Susceptibilities of clinical *Clostridium difficile* isolates to antimicrobials: a systematic review and meta-analysis of studies since 1980

Nagham Khanafer[1,2#], Nick Daneman[3], Tom Greene[4], Andrew Simor[3], Philippe Vanhems[1,2], Matthew Samore[4, 5], Kevin Antoine Brown[3, 4, 5, 6, #]

Author Affiliations: [1]Groupement hospitalier Edouard Herriot, Hospices civils de Lyon, Lyon, France; [2]Université Lyon 1, Lyon, France; [3]Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Canada; [4]Division of Epidemiology, University of Utah, Utah, USA; [5]Salt Lake City Veterans Affairs Health Care System; [6]Public Health Ontario, Ontario, Canada

Citation: Khanafer N, Daneman N, Greene T, et al. Susceptibilities of clinical Clostridium difficile isolates to antimicrobials: a systematic review and meta-analysis of studies since 1970. Clin Microbiol Infect **2018**; 24:110–117.

DOI:10.1016/j.cmi.2017.07.012.
PMID:28750918

Abstract

Objectives: Although exposure to antibiotics can cause *C. difficile* infection, certain antibiotics are used to treat *C. difficile*. The measurements of antimicrobial *Clostridium difficile* activity could help identifying antibiotic risk and emergent resistance. Here, we describe publication patterns relating to *C. difficile* susceptibilities and estimate minimum inhibitory concentrations (MIC) for antibiotic classes in the published literature between January, 1970 and June, 2014.

Methods: We queried PUBMED and EMBASE for studies reporting antibiotic *C. difficile* MIC in English or French. We used mixed-effects models in order to obtain pooled estimates of antibiotic class median MIC (MIC_{50}), 90th percentile of MIC (MIC_{90}), and MIC_{90}:MIC_{50} ratio.

Results: Our search identified 182 articles that met our inclusion criteria, of which 27 were retained for meta-analysis. Aminoglycosides (MIC_{50}: 120mg/L, 95%CI: 62-250), 3rd (MIC_{50}: 75mg/L, 95%CI: 39-130) and 2nd generation cephalosporins (MIC_{50}: 64mg/L, 95%CI: 27-140) had the least *C. difficile* activity. Rifamycins (MIC_{50}: 0.034mg/L, 95%CI: 0.012-0.099) and tetracyclines (MIC_{50}: 0.29mg/L, 95%CI: 0.054-1.7) had the highest level of activity. The activity of 3rd generation cephalosporins was more than 3 times lower than 1st generation agents (MIC_{50}: 19mg/L, 95%CI: 7.0-54). Time-trends in MIC_{50} were increasing for carbapenems (70% increase per 10-years) while decreasing for tetracyclines (51% decrease per 10-years).

Conclusions: We found a 3500-fold variation in antibiotic *C. difficile* MIC_{50}, with aminoglycosides as the least active agents and rifamycins as the most active. Further research is needed to determine how *in vitro* measures can help assess patient *C. difficile* risk and guide antimicrobial stewardship.

Introduction

Clostridium difficile is responsible for substantial morbidity and mortality among people receiving antibiotics in hospitals and in the community. Epidemiologic studies have attempted to classify the differential risks of antibiotic exposures and have found that clindamycin, cephalosporins and fluoroquinolones are associated with the highest incidence of *C. difficile* infection (CDI), while tetracyclines represent the lowest risk antibiotics, and longer courses are associated with higher CDI risk [1-3]. In addition to depleting protective gut flora, one factor that drives antibiotic-associated CDI risk is the lack of activity that these antibiotics have against *C. difficile* bacteria [4]. Some antibiotics, such as piperacillin-tazobactam, demonstrate delayed antibiotic associated effects due to suppression of *C. difficile*. It is possible that, on average, antibiotics with high anti-*C. difficile* activity produce lower *C. difficile* risk. However, quantitative measures of anti-*C. difficile* activity do not exist, which makes testing such hypotheses difficult at the moment.

Antibiotic activity is usually summarized by the minimum inhibitory concentration (MIC), the smallest concentration of an antimicrobial necessary to prevent the growth of a target microorganism. Many studies have reported antibiotic activity against *C. difficile* strains, showing that certain agents including antibiotics in the imidazole and glyocopeptide classes have high activity, while higher risk agents such as cephalosporins and fluoroquinolones have much lower activity against *C. difficile* [5, 6]. Studies considering time-trends in resistance levels have suggested that the emergence of fluoroquinolone resistant 027/NAP1/BI strains of *C. difficile* may be responsible for the increased incidence, morbidity and mortality associated with CDI [7]. However, many of these studies are specific to a given hospital or context.

No published study has ever attempted to compare and combine MIC values from studies around the world, as is frequently done for clinical studies [8, 9]. A meta-analysis of susceptibility data could provide novel insights by quantifying the degree of variation of estimates, the sources of variation, increasing precision of estimates, and, in particular, help identify common weaknesses in the published literature that could be improved.

