β-lactam resistant *Streptococcus pneumoniae* dynamics following treatment: a dose-response meta-analysis

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Running title: Temporal dynamics of drug resistance

Abstract

Background Patient exposure to antibiotics promotes the emergence of drug-resistant pathogens. The aim of this study was to identify whether the temporal dynamics of resistance emergence at the individual-patient level were predictable for specific pathogen-drug classes.

Methods Following a systematic review, a novel robust error meta-regression (REMR) method for dose-response meta-analysis (DRMA) was used to estimate the odds ratio (OR) for carrying resistant bacteria during and following treatment compared to baseline. Probability density functions fitted to the resulting dose-response curves were then used to optimize the period during and/or after treatment when resistant pathogens were most likely to be identified.

Results Studies of *Streptococcus pneumoniae* treatment with β-lactam antibiotics demonstrated a peak in resistance prevalence among patients four days after completing treatment with a 3.32-fold increase in odds (95%CI 1.71 - 6.46). Resistance waned more gradually than it emerged, returning to pre-exposure levels one month after treatment (OR 0.98, 95%CI 0.55 - 1.75). Patient isolation during the peak dose-response period would be expected to reduce the risk that a transmitted pathogen is resistant equivalently to a 50% longer isolation window timed from the first day of treatment.

Conclusions Predictable temporal dynamics of resistance levels have implications both for surveillance and control.

Keywords: antibiotics; drug resistance; penicillin

Background

Since the discovery of penicillin, antibiotics have contributed significantly in extending human life expectancy by 23 years [1, 2]. Widespread resistance among common bacterial pathogens and slow development of replacement compounds or alternative therapies threaten these recent gains [3, 4]. It is estimated that approximately 1.27 million annual deaths are attributable to bacterial antimicrobial resistance [5].

Antibiotic resistance is selected for when bacteria are exposed to sub-therapeutic levels of antibiotics which would otherwise inhibit their growth or kill them [6], making the remedy itself one of the primary drivers and risk factors for antibiotic resistance [7-10]. The relationship between antibiotics and resistance is dose dependent: higher antibiotic consumption correlates with more resistant infections [11, 12]. The association between level of antibiotics administered and resistance development has been demonstrated at the bacterial colony level [13], at the individual patient level [14, 15], and among human populations at the country level [16].

However, resistance is not necessarily a persistent trait of pathogens and decreased resistance rates have been demonstrated following antibiotic withdrawal both at the individual and community level [17, 18]. Prolonged treatment to ensure clearing the infection, therefore, comes at the cost of providing more sustained periods over which resistant pathogens have a competitive advantage. This has led to a recent challenge in the dogma of always completing antibiotic courses [19]. For example, randomized controlled trials have shown that shorter treatment schedules for both hospital- and community-acquired pneumonia yield equivalent outcomes to longer courses, but with fewer infection recurrences and reduced rates of antibiotic resistance [20-22]. Understanding the patient-level temporality of resistance emergence and waning thereby offers important insight into prescriptive practice.

Systematic reviews and meta-analyses have provided useful indication of this temporality. Costelloe *et al*. investigated subsequent antibiotic resistance in individuals prescribed antibiotics in primary care, showing a 2.5 increase in odds of resistance within two months of treatment for urinary tract infections, which waned to 1.3 within 12 months [15]. However, among those treated for respiratory tract infections, the odds of antibiotic resistance remained 2.4 times higher (compared to those not treated with antibiotics) over the whole year [15]. Bakhit *et al*. pooled analyses across bacterial species instead of infection site, showing a 4.2

increase in odds of resistance after receiving penicillin-class treatment for *Streptococcus pneumoniae* within the first week post-treatment waning to a 1.7 increase in odds after 1 month [14]. A similar trend was found for cephalosporin-class treatment of this pathogen: 2.2 increase in odds within the first week waning to 1.6 increase in odds after 1 month [14].

To further refine the temporal dynamics of patient-level resistance emergence and waning, here, the odds of antibiotic resistance are modelled over time using a dose-response meta-analysis (DRMA) framework which incorporates time since antibiotic exposure as a continuous variable [23, 24]. This has the benefit over fixed time intervals (as done in previous meta-analyses) by reducing information loss thus reducing the risk of distorting exposure-outcome relationships [25, 26]. The aim of this study was to examine the relationship between different antibiotic therapies and the emergence of antibiotic resistant pathogens over time. To achieve this aim, the meta-analysis conducted by Bakhit *et al*. [14] was updated and the data re-analysed using a DRMA [24].

