

32. Haase DA, Harding GK, Thomson MJ, Kennedy JK, Urias BA, Ronald AR (1984) **Comparative trial of norfloxacin and trimethoprim-sulfamethoxazole in the treatment of women with localized, acute, symptomatic urinary tract infections and antimicrobial effect on periurethral and fecal microflora** *Antimicrobial agents and chemotherapy* **26**:481–4

33. Harbarth S, von Dach E, Pagani L, Macedo-Vinas M, Huttner B, Olearo F, et al. (2015) **Randomized non-inferiority trial to compare trimethoprim/sulfamethoxazole plus rifampicin versus linezolid for the treatment of MRSA infection** *Journal of antimicrobial chemotherapy* **70**:264–72

34. Hodson ME, Roberts CM, Butland RJ, Smith MJ, Batten JC (1987) **Oral ciprofloxacin compared with conventional intravenous treatment for Pseudomonas aeruginosa infection in adults with cystic fibrosis** *Lancet (london, england)* **1**:235–7

35. Hoepelman IM, Rozenberg-Arska M, Verhoef J (1988) **Comparative study of ceftriaxone monotherapy versus a combination regimen of cefuroxime plus gentamicin for treatment of serious bacterial infections: the efficacy, safety and effect on fecal flora** *Chemotherapy* **34**:21–9

36. Hultén K, Jaup B, Stenquist B, Engstrand L (1997) **Combination treatment with ranitidine is highly efficient against Helicobacter pylori despite negative impact of macrolide resistance** *Helicobacter* **2**:188–93

37. Iravani A, Richard GA, Baer H, Fennell R (1981) **Comparative efficacy and safety of nalidixic acid versus trimethoprim/sulfamethoxazole in treatment of acute urinary tract infections in college-age women** *Antimicrobial agents and chemotherapy* **19**:598–604

38. Jacobs RF, Vats TS, Pappa KA, Chaudhary S, Kletzel M, Becton DL (1993) **Ceftazidime versus ceftazidime plus tobramycin in febrile neutropenic children** *Infection* **21**:223–8

39. Jo JH, Harkins CP, Schwardt NH, Portillo JA, Zimmerman MD, Carter CL, et al. (2021) **Alterations of human skin microbiome and expansion of antimicrobial resistance after systemic antibiotics** *Sci Transl Med* **13**

40. Macnab MF, Bohmer PD, Seager JR (1994) **Evaluation of the 3-drug combination, Rifater, versus 4-drug therapy in the ambulatory treatment of tuberculosis in Cape Town** *South African medical journal* **84**:325–8

41. Markowitz N, Quinn EL, Saravolatz LD (1992) **Trimethoprim-sulfamethoxazole compared with vancomycin for the treatment of Staphylococcus aureus infection** *Annals of internal medicine* **117**:390–8

42. Mavromanolakis E, Maraki S, Samonis G, Tselentis Y, Cranidis A (1997) **Effect of norfloxacin, trimethoprim-sulfamethoxazole and nitrofurantoin on fecal flora of women with recurrent urinary tract infections. Journal of chemotherapy (Florence** *Italy)* **9**:203–7

43. May T, Brel F, Beuscart C, Vincent V, Perronne C, Doco-Lecompte T, et al. (1997) **Comparison of combination therapy regimens for treatment of human immunodeficiency virus-infected patients with disseminated bacteremia due to Mycobacterium avium. ANRS Trial 033 Curavium Group. Agence Nationale de Recherche sur le Sida** *Clinical infectious diseases* **25**:621–9

44. McCarty JM, Tilden SJ, Black P, Craft JC, Blumer J, Waring W, et al. (1988) **Comparison of piperacillin alone versus piperacillin plus tobramycin for treatment of respiratory infections in children with cystic fibrosis** *Pediatric pulmonology* **4**:201–4

45. Menon R, Roberts FE, Barr KW, Howard H, Lord VL, Hegarty MA, et al. (1986) **Comparison of a slow-release trimethoprim with co-trimoxazole: efficacy and selection of resistance in the Enterobacteriaceae** *Journal of antimicrobial chemotherapy* **18**:415–20

46. Miehlke S, Meining A, Lehn N, Höchter W, Weingart J, Simon T, et al. (1998) **Comparison of omeprazole, metronidazole and clarithromycin with omeprazole/amoxicillin dual-therapy for the cure of Helicobacter pylori infection** *Digestion* **59**:646–50

