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

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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 (as defined by the study authors) 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 for careful future use of the meta-analysis methodology, when existing data just don't allow conclusions.

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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. Within-patient antibiotic resistance development, even if rare, 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 (SI section 11.4), for a total of 42 studies, 40 RCTs and two quasi-RCTs, where the allocation method used is not truly random (**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 (**table 1**, SI table S1). Consequently, most studies did not have the statistical power to detect differences in within-patient resistance development even if we assume that the effect on resistance development is large between treatment arms (**figure 2 B**, SI section 8). 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 hematological malignancy patients with four studies (10%) respectively. Twenty-three of the included studies (55%) compared treatment arms 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 section 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%). The overall pooled estimates are based on studies that focus on various clinical conditions/pathogens and compare different antibiotics treatments. To explore the impact of these and other potential sources of heterogeneity on the resistance estimates we performed sub-group analyses and meta-regression. The results for the two resistance outcomes are qualitatively comparable in the sense that individual estimates may differ, but show overall similar absence of evidence to support either benefit, harm or equivalence of treating with a higher number of antibiotics. Therefore, our focus in the following is on the acquisition of resistance (details on emergence of resistance can be found in the SI sections 1-8).

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 3A**), and *MAC* (pooled OR 0.18, 95% CI 0.06 – 0.52; I^2 =26.8%, **figure 3A**), but worse in case of *P. aeruginosa* (pooled OR 3.42, 95% CI 1.03 – 11.43; I^2=1.54%, **figure 3A**). 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 table S3), which could be due to different potencies or resistance prevalences of antibiotics as discussed in SI (SI section 6.1.10). In contrast, when restricting the analysis to studies with at least one common antibiotic in

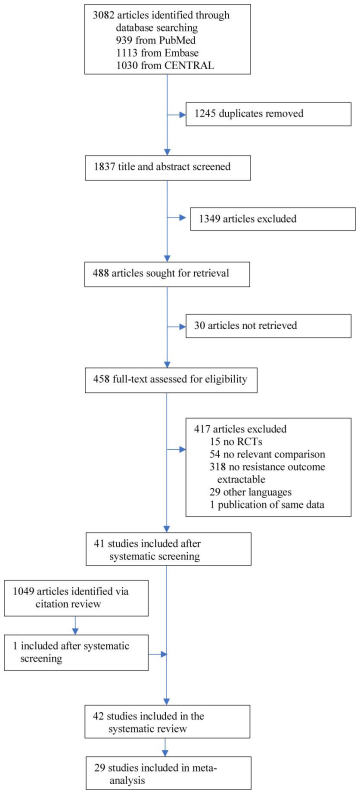

Figure 1.

Study selection

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. Justification for resistance outcome extraction is given in SI table S1.

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. (1994)(23)	RCT	Nosocomial pneumonia, nosocomial sepsis, or severe diffuse peritonitis	Clinical efficacy and tolerance, emergence of resistance and risk of superinfection	Imipenem	Imipenem, and netilmicin	no	Mortality attributable to infection, 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. (continued)

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	yes	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 hematological malignancy patients	Efficacy and safety	Norfloxacin	Vancomycin, and polymyxin	no	Mortality attributable to infection
Winston et al. (1990)(60)	RCT	Prophylaxis for hematological malignancy 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	-

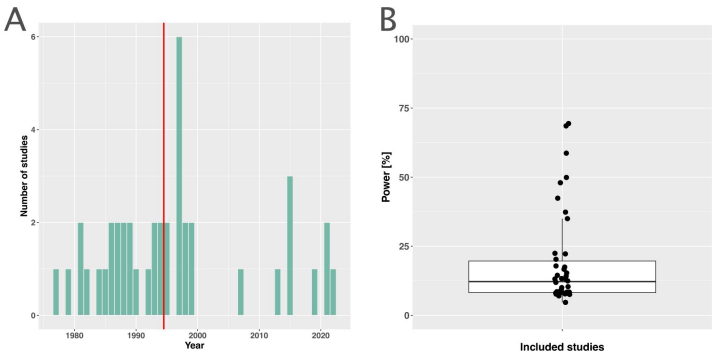

Figure 2.