Methods

The foundation of this study is the systematic review and meta-analysis conducted by Bakhit *et al*. [14] from which the eligibility criteria were adopted along with part of the risk of bias assessment and the included studies. This study was reported according to the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) [27] (checklist available in the Supplementary material S1).

Study search and selection

The study search was updated with a forward citation search using Elsevier's Scopus [28] on 31 October 2019. The basis for the forward citation search consisted of the primary studies included in Bakhit *et al.* [14] and Costelloe *et al.* [15] meta-analyses. No limits were applied to the study search. In case of ambiguities regarding study eligibility at any stage, LY was consulted.

Studies were included if they met the following eligibility criteria: (i) randomized controlled trials (RCTs), quasi-experimental pre-post studies, or prospective cohort studies, (ii) compared patients treated with antibiotics versus controls (i.e. not treated or prior to treatment with antibiotics), (iii) patients were treated in the community or had

community-acquired infections, (iv) patients received an antibiotic therapy of any class, or combination of classes, for a maximum duration of 14 days, and (v) that reported the prevalence or incidence of resistant bacteria among patients, isolates, or specimens over time.

Case reports and conference abstracts were excluded. Studies including patients with hospital-associated, device-related, or persistent infections were excluded; in addition, studies with antibiotic therapies longer than 14 days were excluded.

Data extraction

The data extraction was done by MG and LFK using an Excel spreadsheet, in case of discrepancies LY was consulted. The following items were extracted from the studies: Authors and year of publication, patient characteristics (e.g. symptomatic or asymptomatic patients, age, proportion of females), study characteristics (e.g. study design, recruitment location, duration of study/follow-up), antibiotic exposure (e.g. antibiotic class, duration of antibiotic therapy), and bacterial infection (e.g. type of bacteria, number antibiotic resistant isolates at different time-points).

Some studies reported their case counts relative to the total included patients (pathogen carriers and non-carriers) and others to the respective pathogen carriers. Here, only data from participants carrying pathogens were retained in order to describe the burden of resistance among those with infections. In addition, some studies provided data for resistance against multiple antibiotic classes after treatment, but only studies reporting resistance to the treatment antibiotic (so called primary resistance) were retained.

All antibiotic drugs were classified according to their respective chemical structure. Combined treatments were classified by their active agent in case of an antibiotic and non-antibiotic combination (e.g. amoxicillin-clavulanate classified as beta-lactam). An antibiotic combination was treated as its own class. Studies that were randomized by design but had data extracted from each arm separately were reclassified as “prospective repeated measures cohort studies” (more details in table “study characteristics”), as proposed by Bakhit *et al.* [14].

Risk of bias assessment

The risk of bias assessment was performed by MG using the Cochrane Risk of Bias tool 2.0 (RoB 2) [29] for RCTs and ROBINS-I [30] for non-randomised studies. To evaluate the risk of bias for cohort studies and longitudinal data, the adapted version developed by Bakhit *et al.* [14] of the ROBINS-I for three domains, confounding, missing data, and outcomes was used (S2).

Statistical analyses

The odds ratios (ORs) for carrying resistant bacteria over time were modelled to investigate the temporal relationship between antibiotic intake and resistance. The ORs were estimated as the ratio between the odds of antibiotic resistance at different time points compared to the odds of antibiotic resistance at baseline – i.e. prior to antibiotic therapy or in the control group.

The antibiotic resistance and time data (as a continuous variable) were re-analysed using a robust error meta-regression method (REMR) [31] for DRMA rather than pooling ORs within time categories as in previous meta-analysis [14]. Time was calculated as the difference in days between the start of the antibiotic treatment (day "0") and the resistance measurement. The median was used for time points that were reported as ranges (e.g. 28 to 30 days). For studies specifying measurement time points as "x days after the end of therapy", the time period was added to the therapy duration. To avoid bias, the analysis was additionally sorted by the treatment duration. The REMR method does not require knowledge of the correlation structure of the data within a study, because it stacks included effects as a cluster by study and uses the cluster-robust analysis to obtain a robust standard error, thus treating observations as independent across clusters but correlated within each cluster. Given the results reported in previous meta-analysis, the relationship of resistance over time was not likely to be linear so the REMR DRMA was fitted with a restricted cubic spline (RCS) with 3 knots. The number of knots was decided by assessing the fit of the model through the mean squared error and the R-squared. The DRMAs were run using the *remr* module [32] in Stata SE version 14, Stata Corp, College Station, TX, USA.