Reliable information on *C. difficile* susceptibility to antibiotics could help uncover better treatment agents, describe emerging trends in resistance earlier than single studies, and, in particular, predict the magnitude and timing of *C. difficile* infection risk. In this study, we sought to describe the literature on the susceptibility of clinical *C. difficile* isolates to antibiotics and to assess the variation and predictors of susceptibility, with a specific interest in the role of antibiotic class [10].

Methods

Search strategy

We searched PUBMED (available since Jan 1, 1980) and EMBASE (available since Jan 1, 1970) in June 2014 without restriction in terms of language or year. Our search strategy involved combining terms describing our outcome (i.e. microbial sensitivity, microbial sensitivity test, and minimum inhibitory concentration) with terms for our exposure of interest (i.e. antibiotic, antibacterial, antimicrobial, aminoglyosides, beta-lactams, cephalosporins, clindamycin, fluoroquinolones, macrolides, metronidazole, sulfonamides, and tetracyclines) and terms describing our population (i.e., *C. difficile* or *Clostridium*

difficile). More detailed information on our search strategy can be found in the appendix. We searched reference sections and approached experts to help identify relevant missing articles. We included studies of human *C. difficile* strains (nosocomial or community-acquired) published in French or English. Studies of animal or environmental strains, or strains extracted from gut models were excluded.

Screening and data abstraction

One author (NK) screened articles and identified those appropriate for full text review. For each article that passed full text review, one author (KB) recorded characteristics of the study and design, the number of antibiotics assessed. All studies at this stage were included in a descriptive analysis of study characteristics. We restricted our MIC summary value abstraction to articles that reported at least 9 antibiotic MIC_{50} derived from a minimum of 30 *C. difficile* strains, and our meta-analysis was limited to classes with at least 10 MIC_{50} measurements.

Outcomes

For each antibiotic assessed within a given study, we abstracted the median MIC (MIC_{50}), the 90^{th} percentile (MIC_{90}), and calculated the ratio of MIC_{90} to MIC_{50} (a large dispersion between the MIC_{90} and MIC_{50}, as measured by the MIC_{90} to MIC_{50} ratio, indicates that a given bacteria may readily develop resistance to a given antibiotic) [11]. When studies did not report MIC_{50} or MIC_{90} directly, but instead reported the distribution of strains, we recorded the MIC_{50} and MIC_{90} as the lowest concentration where at least 50% or 90% of strains were inhibited. The values of MIC were in mg/L for results reported in this paper. The resulting dataset had a single record for each study antibiotic, and as such, multiple lines per study.

Variables

Antibiotics were classified into 14 groups: (1) aminoglycosides; (2) carbapenems; (3-5) 1^{st}, 2^{nd} and 3^{rd} generation cephalosporins; (6) fluoroquinolones; (7) glycopeptide; (8) imidazoles; (9) lincosamides; (10) macrolides; (11) broad-spectrum penicillins; (12) narrow-spectrum penicillins; (13) rifamycins; (14) tetracyclines. Antibiotics not fitting this classification were considered separately. The source of isolates was categorized as originating from a single hospital, a regional repository, or an international repository. For studies that pertained to single hospitals or regional repositories, we categorized the geographic source in terms of the continent where the strains were collected (Asia, North America, Europe, or Oceania). We abstracted the different antimicrobial sensitivity testing techniques (agar dilution, gradient strips, or broth dilution) as reported in the included papers and the year the study was published.

Meta-analyses

In order to describe patterns of antibiotic susceptibility, we developed mixed-effects linear regression models with the logarithm of MIC_{50}, MIC_{90}, and MIC_{50}:MIC_{90} ratio as the outcomes (N = 19 x 3). Antibiotic-class specific models (N = 15 x 3) were used to measure the mean antibiotic MIC50 and MIC90 for each class. Each model included a fixed effect for the intercept and a random effect for study to control for study level effects. Antibiotic-class agnostic models (N = 1 x 3) were created in order to understand the relative importance of inter-class, inter-antibiotic, and inter-study variation. These sources of variation were quantified as the proportion of total variance accounted for by the given random effect, also known as the variance partition coefficient (Austin and Merlo, 2017; DOI: 10.1002/sim.7336). Due to our interest in the emergence of resistance to *C. difficile* treatment agents, we developed analogous models (N = 3 x 3) for metronidazole, vancomycin and fidaxomicin. Each model included just the intercept term (no random effect for antibiotic was needed since there was only a single antibiotic within

each model). Each model included a fixed effect for the intercept and a random effect for antibiotic class, antibiotic, and study. For each MIC_{50} model (N = 19 + 1 + 3), we explored factors explaining heterogeneity by creating two more models, one with an additional covariate for study year and one with an additional covariate for study continent.

Analyses were conducted in R; the lme4 package was used for fitting mixed-effects models and to estimate bootstrapped confidence intervals for parameter estimates [12]. P-values for fixed effects parameters were estimated using the Satterthwaite approximation for degrees of freedom using the lmerTest package. Data used for the meta-analysis are publicly available on FigShare (http://dx.doi.org/10.6084/m9.figshare.5131750).