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.

the treatment arms we found no evidence of a difference, only a weak indication that a higher number of antibiotics performs better (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 section 6). The multi-model 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 section 7).

The inspection of the funnel plot and the modified Egger's test showed no indication of a publication bias (SI section 5). The results were largely robust to the choice of the random effects model (SI section 4). 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 section 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 (3, 18) and a priority area for health policy and public health (62).

Our analysis showed that combining antibiotics reduced resistance development for *H. pylori* or MAC, in line with the current standard of care (11, 63). 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 (SI section 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

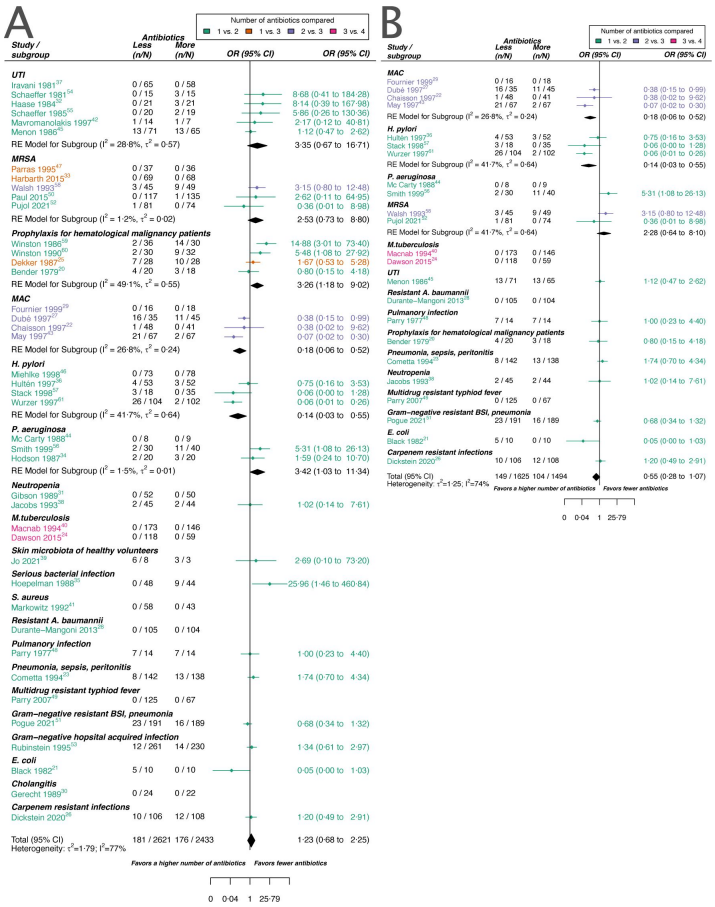

Figure 3.

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.

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 resistance testing of each administered antibiotic. 19 (45%) of our included studies compared treatment arms with no antibiotics in common, and 22 studies (52%) had more than one antibiotic not identical in the treatment arms (**table 1**). 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 (**table 1**). 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 (64). Similarly, today, relevant cohort studies could be analysed collaboratively using various modern statistical methods to address confounding by indication and other biases (65, 66). However, even with appropriate causal inference methods, residual confounding cannot be excluded when using observational data (67). Therefore, RCTs will remain the gold standard to estimate causal relationships.

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 and report systematically the 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 (SI

section 1). 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 (section 11). 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 (as defined by the study authors) 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 (section 9) 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 section 6.2).

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 section 3) (73). To assess publication bias, we visually inspected the funnel plot and a modified Egger's test (SI section 5). We performed sensitivity analyses on the model choice (SI section 4.1), and risk of bias (SI section 4.2), and performed a post-hoc trial sequential analysis (SI section 8.3). 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

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Editors

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 strength of the manuscript is that the article addresses a relevant research question which 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.

Comments on latest version:

The authors adressed all the comments that were shared in the previous peer review. I still believe that both clinical and statistical heterogeneity remains a problem with the interpretation of the meta-analysis. However, as the authors state, this is in line with the original research question as formulated on Prospero.

https://doi.org/10.7554/eLife.93740.2.sa1

Author response:

The following is the authors' response to the original reviews.