The REMR DRMA used time since first antibiotic as a proxy for 'dose' thus producing output that shows how resistance risk increases and then decreases following drug treatment. Fitting these temporal changes in resistance risk to probability distributions enabled estimates for

how risk cumulates over different time spans. We made no *a priori* assumption of the distribution shape and instead fit a range of probability distributions (using a Python library called Reliability [33]) and selected the best fit. These fitted distributions normalise the risk of transmitting resistant pathogens (i.e. ensured the area under the curve summed to one). Knowing how resistance emergence changed over time allowed estimation of how different patient isolation scenarios reduced the risk that a transmitted pathogen was resistant. The first, 'naïve' scenario measured the duration of isolation required to halve risk that a transmitted pathogen was resistant assuming that isolation was initiated from the first day of treatment. The alternative, 'targeted' scenario measured the duration of isolation needed to equivalently impact risk when the isolation window prioritised peak resistance levels.

Results

Yield of search strategy

The forward citation search identified a total of 2173 unique records. The title and abstract screening resulted in the exclusion of 2112 records, and 61 articles were included for the full-text screening. An additional 10 articles were identified by hand search, adding up to 71 full-text articles for screening, of which 16 arti**cles were deemed eligible.**

Bakhit *et al.* [14] included 26 articles, of which one exceeded the maximum therapy of 14 days and was excluded from our study. Therefore, there were a total of 41 [13, 34-72] articles, reporting findings from 35 different studies. At least 10 data points are required for each DRMA (i.e. combination of organism and antibiotic). Studies involving *S. pneumoniae* resistant to either beta-lactams or macrolides, met this requirement and 13 studies [35, 36, 38-52] (n=11049 participants) were included in the analysis (Figure 1).

Study characteristics

The study, patients, and treatment characteristics of the retrieved studies are reported in Table 1. The studies included between 58 and 4782 participants and the study duration ranged between 14 and 180 days. Of the included studies, three were RCTs and ten were prospective cohorts. Nine studies reported data on children, two on adults, and two studies included children and adults as participants. The symptom status of their patients was reported as symptomatic by seven studies, as asymptomatic by three studies, two studies reported on symptomatic and

asymptomatic patients, and one study did not report the symptom status of their patients. All 13 studies reported on respiratory samples. Eleven studies reported the guideline they used to determine the susceptibility and resistance levels for bacteria. Among these 13 studies that examined *S. pneumoniae,* eight studies administered beta-lactam class antibiotics, eight used penicillin, and five studies reported macrolide class antibiotics. The therapy duration was 10 days for beta-lactam antibiotics and one day for macrolide antibiotics. The unit of analysis was at the patient in all of the studies.

Table 1

Quantitative analysis

Eight studies [35, 36, 38-41, 50, 51] (n=3101) reported a total 34 primary resistance data points on beta-lactam antibiotics in *S. pneumoniae* with a maximum follow-up of 60 days and a therapy duration of 10 days. The relationship between resistance to beta-lactams in *S. pneumoniae* and days post-exposure revealed a 3.32-fold increase in odds (95%CI 1.71 - 6.46) of resistance at day 14 followed by a steady decrease to pre-exposure level on day 40 (OR 0.98; 95%CI 0.55 - 1.75) (Figure 2A and Supplementary material S3).

Eight studies [35, 36, 38-41, 50, 51] (n=3101) reported a total 27 primary resistance data points for penicillin treatment of *S. pneumoniae* with a maximum follow-up of 60 days and a therapy duration of 10 days. The results showed a 4.82-fold increase in odds (95%CI 2.57 - 9.01) in resistance at day 14 which steadily decreased to a pre-exposure level on day 40 (OR 0.72, 95%CI 0.41 - 1.25) (Figure 2B and S4). The results for primary resistance data on macrolide antibiotics in *S. pneumoniae* showed a similar trend (see S5), but with greater uncertainty.

Targeting surveillance of resistant pathogens

Consistent patterns across studies emerged from the dose-response analyses whereby odds of resistance increased to a maximum level on day 14 for the beta-lactams (Figure 2). Macrolide treatment studies were also consistent but had peak resistance occurring much later, between days ~30 and 60 (see S5). Knowledge of these temporalities could be used to inform strategically timed sampling to improve estimates of resistance incidence and prevalence. Potentially, this information could also contribute towards temporally targeted isolation of patients with the goal of reducing the risk that transmitted pathogens are drug resistant. The

relative reduction in risk that a transmitted pathogen is resistant when isolating patients from the first day of treatment was compared with isolation during the period in which the odds of resistance was found to be highest in the meta-analysis. Both scenarios for beta-lactams (including sub-group of penicillin) are shown in Figure 3 (For macrolide treatments of *S. pneumoniae* see S5).