Results

Studies

We identified 366 articles in PUBMED and 1172 articles in EMBASE meeting our database search inclusion criteria, with 240 duplicate items, leaving us with a total 1289 articles after the initial database search (Figure 1). Title and abstract screening eliminated 944 articles, leaving 345 articles for the full-text review. Of these, 182 articles met the study inclusion criteria (Figure 1).

The pace of publication increased through the period, from 12 articles published in the 1980 to 1984 period (2.4 per year), to 64 articles published in the 2010 to March 2014 period (14.8 per year). The number of antibiotics investigated per study decreased, from an average of 11.8 antibiotics per study to an average of 6.8 antibiotics per study for the periods described above. The number of isolates per study increased from 39.1 per study to an average of 216.8 per study.

Of the 182 studies meeting the criteria for the descriptive analyses, 27 (15%) articles met the inclusion criteria for the meta-analysis, 9 articles from the 1980s, 2 articles from the 1990s, 5 from the 2000s, and 11 since 2010. Strains originated from Asia (10 articles), North America (8 articles), Europe (7 articles), Oceania (1 article), and a single study based on an international isolate repository.

A total of 409 MIC_{50} and MIC_{90} associated with 104 unique antibiotics were extracted (mean 3.9 susceptibility measures per antibiotic). The most commonly reported antibiotics were fluoroquinolones (N=16), broad-spectrum penicillins (N=11) and 2nd (N=8) and 3rd (N=8) generation cephalosporins (Table 1). Of the 104 antibiotics identified, 49 had MIC_{50} and MIC_{90} values from at least 2 studies (Table 2).

Antibiotic MICs

High MICs (mean $MIC_{50} \geq 8$ mg/L) were observed for aminoglycosides, cephalosporins, lincosamides, and fluoroquinolones (Figure 2, Table 1 and Table 2). Low MICs (mean $MIC_{50} < 1$ mg/L) were observed for glycopeptide, imidazoles, tetracyclines, and rifamycins. The antibiotic classes with the highest MIC_{90}:MIC_{50} ratios were rifamycins (92 mg/L, 95%CI: 9.1-1100), macrolides (9.0 mg/L, 95%CI: 1.8-53), and lincosamides (8.2 mg/L, 95%CI: 1.3, 51).

When we built a model for all antibiotics that included random effects corresponding to antibiotic classes, specific antibiotics, and study. The model estimated that antibiotic classes explained 65.9% of the overall variance of MIC_{50}, while specific antibiotics within classes explained an additional 16.7% of variance. Study-level random-effects explained 2.9% of variance. The remaining variance (14.5%) was unexplained. Similarly, MIC_{90} were largely explained by antibiotic classes (54.1% of variance), antibiotics (17.9% of variance), and study (5.3% of variance). The remaining variance (22.7%) was unexplained.

Time-trends, regions, and testing method

In analyses of time-trends in MIC_{50} stratified by antibiotic class, carbapenems displayed an increasing trend (70% increase per 10 year increment, 95%CI: 30%-220%) and tetracyclines demonstrated a decreasing trend (51% decrease per 10 year increment, 95%CI: 2.7%-73%) of susceptibility. In analyses of continent-level differences in MIC_{50} stratified by antibiotic classes, we observed regional differences in susceptibility to tetracyclines (p=0.02), broad-spectrum penicillins (p=0.04), and fluoroquinolones (p=0.06). Specifically, North American studies demonstrated the highest MICs for tetracyclines (mean MIC_{50}: 1.4 mg/L, 95%CI: 0.19-10) and fluoroquinolones (mean MIC_{50}: 14 mg/L, 95%CI: 5.4-38), while European countries had the highest MIC_{50} for broad-spectrum penicillins (mean MIC_{50}: 4.9 mg/L, 95%CI: 1.7-14).

Across all antibiotic classes combined, we identified no relationship between MIC_{50} and year of study publication (1.0-fold change per 10 years, 95%CI: 0.8-1.2 mg/L), study continent (p=0.71), and testing method (p=0.38).

When we conducted analyses for metronidazole alone (N=22 studies, mean MIC_{50} = 0.44 mg/L), we observed no time-trends in MIC_{50} (slope: 1.0-fold [null] change per 10 year increase, 95%CI: 0.8-1.3 mg/L) though we did observe that studies from North America reported higher MIC_{50} (2.2-fold higher than other study continents, 95%CI: 1.1-4.3 mg/L). For vancomycin (N=17, mean MIC_{50} = 0.73 mg/L), we observed no relationship with either time (slope: 0.8-fold change per 10 year increase, 95%CI: 0.7-1.0 mg/L) or study continent (p=0.75). We identified only 3 studies for fidaxomicin (mean MIC_{50} = 0.27 mg/L) and as such were unable to perform statistical tests for time-trend or regional variation.

Discussion

The emergence of the epidemic strain 027 in the early years of the new millennium, as a result of widespread fluoroquinolone use, has brought more attention to active antimicrobial susceptibility surveillance of *C. difficile* bacteria. In this systematic review and meta-analysis of the published literature on *C. difficile* antimicrobial sensitivity between 1970 and 2014, we found: (1) an increasing trend of publishing on *C. difficile* susceptibility since the late 2000s, (2) that *C. difficile* was least susceptible to aminoglycosides, cephalosporins, and lincosamides, and most susceptible to rifamycins, imidazoles, and glycopeptide, and (3) a large heterogeneity in susceptibility for rifamycins, macrolides, and lincosamides. The remainder of the discussion is organized according to antimicrobial class.