47. Parras F, Guerrero MC, Bouza E, Blázquez MJ, Moreno S, Menarguez MC, et al. (1995) **Comparative study of mupirocin and oral co-trimoxazole plus topical fusidic acid in eradication of nasal carriage of methicillin-resistant Staphylococcus aureus** *Antimicrobial agents and chemotherapy* **39**:175–9

48. Parry MF, Neu HC, Merlino M, Gaerlan PF, Ores CN, Denning CR (1977) **Treatment of pulmonary infections in patients with cystic fibrosis: a comparative study of ticarcillin and gentamicin** *J Pediatr* **90**:144–8

49. Parry CM, Ho VA, Phuong le T, Bay PV, Lanh MN, Tung le T, et al. (2007) **Randomized controlled comparison of ofloxacin, azithromycin, and an ofloxacin-azithromycin combination for treatment of multidrug-resistant and nalidixic acid-resistant typhoid fever** *Antimicrobial agents and chemotherapy* **51**:819–25

50. Paul M, Bishara J, Yahav D, Goldberg E, Neuberger A, Ghanem-Zoubi N, et al. (2015) **Trimethoprim-sulfamethoxazole versus vancomycin for severe infections caused by meticillin resistant Staphylococcus aureus: randomised controlled trial** *BMJ (Clinical research ed)* **350**

51. Pogue JM, Rybak MJ, Stamper K, Marchaim D, Thamlikitkul V, Carmeli Y, et al. (2021) **Emergence of Colistin Resistance in the OVERCOME Trial: Impact of Combination Therapy with Meropenem** *Open Forum Infectious Diseases* **8**:S418–S9

52. Pujol M, Miró JM, Shaw E, Aguado JM, San-Juan R, Puig-Asensio M, et al. (2021) **Daptomycin Plus Fosfomycin Versus Daptomycin Alone for Methicillin-resistant Staphylococcus aureus Bacteremia and Endocarditis: A Randomized Clinical Trial** *Clin Infect Dis* **72**:1517–25

53. Rubinstein E, Lode H, Grassi C, Castelo A, Ward K, Alanko K, et al. (1995) **Ceftazidime monotherapy vs. Ceftriaxone/tobramycin for serious hospital-acquired gram-negative infections** *Clinical Infectious Diseases* **20**:1217–28

54. Schaeffer AJ, Flynn S, Jones J (1981) **Comparison of cinoxacin and trimethoprim-sulfamethoxazole in the treatment of urinary tract infections** *Journal of urology* **125**:825–7

55. Schaeffer AJ, Sisney GA (1985) **Efficacy of norfloxacin in urinary tract infection biological effects on vaginal and fecal flora** *Journal of urology* **133**:628–30

56. Smith AL, Doershuk C, Goldmann D, Gore E, Hilman B, Marks M, et al. (1999) **Comparison of a β-lactam alone versus β-lactam and an aminoglycoside for pulmonary exacerbation in cystic fibrosis** *Journal of Pediatrics* **134**:413–21

57. Stack WA, Knifton A, Thirlwell D, Cockayne A, Jenkins D, Hawkey CJ, et al. (1998) **Safety and efficacy of rabeprazole in combination with four antibiotic regimens for the eradication of Helicobacter pylori in patients with chronic gastritis with or without peptic ulceration** *American journal of gastroenterology* **93**:1909–13

58. Walsh TJ, Standiford HC, Reboli AC, John JF, Mulligan ME, Ribner BS, et al. (1993) **Randomized double-blinded trial of rifampin with either novobiocin or trimethoprim-sulfamethoxazole against methicillin-resistant Staphylococcus aureus colonization: prevention of antimicrobial resistance and effect of host factors on outcome** *Antimicrobial agents and chemotherapy* **37**:1334–42

59. Winston DJ, Ho WG, Nakao SL, Gale RP, Champlin RE (1986) **Norfloxacin versus vancomycin/polymyxin for prevention of infections in granulocytopenic patients** *Am J Med* **80**:884–90

60. Winston DJ, Ho WG, Bruckner DA, Gale RP, Champlin RE (1990) **Ofloxacin versus vancomycin/polymyxin for prevention of infections in granulocytopenic patients** *American journal of medicine* **88**:36–42

61. Wurzer H, Rodrigo L, Stamler D, Archambault A, Rokkas T, Skandalis N, et al. (1997) **Short-course therapy with amoxycillin-clarithromycin triple therapy for 10 days (ACT-10) eradicates Helicobacter pylori and heals duodenal ulcer** *ACT-10 Study Group. Alimentary pharmacology & therapeutics* **11**:943–52

62. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, et al. **Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis** *The Lancet*

63. Joshi LT (2021) **The G7 Summit 2021: time for our world leaders to step up to the challenge of antimicrobial resistance** *Access Microbiology* **3**

64. Christopher A Kerantzas, William R Jacobs, Eric J Rubin, Collier RJ **Origins of Combination Therapy for Tuberculosis: Lessons for Future Antimicrobial Development and Application** *mBio* **8**:e01586–16

65. Fish DN, Piscitelli SC, Danziger LH (1995) **Development of resistance during antimicrobial therapy: a review of antibiotic classes and patient characteristics in 173 studies** *Pharmacotherapy* **15**:279–91

66. Hernán MA, Wang W, Leaf DE (2022) **Target Trial Emulation: A Framework for Causal Inference From Observational Data** *JAMA* **328**:2446–7

67. Hernán MA (2021) **Methods of Public Health Research — Strengthening Causal Inference from Observational Data** *New England Journal of Medicine* **385**:1345–8

68. Schulz KF, Altman DG, Moher D, for the CG (2010) **CONSORT 2010 Statement: Updated Guidelines for Reporting Parallel Group Randomised Trials** *PLOS Medicine* **7**

69. Bramer WM, Giustini D, de Jonge GB, Holland L, Bekhuis T (2016) **De-duplication of database search results for systematic reviews in EndNote** *J Med Libr Assoc* **104**:240–3

70. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. (2021) **The PRISMA 2020 statement: an updated guideline for reporting systematic reviews** *Systematic Reviews* **10**

71. Jackson D, Law M, Stijnen T, Viechtbauer W, White IR (2018) **A comparison of seven random-effects models for meta-analyses that estimate the summary odds ratio** *Stat Med* **37**:1059–85

72. Deeks JJ, Higgins JPT, Altman DG (2008) **Analysing Data and Undertaking Meta-Analyses** :243–96

73. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. (2019) **RoB 2: a revised tool for assessing risk of bias in randomised trials** *BMJ* **366**

74. Viechtbauer W (2010) **Conducting Meta-Analyses in R with the metafor Package** *Journal of Statistical Software* **36**:1–48

75. Bartoń K. (2020) **MuMIn: Multi-Model Inference**

Article and author information

Berit Siedentop

Institute of Integrative Biology, Department of Environmental Systems Science, ETH Zürich, Zurich, Switzerland, Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zürich, University of Zürich, Zürich, Switzerland
For correspondence: berit.siedentop@env.ethz.ch
ORCID iD: 0000-0003-0802-7681

Viacheslav N. Kachalov

Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zürich, University of Zürich, Zürich, Switzerland, Institute of Medical Virology, University of Zurich, Zurich, Switzerland
ORCID iD: 0000-0002-0310-3764

Christopher Witzany
Institute of Integrative Biology, Department of Environmental Systems Science, ETH Zürich, Zurich, Switzerland
ORCID iD: 0000-0002-7128-6419

Matthias Egger
Institute of Social and Preventive Medicine (ISPM), University of Bern, Bern, Switzerland, Population Health Sciences, University of Bristol, Bristol, UK, Centre for Infectious Disease Epidemiology and Research, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa
ORCID iD: 0000-0001-7462-5132

Roger D. Kouyos
Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zürich, University of Zürich, Zürich, Switzerland, Institute of Medical Virology, University of Zurich, Zurich, Switzerland
ORCID iD: 0000-0002-9220-8348

Sebastian Bonhoeffer
Institute of Integrative Biology, Department of Environmental Systems Science, ETH Zürich, Zurich, Switzerland
For correspondence: seb@env.ethz.ch
ORCID iD: 0000-0001-8052-3925

Copyright

Editors

Reviewing Editor
Marc Bonten
University Medical Center Utrecht, Utrecht, Netherlands

Senior Editor
Diane Harper
University of Michigan, Ann Arbor, United States of America

Reviewer #1 (Public Review):

Summary:
The investigators have performed a state-of-the art systematic review and meta-analysis of studies that may help to answer the research question: if administration of multiple antibiotics simultaneously prevents antibiotic resistance development in individuals. The amount of studies eligible for analysis is very low, and within that low number, there is huge variability in bug-drug combinations studied and most studies had a high risk of bias, further limiting the capability of meta-analysis to answer the research question. In addition, based on I2 values there is also huge statistical heterogeneity between outcomes of studies compared, further limiting the predictive value of meta-analysis. In fact, the only 2 studies meeting all

eligibility criteria addressed the treatment of mycobacterium tuberculosis, for which the research question is hardly applicable. The authors, therefore, conclude that "our analysis could not identify any benefit or harm of using a higher or a lower number of antibiotics regarding within-patient resistance development." Apart from articulating this knowledge gap, the findings will not have consequences for patient care, but may stimulate the scientific community to better address this research question in future studies.