Relative to a 'naïve' approach, a targeted approach reduced the isolation time by about one-third for beta-lactam treatments (requiring isolation from day 9-19 instead of from day 0-15), and by 12.5 days for macrolide treatments of *S. pneumoniae*.

Conclusions

Antibiotic resistance incurs a huge and growing toll in terms of morbidity, mortality and societal costs [73]. Previous studies have provided evidence of non-linear temporal trends in the emergence of resistance among patients following exposure to antibiotics [14, 15]. Using a novel meta-analytical approach [31] this study sought to refine our understanding of the temporality of resistance emergence and waning. After pooling the evidence from eight studies (n= 3101 participants) an increased risk of resistant *S. pneumoniae* among patients was found, peaking at day 14 for beta-lactams and for the penicillin sub-group (four days after treatment cessation). Evidence is shown for an eventual waning in resistance 30 days following cessation of the antibiotics course, corroborating findings from earlier studies [3, 17, 18].

Identifying consistent dynamics in resistance emergence and waning offers new opportunities for understanding the epidemiology of antibiotic resistance. Surveillance is crucial for tracking resistance spread and in targeting its control. It is one of the five strategic priorities of the Global Action Plan (GAP) on antimicrobial resistance [74], and research on resistance is dominated by surveillance reports [75]. A recent report from the Interagency Coordination Group on Antimicrobial Resistance [76] describes several ways in which surveillance can support efforts to reduce antimicrobial resistance: improve detection of the emergence and prevalence of antimicrobial resistance; help guide patient treatment; identify populations at risk; inform policy development; assess the impact of interventions. Hence, identifying the precise window when patients are most likely to have detectable resistant pathogens improves their detectability and can potentially assist with all these key features of resistance surveillance.

A more refined understanding of patient-level resistance dynamics also provides new opportunities for strategizing interventions. Stewardship has been the primary means of combatting the spread of resistance, and, while it has proven effective in some settings [77, 78] only a quarter of studies included in a systematic review of interventions to change prescriptive practices in hospitals showed evidence of decreased resistance as a result [79]. Alternative strategies for combatting resistance are needed and temporally targeted isolation windows may comprise a novel approach. Transmission-based precautions often require physical patient isolation which may include single-room isolation, an entire isolation ward, or cohorting of a group of patients [80]. Owing to its exaggerated expense, this infection prevention and control strategy is normally reserved for patients infected with multidrug-resistant microorganisms to limit nosocomial transmission to other patients or to healthcare workers [81]. Resistant pathogen transmission risk is a compound of several factors including pathogen burden and patient behaviour. Our new findings add a new layer of understanding of how the transmission risk of resistant pathogens changes over the course of infection. Future work should explore combining these factors to inform resistant-infection prevention and control strategies. If predictable temporal dynamics of resistance risk among patients could be exploited to reduce the time required to isolate patients, this would not only reduce costs associated with isolation but the many adverse impacts that isolation are reported to have [82] including on patient mental health [83]. Since most patients with *S. pneumoniae* infections are not hospitalized, it is possible that the reduction of transmission risk could occur by mask use or "social distancing" during the period of greatest risk.

Strengths of this study include the novel statistical approach which allowed for time to be treated as a continuous variable instead of being categorized (e.g., before vs after, or intervention vs control). This meant that the multiple, longitudinal observations per study could be capitalized upon more effectively for analysis [26, 84]. Limitations of this study include the fact that extracted data from the reviewed studies were insufficient to analyze pathogens other than *S. pneumoniae* and this analysis was restricted to a single antibiotic class with two subclasses. It was also not possible to assess the differences between high- and low-dose of antibiotics, or between adults and children.

This study identified consistent temporal dynamics in the emergence and waning of drug-resistance for specific drug-pathogen combinations. Acknowledging the shortfall in the development of new drugs, the World Health Organization recently reiterated the critical importance of alternative infection control strategies [74]. Implications of predictable dynamics extend beyond improved targeting for future surveillance and highlight a potential novel strategy of temporally optimized patient isolation to reduce transmission of resistant pathogens. Future work will explore alternative data sources beyond published research (e.g. hospital records) to investigate the generalizability of the new methods and results presented here to other pathogen-drug class combinations.

NOTES

Author contributions

LY conceived the study. LY and LFK directed the study's implementation, designed the analytical strategy and helped to interpret the findings. MG led the literature review, the analysis, the risk of bias analysis and helped to prepare the text. All authors contributed to results interpretations and manuscript writing.

Data availability

All data were obtained freely from previously published articles.