> ***Reviewer #1 (Public Review):***
>
> *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.*

Thank you for raising these important points and highlighting the need for clarification. We understand that the reviewer has concerns regarding the following points:

(1) The structure of presenting our analyses, i.e. main analyses and sub-group analyses and their corresponding discussion and interpretation

Our primary interest was whether combining antibiotics has an overarching effect on resistance and to identify factors that explain potential differences of the effect of combining antibiotic across pathogens/drugs. Therefore, pooling all studies, and thereby all combinations of antibiotics, is one of our main analyses. The decision to pool all studies that compare a lower number of antibiotics to a higher number of antibiotics was hence predefined in our previously published study protocol (PROSPERO CRD42020187257).

We indeed, find that heterogeneity is high in our statistical analyses. As planned in our study protocol, we did perform several prespecified sub-group analyses and added additional ones. We now emphasize that several sub-group analyses were performed to investigate heterogeneity (L 119ff): “The overall pooled estimates are based on studies that focus on various clinical conditions/pathogens and compare different antibiotics treatments. To explore the impact of these and other potential sources of heterogeneity on the resistance estimates we performed various sub-group analyses and metaregression.”

The performed sub-group analyses specifically focused on specific pathogens/clinical conditions (figure 3) or explored heterogeneity due to different antibiotics in comparator arms – as suggested by the reviewer (figure 3B, SI section 6). We find that the heterogeneity remains high even if only resistances to antibiotics common to both arms are considered (SI section 6.1.8). With this analysis we excluded comparisons of different antibiotics (e.g., A vs B+C), such as those between vancomycin and cotrimoxazole named by the reviewer. While we aimed to explore heterogeneity and investigate potential factors affecting the effect of combining antibiotic on resistance, limitations arose due to limited evidence and the nature of data provided by the identified studies. Therefore, interpretability remains also limited for the subgroup analyses, which we highlight in the discussion. (L 186 ff: We accounted for many sources of heterogeneity using stratification and meta-regression, but analyses were limited by missing information and sparse data.) Further, specific subgroup analyses are discussed in more detail in the SI.

(2) Difference in resistance development due to the type of the antibiotics or due to combination therapy?

The reviewer raises an important point, which we also try to make: future studies should be systematically designed to compare antibiotic combination therapy, i.e. identical antibiotics in treatment arms should be used, except for additional antibiotics used in both treatment arms. We already mentioned this point in our discussion but highlight this now by emphasizing how many studies did not have identical antibiotics in their treatment arms. We write in L194ff: “19 (45%) of our included studies compared treatment arms with no antibiotics in common, and 22 studies (52%) had more than one antibiotic not identical in the treatment arms (table 1). 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 (table 1).”

Furthermore, we investigated the importance of the type of antibiotics with several subgroup analyses (e.g. SI sections 6.1.8 and 6.1.10). We now further highlight the concern of the type of antibiotics in the result section of the main manuscript, where we discuss the sub-group analysis with no common antibiotics in the treatment arms 131 ff: “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; $I2$=37%, SI table S3),

which could be due to different potencies or resistance prevalences of antibiotics as discussed in SI (SI section 6.1.10)." As mentioned above we also perform sub-group analyses, where only resistances of antibiotics common to both arms are considered (SI section 6.1.8). However, as discussed in the corresponding sections, the systematic assessment of antibiotic combination therapy remains challenging as not all resistances against antibiotics used in the arms were systematically measured and reported. Furthermore, the power of these sub-group analyses is naturally a concern, as they include fewer studies.

> *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.*

Thank you for pointing out this potential ambiguity. The definition of acquisition of resistance reads now (L 275 ff): "A patient was considered to have acquired resistance if, at the follow-up culture, a resistant bacterium (as defined by the study authors) was detected that was not present in the baseline culture." We also changed the definition accordingly in the abstract (L 36 ff). We hope that the definition of acquisition is now clearer. Our definition of "acquisition of resistance" is agnostic to bacterial species and hence intrinsically resistant species, as the example raised by the reviewer, can be included if they were only detected during the follow-up culture by the studies. Generally, it was not always clear from the studies, which pathogens were screened for and whether the selection of intrinsically resistant bacteria was reported or not. Therefore, we rely on the studies' specifications of resistant and non-resistant without further distinction from our side, i.e. classifying data into intrinsic and non-intrinsic resistance. Overall, the outcome "acquisition of resistance" can be interpreted as 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 detected as more resistant during or after treatment.