Strengths:
The systematic and rigorous approach for the review and meta-analysis.

Weaknesses:
None identified.

https://doi.org/10.7554/eLife.93740.1.sa1

Reviewer #2 (Public Review):

Summary:
The authors performed a systematic review and meta-analysis to investigate whether the frequency of emergence of resistance is different if combination antibiotic therapy is used compared to fewer antibiotics. The review shows that there is currently insufficient evidence to reach a conclusion due to the limited sample size. High-quality studies evaluating appropriate antimicrobial resistance endpoints are needed.

Strengths:
The strengths of the manuscript are that the article addresses a relevant research question that is often debated. The article is well-written and the methodology used is valid. The review shows that there is currently insufficient evidence to reach a conclusion due to the limited sample size. High-quality studies evaluating appropriate antimicrobial resistance endpoints are needed. I have several comments and suggestions for the manuscript.

Weaknesses:
Weaknesses of the manuscript are the large clinical and statistical heterogeneity and the lack of clear definitions of acquisition of resistance. Both these weaknesses complicate the interpretation of the study results.

Major comments:
My main concern about the manuscript is the extent of both clinical and statistical heterogeneity, which complicates the interpretation of the results. I don't understand some of the antibiotic comparisons that are included in the systematic review. For instance the study by Paul et al (50), where vancomycin (as monotherapy) is compared to co-trimoxazole (as combination therapy). Emergence (or selection) of co-trimoxazole in S. aureus is in itself much more common than vancomycin resistance. It is logical and expected to have more resistance in the co-trimoxazole group compared to the vancomycin group, however, this difference is due to the drug itself and not due to co-trimoxazole being a combination therapy. It is therefore unfair to attribute the difference in resistance to combination therapy. Another example is the study by Walsh (71) where rifampin + novobiocin is compared to rifampin + co-trimoxazole. There is more emergence of resistance in the rifampin + co-trimoxazole group but this could be attributed to novobiocin being a different type of antibiotic than co-trimoxazole instead of the difference being attributed to combination therapy. To improve interpretation and reduce heterogeneity my suggestion would be to limit the primary analyses to regimens where the antibiotics compared are the same but in one group one or more antibiotic(s) are added (i.e. A versus A+B). The other analyses are problematic in their interpretation and should be clearly labeled as secondary and their interpretation discussed.

Another concern is about the definition of acquisition of resistance, which is unclear to me. If for example meropenem is administered and the follow-up cultures show Enterococcus species (which is intrinsically resistant to meropenem), does this constitute acquisition of resistance? If so, it would be misleading to determine this as an acquisition of resistance, as many people are colonized with Enterococci and selection of Enterococci under therapy is very common. If this is not considered as the acquisition of resistance please include how the acquisition of resistance is defined per included study. Table S1 is not sufficiently clear because it often only contains how susceptibility testing was done but not which antibiotics were tested and how a strain was classified as resistant or susceptible.

Line 85: "Even though within-patient antibiotic resistance development is rare, it may contribute to the emergence and spread of resistance."
Depending on the bug-drug combination, there is great variation in the propensity to develop within-patient antibiotic resistance. For example: within-patient development of ciprofloxacin resistance in Pseudomonas is fairly common while within-patient development of methicillin resistance in S. aureus is rare. Based on these differences, large clinical heterogeneity is expected and it is questionable where these studies should be pooled.