Ethics approval

No ethical clearance was needed for this publication because all data were previously published and were anonymised.

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Conflict of interest

DLP reports contracts or grants unrelated to this work and paid to institution from Shionogi, Merck, and Pfizer; consulting fees paid to author from Spero Therapeutics, Merck, AMR Action Fund, and QPex; and payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events, paid to author, from Pfizer, BioMerieux, and Merck. None other declared.

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Table 1: Characteristics of Included Studies - Design and Patients

Author & Year of Publication	Country	N	Design	Female Proportion	Age Group / Symptoms	Study Duration	Sample Site	MoM Resistance / Guideline	Pathogen Examined	Antibiotic Treatment	Treatment Duration	Unit of Analysis
Chern et al. (1999) [46]	Nepal / PED	122	RCT	n.a.	child / SaAS	14	respiratory	Etest / n.a.	*S. pneumoniae*	azithromycin	1	patient
Cohen et al. (1999) [38]	France / PED	513	COS-RT	n.a.	child / S	42	respiratory	agar / NCCLS	*S. pneumoniae*	amoxicillin-clavulanate	10	patient
Conradi et al. (2007) [39]	Spain / hER	134	COS	0.48	child / S	44	respiratory	agar / NCCLS	*S. pneumoniae*	amoxicillin	10	patient
Dabernat, H. (1998) [40]	France / PED & ENT	426	COS-RT	0.46	child / S	40	respiratory	disk / NCCLS/EUCAST	*S. pneumoniae*	cefixime, co-amoxiclav	10, 10	patient
Ghaffar et al. (1999)** [41-43]	USA / PED	160	COS-RT	0.45	child / SaAS	60	respiratory	Etest & disk / NCCLS	*S. pneumoniae*	amoxicillin-clavulanate	10	patient
Schrag et al. (2001) [50]	Dom.Rep. / hOC	795	COS-RT	0.45	child / S	28	respiratory	Etest / NCCLS	*S. pneumoniae*	amoxicillin	10	patient
Toltzis et al. (2005)** [51, 52]	USA 05 / PED	1009	COS-RT	n.a.	child / S	30	respiratory	Etest / NCCLS	*S. pneumoniae*	amoxicillin	10	patient
Batt et al. (2003)* [44]	Tanzania / V	4782	COS	0.56	child / n.a.	180	respiratory	Etest / n.a.	*S. pneumoniae*	azithromycin	1	patient
Brook et al. (2005)* [35]	USA / OC	58	COS	0.34	adult / S	14	respiratory	broth / NCCLS	*S. pneumoniae*	amoxicillin, amoxicillin-clavulanate	10, 10	patient
Brook and Gober (2004)* [36]	USA / n.a.	60	COS	0.28	child / S	14	respiratory	broth / NCCLS	*S. pneumoniae*	amoxicillin-clavulanate	10	patient
Burr et al. (2014)*	Gambia / V	417	COS	0.5	child&adult /	180	respiratory	Etest / CLSI	*S. pneumoniae*	azithromycin	1	patient

[45]					AS[38]								
Guchev et al. (2004)* [47, 48]	Russia / M or Vol	1798	RCT	0	child&adult / AS	154	respiratory	broth / NCCLS	*S. pneumoniae*	azithromycin	1	patient
Roca et al.(2016)* [49]	Gambia / hCC	829	RCT	1	adult / AS	28	respiratory	disk & Etest / CLSI	*S. pneumoniae*	azithromycin	1	patient

*Studies retrieved by the study update

**Articles collated into a single study

PED: Paediatric clinics; PEP: Paediatric practice; hOC: Hospital outpatient clinic; GP: General practices; S: School; OC: Outpatient clinic; hCC: Health care centre; hER: Hospital emergency department; V: Villages; Vol: Volunteers; PC: Primary care; ENT: Ear, nose and throat;

RCT: Randomised-controlled trials; RT Randomised trial; COS Prospective cohort study design; COS-C with a control group; COS-RT: COS nested in RT;

S: Symptomatic; AS: Asymptomatic; SaAS: Symptomatic and asymptomatic;

MoM: Method of Measurement; agar: Agar dilution; disk: Disk diffusion; etest: E Test, paper: Paper disk testing; broth: Broth-dilution method, ASS: automated antimicrobial susceptibility testing systems)

NCCLS: NCCLS/CLSI the Clinical and Laboratory Standards Institute; EUCAST: European Committee on Antimicrobial Susceptibility Testing, N.A.: Not reported)

1

Figure 1: Flow chart for the study screening process

Figure 2: Odds (with 95% confidence intervals) of *S. pneumoniae* antibiotic resistance to A) beta-lactams and B) penicillin over time, as determined by REMR.