The information, which antibiotic susceptibility tests were performed in each individual study can be found in the main text in table 1. However, we agree that this information should be better linked and highlighted again in table S1. We therefore now refer to table 1 in the table description of table S1. L134 ff.: "See table 1 in the main text for which antibiotics the antibiotics tested and reported extractable resistance data". Furthermore, we added the breakpoints for resistant and susceptible classification if specifically stated in the main text of the study. However, we did not do further research into old guidelines, manufactures manuals or study protocols in case the breakpoints are not specifically stated in the main text as the main goal of this table, in our opinion, is to show a justification, why the studies could be considered for a resistance outcome. We therefore decided against further breakpoint investigations for studies, where the breakpoint is not specifically stated in the main text.

> *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 changed our statement in L 86 to: “Within-patient antibiotic resistance development, even if rare, may 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. As mentioned above, we now highlight that we performed several subgroup analyses to investigate heterogeneity. (L 116ff): “The overall pooled estimates are based on studies that focus on various clinical conditions/pathogens and compare different antibiotics treatments. To explore the impact of these and other potential sources of heterogeneity on the resistance estimates we performed various subgroup analyses and meta-regression.” Nevertheless, these analyses faced limitations due to the scarcity of evidence and often still showed a high amount of heterogeneity. 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. Therefore, we think that the presentation of the overall pooled estimate is appropriate, as it was predefined, and potential heterogeneity is furthermore explored in the subgroup analyses.

> ***Reviewer #1 (Recommendations For The Authors):***
>
> *I want to congratulate the investigators for the rigorous approach followed and the - in my opinion - correct interpretation of the data and analysis. The disappointing outcome is independent of the quality of the approach used. Yet, the consequences of that outcome are rather limited, and will not be surprising for - at least - some in the field of antibiotic resistance.*

Thank you for your positive and differentiated feedback.

> ***Reviewer #2 (Recommendations For The Authors):***
>
> *Line 93: "The screening of the citations of the 41 studies identified one additional eligible study, for a total of 42 studies".*
>
> *Why was this study missed in the search strategy?*
>
> *What is the definition of "quasi-RCTs"? Why were these included in the analysis?*

Thank you for pointing out this lack of clarity. The additional study, which was found through screening the references of included studies, was not identified with our search strategy as neither the abstract nor database specific identifiers provided any indications that resistance was measured in this study. We added an explanation in the supplementary materials L 792 ff. and refer to this explanation in the main manuscript (L 95).

Quasi-randomized trials are trials that use allocation methods, which are not considered truly random. We added this specification in L 95. It now reads: "….two quasi-RCTs, where the allocation method used is not truly random" and in L 252 ff: "Studies were classified as quasi-RCTs if the allocation of participants to study arms was not truly random." For instance, the study Macnab et al. (1994) assigned patients alternately to the treatment arms. Quasi-randomized controlled trials can lead to biases and especially old studies are more likely to have used quasi-random allocation methods. This can also be seen in our study, where the two quasi-randomized controlled trials were published in 1994 and 1997. The bias is considered in the risk of bias assessment and in our conducted sensitivity analysis regarding the impact of risk of bias on our estimates (supplementary information sections 3.0 and 4.2). Furthermore, one of the two previous conducted meta-analyses comparing beta-lactam monotherapy to beta-lactam and aminoglycoside, which assessed resistance development also included quasi-randomized controlled trials Paul et al 2014. Overall, while designing the study, we decided to include quasi-randomized controlled trials to increase statistical power as we expected that limited statistical power might be a concern and decided to assess potential biases in the risk of bias assessment.

> *Line 100: "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).".*
>
> *Small studies actually have more power to detect large effects while smaller power to detect small effects. Please rephrase.*

Thank you for pointing out this lack of clarity. We rephrased the sentence in order to emphasize our point that the studies are underpowered even if we assume in our power analysis a large effect on resistance development between treatment arms. In this context "the small" studies include too few patients to detect a large difference in resistance development. As resistance development is a rare event, generally studies have to include a larger number of patients to estimate the effect of intervention. We rephrased the sentence in L 101ff to: "Consequently, most studies did not have the statistical power to detect differences in within-patient resistance development even if we assume that the effect on resistance development is large between treatment arms."