Anaerobic bacteria are naturally resistant to aminoglycosides because they lack the oxygen-dependent transport mechanism required for cellular uptake of this family of antibiotics [13]. The high MIC_{50} values we observed, and the low heterogeneity of MIC_{50} values, confirmed the universality of this resistance

mechanism. Despite elevated MIC_{50}, aminoglycosides have not frequently been associated with higher risk of CDI, which may be due to a lack of impact on other protective anaerobic gut flora.

Carbapenems are generally thought to have good activity against anaerobes; but the estimated MIC_{50} showed a wide variability in their in vitro activities with meropenem and doripenem having excellent activity while imipenem had low activity. These discrepancies in susceptibility may be, in part, explained by antibiotic pressure since imipenem is frequently prescribed. Several authors have described resistance to imipenem in PCR ribotypes 046 (100%), 014/020 (50%) and 002 (45%), however, resistance to meropenem has not been documented [14, 15].

Cephalosporins are a group of broad spectrum, semi-synthetic beta-lactam antibiotics with the same mechanism of action as penicillins and are known to have a high propensity to precipitate CDI due to their major disturbance of the colonic microflora and low anti-*C. difficile* activity [16]. Successive generations of cephalosporins have both increasing Gram-negative activity with decreasing activity against Gram-positive organisms, which explains the trend of decreasing *C. difficile* activity levels from the 1st to the 3rd generation. Nevertheless, there was some variability among analyzed studies. Karlowsky et al. described a high rate of resistance to ceftriaxone among isolates of NAP2 [17]. However, Samore et al. detected a lower prevalence of resistance which may be explained by the close correspondence between genotypic strain grouping and phenotypic characteristics or by the use of different methods to test susceptibility [18]. Our results showed that the activity of narrow-spectrum penicillins was substantially higher (MIC_{50}: 1.5 mg/L) compared to that of broad spectrum penicillins (MIC_{50}: 4.9 mg/L). The observed values may be explained by the common use of penicillins in clinical practice [19-21]. A 15-year study of isolates from Sweden permitted to detect decreasing *C. difficile* activity for common β-lactam agents that correlated with the emergence of resistant strains, including ribotype 012 [5].

Susceptibility *of C. difficile* to fluoroquinolones has always been low, particularly for 1st and 2nd generation agents. Resistance to ciprofloxacin was observed in all included papers while resistance to moxifloxacin varied between 2% and 82% [6, 22]. The risk thought to be associated with moxifloxacin usage has been attributed to its extended anti-anaerobe spectrum and, thus, its propensity to disrupt a major part of the colonic flora [23]. The epidemic strain 027 is associated with universal high-level resistance to the fluoroquinolones and clindamycin, in contrast to that of the 027 isolates predating 2001. Noren et al. reported that resistance to moxifloxacin increased from 2% in 1993, before the drug was marketed, to 4–23% among isolates from 2004 to 2007 [5]. Numerous studies has documented a relationship between strain and fluoroquinolone susceptibility [5, 6, 17, 22, 24]. Levels of fluoroquinolone-resistant *C. difficile* are higher among patients with previous fluoroquinolone use [25].

Taken together glycopeptide (vancomycin, teicoplanin and ramoplanin) had high *C. difficile* activity (MIC_{50}: 0.31 mg/L, MIC_{90}: 0.50 mg/L) but with an MIC_{90} of just 0.5 mg/L lower than the breakpoint [26]. Teicoplanin, in particular, had the lowest MIC_{90} (0.31 mg/L). Teicoplanin was reported as superior to vancomycin and metronidazole for initial bacteriologic response but its use for CDI treatment is limited by lack of availability in many countries and great cost relative to the other options [27].

Included papers showed low MICs with respect to imidazoles, which were largely represented by metronidazole. However, the highest MIC_{90} reported at 16 mg/L was far higher than the breakpoint of ≤2 mg/L recommended by the European Committee on Antimicrobial Susceptibility. This study by Snydman et al. reported that approximately one quarter of their stains had elevated MICs to metronidazole but MICs

were calculated on resistant strains to underscore the activity of a new antibiotic [28]. The elevated MICs observed in his study could cause therapeutic concern, especially considering the low gut concentrations of metronidazole. Such resistance to metronidazole has been described in vitro but the clinical relevance of this is unknown. Some authors have described cases of reduced susceptibility, especially for PCR ribotype 001, and heteroresistant populations to metronidazole [29]. Further, it should be noted that heteroresistance to metronidazole can be lost in frozen strains when they are studied immediately after thawing, which may impact many of the MIC measurements in this review [5].