Line 114: "The overall pooled OR for acquisition of resistance comparing a lower number of antibiotics versus a higher one was 1.23 (95% CI 0.68 - 2.25), with substantial heterogeneity between studies (I2=77.4%)"
What consequential measures did the authors take after determining this high heterogeneity? Did they explore the source of this large heterogeneity? Considering this large heterogeneity, do the authors consider it appropriate to pool these studies?

https://doi.org/10.7554/eLife.93740.1.sa0

Author Response

Reviewer #1 (Public Review):

Summary:

The investigators have performed a state-of-the art systematic review and meta-analysis of studies that may help to answer the research question: if administration of multiple antibiotics simultaneously prevents antibiotic resistance development in individuals. The amount of studies eligible for analysis is very low, and within that low number, there is huge variability in bug-drug combinations studied and most studies had a high risk of bias, further limiting the capability of meta-analysis to answer the research question. In addition, based on I2 values there is also huge statistical heterogeneity between outcomes of studies compared, further limiting the predictive value of meta-analysis. In fact, the only 2 studies meeting all eligibility criteria addressed the treatment of mycobacterium tuberculosis, for which the research question is hardly applicable. The authors, therefore, conclude that "our analysis could not identify any benefit or harm of using a higher or a lower number of antibiotics regarding within-patient resistance development." Apart from articulating this knowledge gap, the findings will not have consequences for patient care, but may stimulate the scientific community to better address this research question in future studies.

Strengths:

The systematic and rigorous approach for the review and meta-analysis.

Weaknesses:

> *None identified.*

We thank the reviewer for this thoughtful and positive appraisal of our work.

> ***Reviewer #2 (Public Review):***
>
> *Summary:*

The authors performed a systematic review and meta-analysis to investigate whether the frequency of emergence of resistance is different if combination antibiotic therapy is used compared to fewer antibiotics. The review shows that there is currently insufficient evidence to reach a conclusion due to the limited sample size. High-quality studies evaluating appropriate antimicrobial resistance endpoints are needed.

> *Strengths:*
>
> *The strengths of the manuscript are that the article addresses a relevant research question that is often debated. The article is well-written and the methodology used is valid. The review shows that there is currently insufficient evidence to reach a conclusion due to the limited sample size. High-quality studies evaluating appropriate antimicrobial resistance endpoints are needed. I have several comments and suggestions for the manuscript.*
>
> *Weaknesses:*
>
> *Weaknesses of the manuscript are the large clinical and statistical heterogeneity and the lack of clear definitions of acquisition of resistance. Both these weaknesses complicate the interpretation of the study results.*

We thank the reviewer for the positive comments and pointing out where our work can be improved.

> *Major comments:*
>
> *My main concern about the manuscript is the extent of both clinical and statistical heterogeneity, which complicates the interpretation of the results. I don't understand some of the antibiotic comparisons that are included in the systematic review. For instance the study by Paul et al (50), where vancomycin (as monotherapy) is compared to co-trimoxazole (as combination therapy). Emergence (or selection) of co-trimoxazole in S. aureus is in itself much more common than vancomycin resistance. It is logical and expected to have more resistance in the co-trimoxazole group compared to the vancomycin group, however, this difference is due to the drug itself and not due to co-trimoxazole being a combination therapy. It is therefore unfair to attribute the difference in resistance to combination therapy. Another example is the study by Walsh (71) where rifampin + novobiocin is compared to rifampin + co-trimoxazole. There is more emergence of resistance in the rifampin + co-trimoxazole group but this could be attributed to novobiocin being a different type of antibiotic than co-trimoxazole instead of the difference being attributed to combination therapy. To improve interpretation and reduce heterogeneity my suggestion would be to limit the primary analyses to regimens where the antibiotics compared are the same but in one group one or more antibiotic(s) are added (i.e. A versus A+B). The other analyses are problematic in their interpretation and should be clearly labeled as secondary and their interpretation discussed.*

We acknowledge the presence of statistical and clinical heterogeneity in our overall analysis. The decision to pursue this comprehensive examination was predefined in our previously

published study protocol (PROSPERO CRD42020187257) and driven by our interest whether, despite some differences, we could either identify an overarching effect of combination therapy on resistance or identify factors that explain potential differences of the effect of combination therapy across pathogens/drugs. We indeed, find that heterogeneity is high, however identifying the driving factors of this heterogeneity is difficult as evidence is limited.

We carried out several subgroup analyses, e.g. explicitly focusing on specific pathogen groups and medical conditions or exploring heterogeneity in treatment arms (figure 3, supplementary materials section 6). However, it is important to highlight that the number of studies available for these subgroup analyses was low. Additionally, recognizing the high heterogeneity within treatment arms, we performed a subgroup analysis focusing solely on resistances of antibiotics common to both arms (supplementary material section 6.1.8; which would avoid comparisons such as the one between vancomycin and co-trimoxazole raised by the reviewer). Unfortunately, this also revealed substantial heterogeneity. While we aimed to address heterogeneity through these subgroup analyses, limitations arose due to the number of studies meeting specific criteria and the nature of data provided by these studies.