> *Line 108: "... and prophylaxis for blood cancer patients with four studies (10%) respectively.".*
>
> *I would suggest using the medical term hematological malignancy patients.*

Thank you for the suggestion, we changed it as suggested to hematological malignancy patients, also accordingly in the figures, and table 1.

> *Line 117: "Since the results for the two resistance outcomes are comparable, our focus in the following is on the acquisition of resistance".*
>
> *The first OR is 1.23 and the second is 0.74, why do you consider these outcomes as comparable?*

Thank you for pointing out our unprecise formulation. Due to the lack of power the exact estimates need to be interpreted with care. Here, we wanted to make the point that qualitatively the results of both outcomes do not differ in the sense that our analysis shows no substantial difference between a higher and a lower number of antibiotics. We rephrased the sentence to be more precise (L 123ff): "The results for the two resistance outcomes are qualitatively comparable in the sense that individual estimates may differ, but show similar absence of evidence to support either the benefit, harm or equivalence of treating with a

higher number of antibiotics. Therefore, our …". More detailed discussion about differences in estimates can be found in the SI, when the estimates of emergence of resistance are presented (e.g. SI section 2.1).

> *Line 123: "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).".*
>
> *How do you explain this? What does this mean?*

We now added a more detailed explanation in the supplement (L 376ff.): "The result that if the treatment arms had no antibiotics in common a lower number of antibiotics performed better than a higher number of antibiotics could be due to different potencies of antibiotics or resistance prevalences. Further, there could be a bias to combine less potent antibiotics or antibiotics with higher resistance prevalence to ensure treatment efficacy, which couldlead to higher chances to detect resistances in the treatment arm with higher number of antibiotics, e.g. by selecting pre-existing resistance due to antibiotic treatment (see also section 6.1.9)." We furthermore already specifically mention this point in the main manuscript and refer then to the detailed explanation in the SI (L134 ff, "which could be due to different potencies or resistance prevalences of antibiotics as discussed in SI (SI section 6.1.10)")

Overall, we want to point out that these results need to be interpreted with caution as overall the statistical power is limited to confidently estimate the difference in effect of a higher and lower number of antibiotics.

> *Line 125: ". 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".*
>
> *The difference was not statistically significant but there does seem to be an indication of a difference, please rephrase.*

We rephrased the sentence to (L135 ff.): "In contrast, when restricting the analysis to studies with at least one common antibiotic in the treatment arms we found no evidence of a difference, only a weak indication that a higher number of antibiotics performs better (pooled OR 0.55, 95% CI 0.28 – 1.07; *I2* =74%, figure 3B)."

> *Line 190: "Similarly, today, relevant cohort studies could be analysed collaboratively using various modern statistical methods to address confounding by indication and other biases (66, 67)".*
>
> *However, residual confounding by indication is likely. Please also mention the disadvantages of observational studies compared to RCTs.*

We now highlight that causal inference with observational data comes with its own challenges and stress that randomized controlled trials are still considered the gold standard. L 204ff now reads: "However, even with appropriate causal inference methods, residual confounding cannot be excluded when using observational data (67). Therefore, will remain the gold standard to estimate causal relationships."

> *Line 230: "Gram-negative bacteria have an outer membrane, which is absent in grampositive bacteria for instance, therefore intrinsic resistance against antibiotics can be observed in gram-negative bacteria (11)".*
>
> *Intrinsic resistance is not unique for Gram-negative bacteria but also exists for Grampositive bacteria.*

We agree with the reviewer that intrinsic resistance is not unique to gram-negative bacteria and refined our writing. We additionally added that differences between gram-negative and gram-positive bacteria are not only to be expected due to differing intrinsic resistances but also due to potential differences in the mechanistic interactions of antibiotics, i.e., synergy or antagonism. The paragraph reads now (SI L289): “The gram status of a bacterium may potentially determine how effective an antibiotic, or an antibiotic combination is. Differences between gram-negative and gram-positive bacteria such as distinct bacterial surface organisation can lead to specific intrinsic resistances of gram-negative and grampositive bacteria against antibiotics (55). These structural differences can lead to varying effects of antibiotic combinations between gram-negative and gram-positive bacteria (56).”

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