In our study, lincosamides were mainly represented by clindamycin, one of the antibiotics most associated with CDI. Clindamycin is active against anaerobic bacteria; however, *C. difficile* is frequently resistant to clindamycin as shown by the high MIC observed in our study. The variability could be explained by the difference among genotypes and/or toxigenicity. The NAP1 strain seems susceptible, while NAP2 is highly resistant to clindamycin [6, 17]. A reduced susceptibility was described among the following PCR ribotypes: 014/020, 017, 078, 012 and 126. Interestingly, in one study, a low proportion of PCR ribotype 002 strains showed resistance to clindamycin [16]. Resistance to clindamycin seems also correlated with previous exposure to this antibiotic. A plausible explanation for this association is that, during the period of antibiotic treatment, there is selective pressure against clindamycin-susceptible strains due to antimicrobial activity within the gastrointestinal tract [18, 30-32]. In one study resistance was restricted to strains isolated in diarrheic patients but this finding must be interpreted with caution given that asymptomatic patients were mainly infants [31]. Others have claimed that resistant strains were more frequent in elderly than in young people [33]. This association could be simply correlated with increasing incidence of CDI in elderly adults usually sick and frequently exposed to antibiotics.

Macrolides have a similar spectrum of activity to that of lincosamides. Cross resistance between clindamycin and macrolides is well described and is most likely due to the presence of *ermB* genes (erythromycin ribosomal methylase B) [15, 34]. Roberts et al. described that 95% of strains were susceptible to clarithromycin whereas 85% of strains tested by Bourgeault et al. were resistant. The resistance was less frequent in strains other than NAP1 and NAP2 [6, 22]. Samore et al. reported that all isolates that were clindamycin resistant were also erythromycin resistant and vice versa [18]. This close correspondence between genotypic strain grouping and resistance to macrolides, clindamycin and fluoroquinolones is well described in the literature [6, 15, 35]. Erythromycin resistance was found mainly with isolates producing binary toxin, especially those belonging to ribotype 078. Furthermore, ribotype 001 has been associated with resistance to both erythromycin and fluoroquinolones [24]. As such, although macrolides are generally considered to be associated with an intermediate risk for CDI, that risk may be heterogeneous [7].

Rifamycins were the most active agents in vitro, inhibiting *C. difficile* strains at very low concentrations. However, our results showed a wide range of susceptibility with elevated MIC_{90}. For rifampicin, the *C. difficile* isolates were either highly susceptible or highly resistant [5, 35]. Resistance may explain the poor results in the past studies that have used rifampicin for CDI treatment. Analysis of drug cross-resistance revealed an association between resistance of clindamycin, erythromycin and rifampicin especially in strains belonged to PCR ribotype 012 [5]. Increased MICs for rifampicin with co-resistance to fluoroquinolones seems frequent in isolates of PCR ribotype 017 [14, 36]. This resistance is based on coexisting amino acid substitutions in Gyr A and RpoB, the b subunit of RNA polymerase [37]. Rifamycins have been tested for treatment of CDI relapse, but rapid development of resistance suggests that this approach is likely to fail [5].

The activity of tetracyclines was generally high, with 90% of isolates displaying MICs ≤2.3 mg/L, however variability in MIC was also high. Resistance to tetracycline has been observed in both historic and recent isolates. The resistance to tetracycline is usually due to the presence of a *tet*(M) gene carried by the conjugative transposon Tn5397 [37]. Noren et al. reported that the numbers of highly resistant isolates doubled between 2004 and 2007 [5]. A high proportion of resistance was described among PCR ribotypes 046, 012, 017 and 078 [14]. Moreover, a certain correlation could be found between toxigenicity and tetracycline sensitivity [33]. Tigecycline had a low MIC (MIC_{50}: 0.05 mg/L and MIC_{90}: 0.10 mg/L) which may explain the variability of susceptibility among this family of antibiotics. Tigecycline has been suggested as a possible alternative to treat severe and/or refractory CDI [38].

This study has a number of limitations. First, not all studies reporting *C. difficile* MICs could be analyzed, due to the sheer quantity of studies; we chose to analyze studies with selected criteria, due to the increased statistical reliability, however this approach may have biased the activity levels upwards since small, outbreak-specific strains may have been underrepresented. Second, measurement of the antimicrobial activity of *C. difficile* depends on the methodologies and breakpoints used and may fluctuate from one laboratory to another; we attempted to account for this by recording the type of testing methods used and found no differences between laboratory methods. Third, the MICs we report did not take into consideration the concentrations of antibiotic achieved in the gut, nor the pharmacodynamics, and as such cannot be directly translated into measures of risk related to CDI or potential utility for CDI treatment. Finally, because studies do not report isolate-level results, our analysis was limited to MIC_{50}, MIC_{90}, and MIC_{90}:MIC_{50} values across groups of *C. difficile* strains of diverse composition.