Figure 3: Strategically timed isolation of patients treated for *S. pneumoniae* infection can reduce the risk that transmitted pathogens are resistant. Alternative isolation windows are shown: time extending from first day of treatment ('naïve', black, solid line) or during windows of highest resistance risk as identified in the meta-analysis ('targeted', blue, broken line). The dotted lines denote the durations of isolation required to halve risk that transmitted *S. pneumoniae* is drug resistant under the alternative isolation windows.

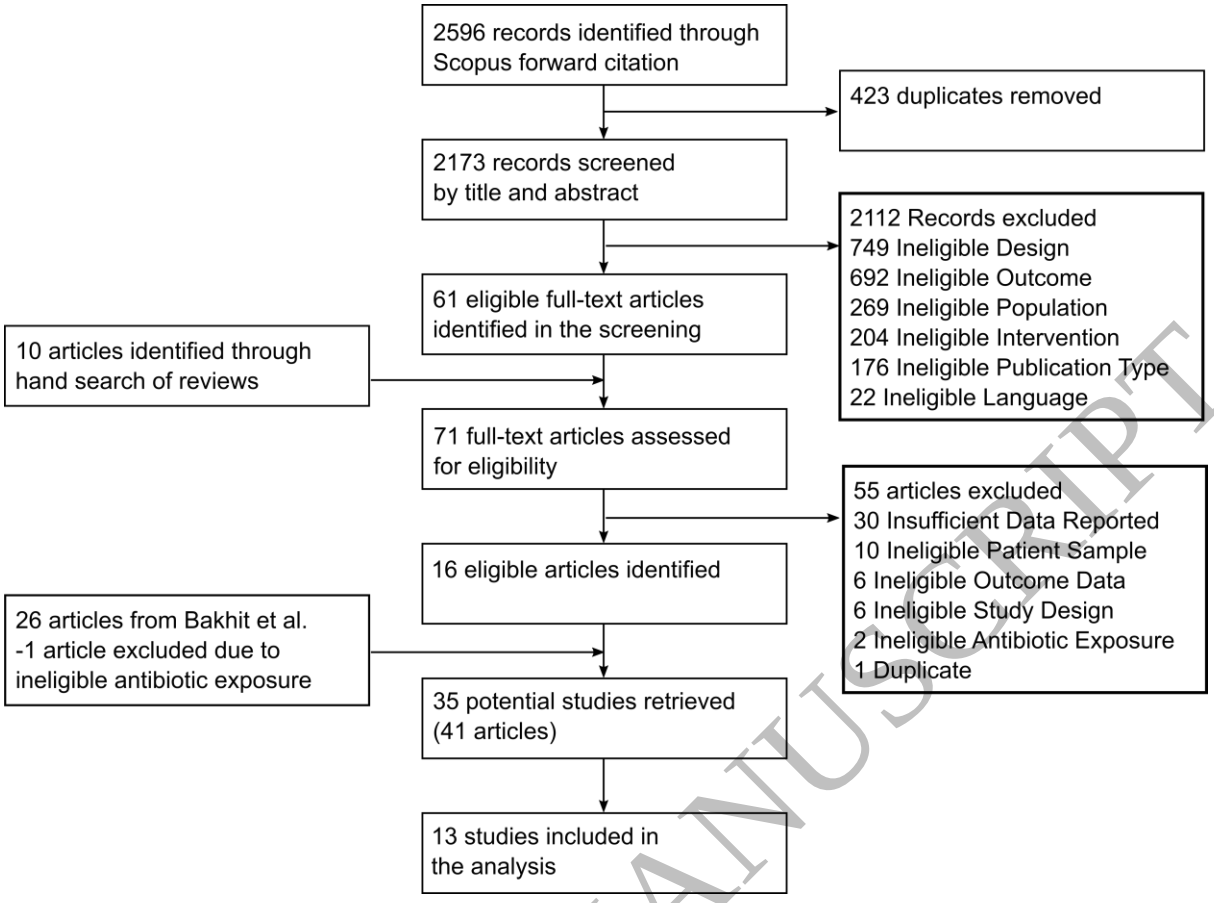

Figure 1

159x118 mm (8.8 x DPI)