Moreover, regarding the concern on interpretation of co-trimoxazole as combination therapy, we acknowledge the confusion surrounding its classification as one or two antibiotics. Despite the common contemporary view of co-trimoxazole as a single antibiotic, we chose to consider it as two antibiotics due to historical practices, as observed in Black et al. (1982), where trimethoprim was compared to trimethoprim and sulfamethoxazole. We recognize that this decision may lead to confusion and we consider conducting a further sensitivity analysis in the future version of this manuscript, exploring the possibility of considering co-trimoxazole as a single antibiotic. We agree that the slight trend of less antibiotics performing better overserved for MRSA, should not be over interpreted as this is driven by the two studies Walsh et al 1993 and Paul et al 2015 as pointed out by the reviewer. In lines 183-186 we discuss this issue that for better evaluation of antibiotic combination therapy, more studies which use identical antibiotics (i.e. A versus A+B) are needed. We will try to clarify and highlight this in the future version of the manuscript.

> *Another concern is about the definition of acquisition of resistance, which is unclear to me. If for example meropenem is administered and the follow-up cultures show Enterococcus species (which is intrinsically resistant to meropenem), does this constitute acquisition of resistance? If so, it would be misleading to determine this as an acquisition of resistance, as many people are colonized with Enterococci and selection of Enterococci under therapy is very common. If this is not considered as the acquisition of resistance please include how the acquisition of resistance is defined per included study.*

Thank you for pointing out this potential ambiguity. Our definition of “acquisition of resistance” is agnostic to bacterial species and hence intrinsically resistant species can be included if they were only detected during the follow-up culture by the studies. We will clarify this in the definition of “acquisition of the resistance” in the manuscript (see l. 259-260). However, it was not always clear from the studies which pathogens were acquired or whether intrinsically resistant species were not reported. Therefore, we rely on the studies' specifications of resistant and non-resistant without further classifying data into intrinsic and non-intrinsic resistance. The outcome “acquisition of resistance” can be seen more of a risk assessment for having any resistant bacterium during or after treatment. In contrast, the outcome “emergence of resistance” is more rigorous, demanding the same species to be measured as more resistant during or after treatment.

> *Table S1 is not sufficiently clear because it often only contains how susceptibility testing was done but not which antibiotics were tested and how a strain was classified as resistant or susceptible.*

In Table S1, we omitted the listing of antibiotics for which susceptibility testing was performed, as this information is already presented in the main text (Table 1). However, we agree that linking this information better in a future version would benefit the understanding. Given the variability in methods used to assess resistance and the variability in drugs, the comparability of breakpoints is limited. Hence, we decided not to provide further details on this aspect so far.

> *Line 85: "Even though within-patient antibiotic resistance development is rare, it may contribute to the emergence and spread of resistance."*
>
> *Depending on the bug-drug combination, there is great variation in the propensity to develop within-patient antibiotic resistance. For example: within-patient development of ciprofloxacin resistance in Pseudomonas is fairly common while within-patient development of methicillin resistance in S. aureus is rare. Based on these differences, large clinical heterogeneity is expected and it is questionable where these studies should be pooled.*

We agree that our formulation neglects differences in prevalence of within-host resistance emergence depending on bug-drug combinations. We will correct this in our upcoming version. (i.e. we will correct our statement to: “Within-patient antibiotic resistance development, even if rare, can contribute to the emergence and spread of resistance.”)

> *Line 114: "The overall pooled OR for acquisition of resistance comparing a lower number of antibiotics versus a higher one was 1.23 (95% CI 0.68 - 2.25), with substantial heterogeneity between studies (I2=77.4%)"*
>
> *What consequential measures did the authors take after determining this high heterogeneity? Did they explore the source of this large heterogeneity? Considering this large heterogeneity, do the authors consider it appropriate to pool these studies?*

Thank you for highlighting this lack of clarity. In our upcoming version, we will emphasize the sub-analyses conducted to explore heterogeneity (i.e., figure 3 and supplementary materials section 6). Nevertheless, these analyses faced limitations due to the scarcity of evidence and the data provided by the studies. Given the lack of appropriate evidence, it is hard to identify the source of heterogeneity. The decision to pool all studies was pre-specified in our previously published study protocol (PROSPERO CRD42020187257) and was motivated by the question whether there is a general effect of combination therapy on resistance development or identify factors that explain potential differences of the effect of combination therapy across bug-drug combinations.