Our study identified over 180 articles reporting on *C. difficile* susceptibility testing and our analysis of a subset of these studies has demonstrated that *C. difficile* was least susceptible to aminoglycosides, cephalosporins, and lincosamides, and most susceptible to rifamycins, imidazoles, and glycopeptides; decreasing susceptibility to carbapenems and regional variations in susceptibility were observed. While the impact of antibiotics against commensal gut flora is likely a primary driver of antibiotic-associated *C. difficile* risk, we believe that other characteristics of the antibiotic could play a role, including the route of administration, pharmacokinetics, and, as summarized here, anti-*C. difficile activity*. Surveillance of time-trends and regional patterns in *C. difficile* susceptibility are important for monitoring the evolution of resistance and understanding the epidemiology of *C. difficile.*

Funding and Transparency statement

This study was funded through the Veterans Affairs Fellowship Program in Medical Informatics and a Canadian Institute for Health Research Doctoral Fellowship. The funders had no role in the design, analysis, writing, or decision to submit this manuscript.

No authors have conflicts related to this work. However, several authors have received funding for work not related to this manuscript. AS received speaking fees from Cubist pharmaceuticals and Merck Canada. KAB received grant funds from AstraZeneca. NK reports a PhD grant from Sanofi Pasteur, non-financial support (travel expenses) from Alere and Sanofi Pasteur and personal fees from Astellas grants. PV reports grants and personal fees from Sanofi Pasteur, Biomérieux, GSK, Astellas and Sanofi MSD. TG is a consultant for Jansen Pharmaceuticals, and received grant funds from Keryx biopharmaceuticals, Genkyotex, and Pharmalink. MS and ND report no conflicts.

Author contributions

NK and KAB designed the study. NK and KAB extracted the data. KAB and TG analyzed the results. NK and KB drafted the manuscript. ND, AS, TG, PV, and MS provided feedback on drafts of the manuscript.

References

[1] Brown KA, Khanafer N, Daneman N, Fisman DN. Meta-analysis of antibiotics and the risk of community-associated *Clostridium difficile* infection. Antimicrobial agents and chemotherapy 2013;57(5):2326–32.

[2] Slimings C, Riley TV. Antibiotics and hospital-acquired *Clostridium difficile* infection: update of systematic review and meta-analysis. The Journal of antimicrobial chemotherapy 2014;69(4):881–91.

[3] Stevens V, Dumyati G, Fine LS, Fisher SG, van Wijngaarden E. Cumulative antibiotic exposures over time and the risk of *Clostridium difficile* infection. Clinical infectious diseases 2011;53(1): 42–8.

[4] Owens RC, Jr., Donskey CJ, Gaynes RP, Loo VG, Muto CA. Antimicrobial-associated risk factors for *Clostridium difficile* infection. Clinical infectious diseases 2008;46 Suppl 1:S19–31.

[5] Noren T, Alriksson I, Akerlund T, Burman LG, Unemo M. In vitro susceptibility to 17 antimicrobials of clinical *Clostridium difficile* isolates collected in 1993-2007 in Sweden. Clinical microbiology and infection 2010;16(8):1104–10.

[6] Bourgault AM, Lamothe F, Loo VG, Poirier L, Group C-CS. In vitro susceptibility of *Clostridium difficile* clinical isolates from a multi-institutional outbreak in Southern Quebec, Canada. Antimicrobial agents and chemotherapy 2006;50(10):3473–5.

[7] Pepin J, Saheb N, Coulombe MA, Alary ME, Corriveau MP, Authier S, et al. Emergence of fluoroquinolones as the predominant risk factor for *Clostridium difficile*-associated diarrhea: a cohort study during an epidemic in Quebec. Clinical infectious diseases 2005;41(9):1254–60.

[8] Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000;283(15):2008–12.

[9] Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Annals of internal medicine 2009;151(4):264–9.

[10] Turnidge J, Paterson DL. Setting and revising antibacterial susceptibility breakpoints. Clinical microbiology reviews 2007;20(3):391–408.

[11] Higgins PG, Coleman K, Amyes SG. Bactericidal and bacteriostatic activity of gemifloxacin against Acinetobacter spp. in vitro. The Journal of antimicrobial chemotherapy 2000;45 Suppl 1:71–7.

[12] Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MH, et al. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in ecology & evolution 2009;24(3):127–35.

[13] Baron S. Medical microbiology. 4th ed. Texas: Galveston, Tex: University of Texas, Medical Branch at Galveston, 1996.

[14] Pirs T, Avbersek J, Zdovc I, Krt B, Andlovic A, Lejko-Zupanc T, et al. Antimicrobial susceptibility of animal and human isolates of *Clostridium difficile* by broth microdilution. Journal of medical microbiology 2013;62(Pt 9):1478–85.

[15] John R, Brazier JS. Antimicrobial susceptibility of polymerase chain reaction ribotypes of *Clostridium difficile* commonly isolated from symptomatic hospital patients in the UK. The Journal of hospital infection 2005;61(1):11–4.

[16] Foye WO, Lemke TL, Williams DA. Foye's principles of medicinal chemistry. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins, 2013.

[17] Karlowsky JA, Zhanel GG, Hammond GW, Rubinstein E, Wylie J, Du T, et al. Multidrug-resistant North American pulsotype 2 *Clostridium difficile* was the predominant toxigenic hospital-acquired strain in the province of Manitoba, Canada, in 2006-2007. Journal of medical microbiology 2012;61(Pt 5):693–700.