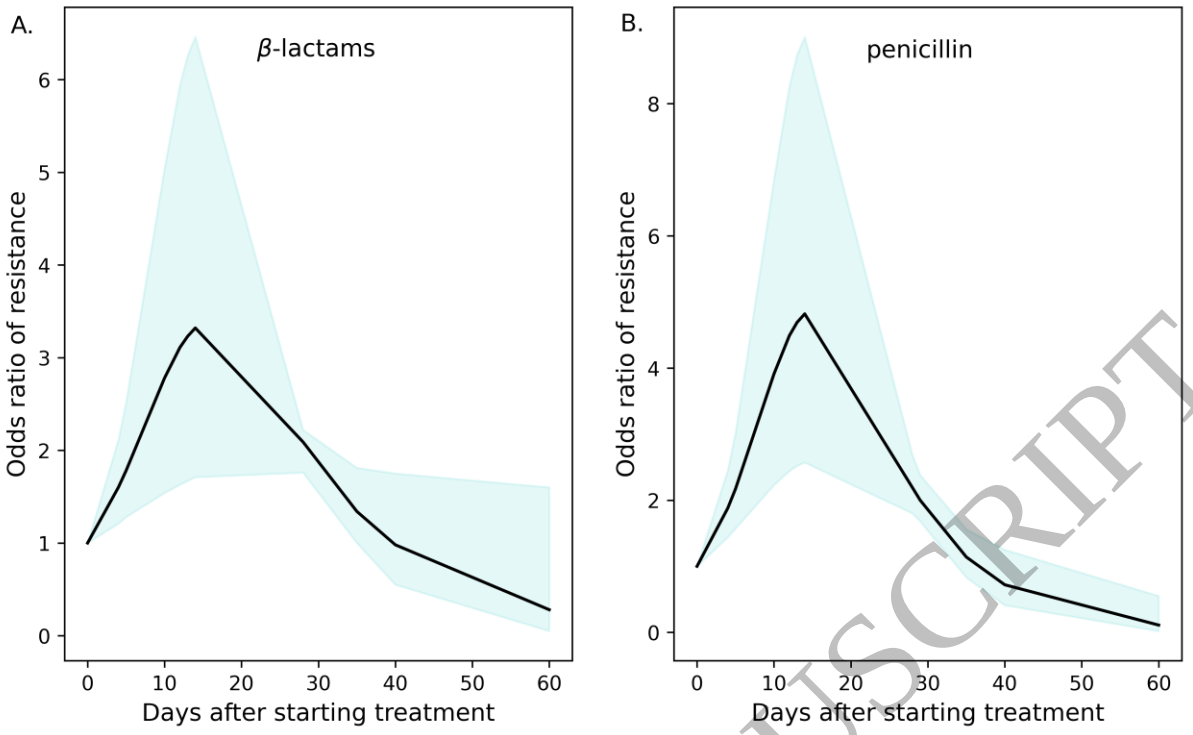

Figure 2

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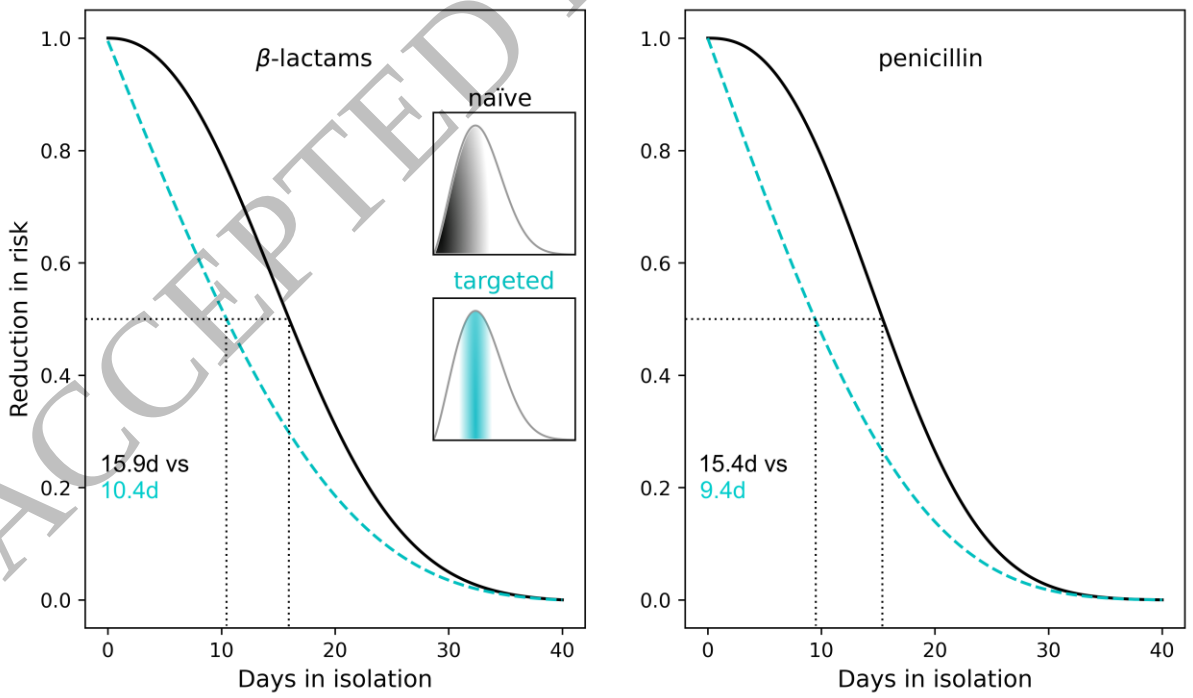