[18] Samore MH, Venkataraman L, DeGirolami PC, Merrigan MM, Johnson S, Gerding DN, et al. Genotypic and phenotypic analysis of *Clostridium difficile* correlated with previous antibiotic exposure. Microbial drug resistance 2006;12(1):23–8.

[19] Versporten A, Coenen S, Adriaenssens N, Muller A, Minalu G, Faes C, et al. European Surveillance of Antimicrobial Consumption (ESAC): outpatient penicillin use in Europe (1997-2009). The Journal of antimicrobial chemotherapy 2011;66 Suppl 6: vi13–23.

[20] Currie CJ, Berni E, Jenkins-Jones S, Poole CD, Ouwens M, Driessen S, et al. Antibiotic treatment failure in four common infections in UK primary care 1991-2012: longitudinal analysis. BMJ 2014;349: g5493.

[21] Van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, Laxminarayan R. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. The Lancet Infectious diseases 2014;14(8):742–50.

[22] Roberts S, Heffernan H, Al Anbuky N, Pope C, Paviour S, Camp T, Swager T. Molecular epidemiology and susceptibility profiles of *Clostridium difficile* in New Zealand, 2009. The New Zealand medical journal 2011;124(1332):45–51.

[23] Deshpande A, Pant C, Jain A, Fraser TG, Rolston DD. Do fluoroquinolones predispose patients to *Clostridium difficile* associated disease? A review of the evidence. Current medical research and opinion 2008;24(2):329–33.

[24] Weber I, Riera E, Deniz C, Perez JL, Oliver A, Mena A. Molecular epidemiology and resistance profiles of *Clostridium difficile* in a tertiary care hospital in Spain. International journal of medical microbiology 2013;303(3):128–33.

[25] Novak A, Spigaglia P, Barbanti F, Goic-Barisic I, Tonkic M. First clinical and microbiological characterization of *Clostridium difficile* infection in a Croatian University Hospital. Anaerobe 2014;30:18–23.

[26] The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Available at: www.eucast.org.

[27] Nelson RL, Kelsey P, Leeman H, Meardon N, Patel H, Paul K, et al. Antibiotic treatment for *Clostridium difficile*-associated diarrhea in adults. The Cochrane database of systematic reviews 2011;(9): CD004610.

[28] Snydman DR, Jacobus NV, McDermott LA. Activity of a novel cyclic lipopeptide, CB-183,315, against resistant *Clostridium difficile* and other Gram-positive aerobic and anaerobic intestinal pathogens. Antimicrobial agents and chemotherapy 2012;56(6):3448–52.

[29] Baines SD, O'Connor R, Freeman J, et al. Emergence of reduced susceptibility to metronidazole in *Clostridium difficile*. The Journal of antimicrobial chemotherapy 2008;62(5):1046–52.

[30] Dzink J, Bartlett JG. In vitro susceptibility of *Clostridium difficile* isolates from patients with antibiotic-associated diarrhea or colitis. Antimicrobial agents and chemotherapy 1980;17(4):695–8.

[31] Shuttleworth R, Taylor M, Jones DM. Antimicrobial susceptibilities of *Clostridium difficile*. Journal of clinical pathology 1980;33(10):1002–5.

[32] Kim J, Kang JO, Pai H, Choi TY. Association between PCR ribotypes and antimicrobial susceptibility among *Clostridium difficile* isolates from healthcare-associated infections in South Korea. International journal of antimicrobial agents 2012;40(1):24–9.

[33] Nakamura S, Nakashio S, Mikawa M, Yamakawa K, Okumura S, Nishida S. Antimicrobial susceptibility of *Clostridium difficile* from different sources. Microbiology and immunology 1982;26(1):25–30.

[34] Tang-Feldman YJ, Henderson JP, Ackermann G, Feldman SS, Bedley M, Silva J, Jr., Cohen SH. Prevalence of the ermB gene in *Clostridium difficile* strains isolated at a university teaching hospital from 1987 through 1998. Clinical infectious diseases 2005;40(10):1537–40.

[35] Wust J, Hardegger U. Studies on the resistance of *Clostridium difficile* to antimicrobial agents. Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene 1988;267(3):383–94.

[36] Huang H, Weintraub A, Fang H, Wu S, Zhang Y, Nord CE. Antimicrobial susceptibility and heteroresistance in Chinese *Clostridium difficile* strains. Anaerobe 2010;16(6):633–5.

[37] Spigaglia P, Barbanti F, Mastrantonio P, European Study Group on *Clostridium difficile*. Multidrug resistance in European *Clostridium difficile* clinical isolates. The Journal of antimicrobial chemotherapy 2011;66(10):2227–34.

[38] Herpers BL, Vlaminckx B, Burkhardt O, Blom H, Biemond-Moeniralam HS, Hornef M, et al. Intravenous tigecycline as adjunctive or alternative therapy for severe refractory *Clostridium difficile* infection. Clinical infectious diseases 2009;48(12):1732–5.