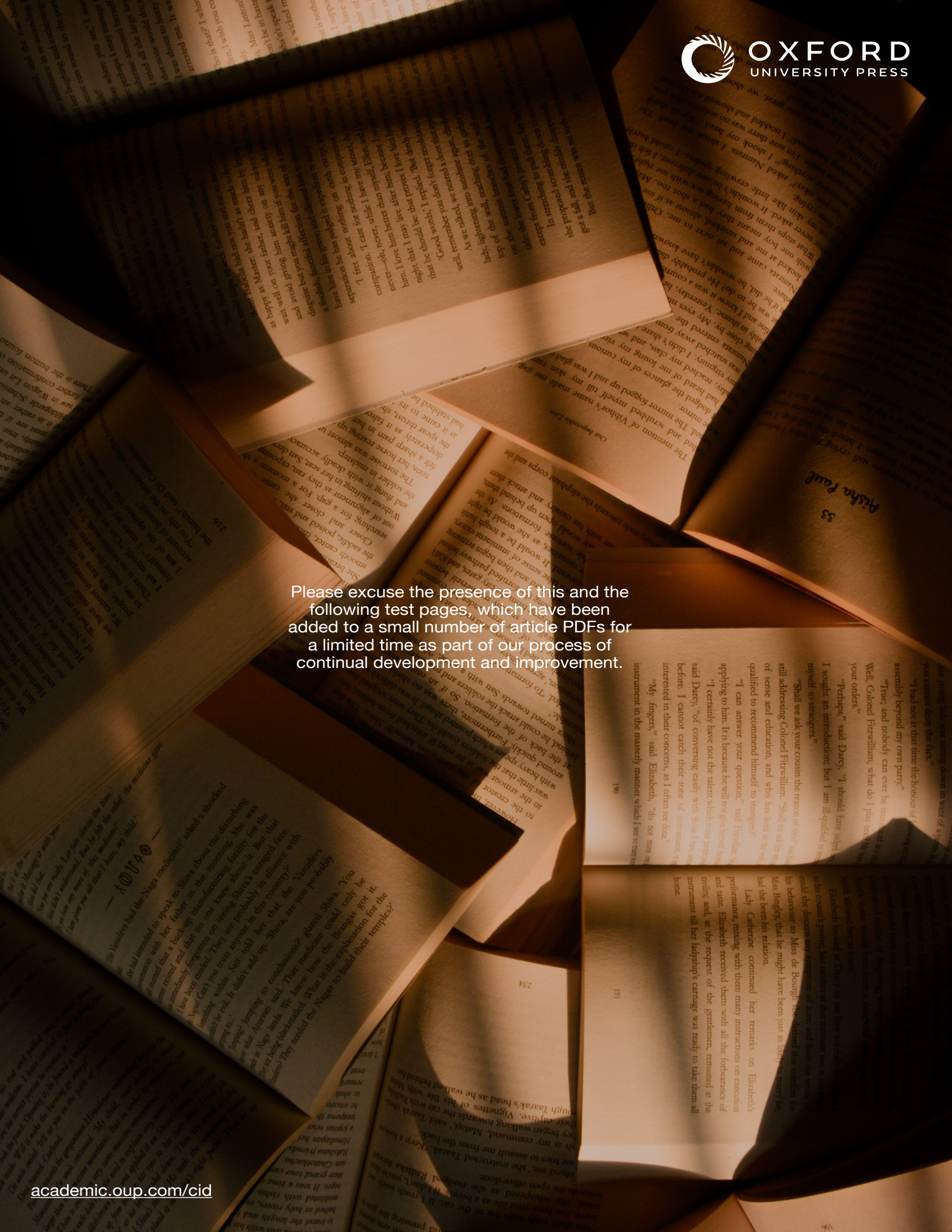


Figure 3

159x94 mm (8.8 x DPI)



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