Table 1. Estimated antibiotic class MIC_{50}, MIC_{90}, and MIC_{90}:MIC_{50} ratio for *C. difficile* bacteria

	Antibiotics (N)	**N[a]**	**MIC_{50} Estimate (95%CI)**	**MIC_{90} Estimate (95%CI)**	**MIC_{90}:MIC_{50} Estimate (95%CI)[b]**
Aminoglycosides	4	10	120 (62-250)	200 (78-490)	1.5 (1.2-1.9)
Carbapenems	5	26	3.5 (1.8-8.1)	6.3 (2.4-15)	1.8 (1.3-2.5)
Cephalosporins					
1st Generation	4	12	19 (7.0-54)	51 (22-120)	2.3 (1.1-4.3)
2nd Generation	8	31	64 (27-140)†	130 (76-260)	2.0 (1.4-3.1)
3rd Generation	8	31	75 (39-130)	130 (93-180)	1.8 (1.3-2.4)
Fluoroquinolones	16	51	8.0 (3.1-19)†	26 (13-51)	3.2 (2.0-5.5)
Glycopeptide	3	28	0.31 (0.11-0.95)	0.50 (0.15-1.9)	1.7 (1.2-2.4)
Imidazoles	3	29	0.33 (0.18-0.57)	0.79 (0.24-2.6)	2.0 (1.5-2.8)
Lincosamides	2	28	18 (2.3-130)†	120 (44-350)	11 (4.6-28)
Macrolides	5	15	4.5 (1.2-19)	46 (11-180)	9.0 (1.8-53)
Penicillins					
Broad Spectrum	11	54	4.9 (1.6-14)‡	12 (4.6-36)‡	2.5 (1.6-3.7)
Narrow Spectrum	4	17	1.5 (0.35-8.0)‡	5.0 (1.6-19)	2.8 (1.8-4.7)
Rifamycins	4	12	0.034 (0.012-0.099)	3.9 (0.34-45)	92 (9.1-1100)
Tetracyclines	3	19	0.29 (0.054-1.7)†	2.2 (0.050-62)‡	8.2 (1.3-51)‡
Other	24	52	7.4 (2.5-27)‡	14 (4.8-43)‡	1.9 (1.4-2.7)

All table values are rounded to 2 significant digits, † medium inter-antibiotic heterogeneity (inter-antibiotic standard deviation: 3-fold to 3.9-fold), ‡ high inter-antibiotic heterogeneity (inter-antibiotic standard deviation: 4-fold or more), [a]note that a single study could contribute multiple MIC_{50} measures (e.g. for fluoroquinolones the average study contributed 51/21 = 2.4 MIC_{50} and MIC_{90} measures), [b]MIC_{90}:MIC_{50} is the ratio of MIC_{90} to MIC_{50} is a measure of the dispersion of the distribution of MICs for a given antibiotic.

Table 2a. Estimated antibiotic MIC_{50}, MIC_{90}, and MIC_{90}:MIC_{50} ratio for *C. difficile* bacteria for antibiotics with at least 2 observations (N=66 antibiotics)

Antibiotic	**N**	**MIC_{50} Estimate (95%CI)**	**MIC_{90} Estimate (95%CI)**	**MIC_{90}:MIC_{50} Estimate (95%CI)**
Aminoglycosides				
Gentamicin	5	100 (54,290)	130 (61,650)	1.5 (1.2,2.0)
Neomycin	2	120 (50,340)	180 (57,860)	1.5 (1.1,2.0)
Streptomycin	2	150 (47,320)	330 (49,900)	1.5 (1.1,2.1)
Carbapenems				
Doripenem	2	2.5 (1.1,17)	3.9 (1.3,28)	1.8 (1.2,2.8)
Imipenem	12	7.3 (0.90,16)	14 (1.4,29)	1.8 (1.3,2.5)
Meropenem	10	1.6 (1.1,15)	2.7 (1.1,32)	1.8 (1.2,2.5)
Cephalosporins				
1st Generation				
Cefalexin	5	37 (4.3,76)	51 (23,120)	2.2 (1.1,4.8)
Cefazolin	2	18 (4.1,86)	51 (23,120)	2.2 (0.82,5.9)
Cephalothin	4	15 (4.9,78)	51 (23,120)	2.3 (1.1,5.2)
2nd Generation				
Cefaclor	2	35 (8.6,480)	130 (43,430)	2.8 (1.0,4.5)
Cefamandole	3	18 (7.2,510)	91 (38,540)	3.4 (0.97,4.6)
Cefmetazole	2	26 (8.5,490)	81 (35,490)	2.4 (0.94,4.1)
Cefotetan	3	28 (9.7,480)	53 (40,530)	1.8 (1.0,4.2)
Cefotiam	2	220 (8.2,500)	270 (40,570)	1.7 (0.99,4.2)
Cefoxitin	13	100 (8.6,440)	150 (34,530)	1.5 (0.91,5.0)
Cefuroxime	5	270 (7.6,630)	290 (39,560)	1.4 (0.90,4.6)
3rd Generation				
Cefmenoxime	2	46 (16,340)	120 (77,210)	1.8 (1.2,2.7)
Cefoperazone	4	30 (19,310)	120 (74,200)	1.8 (1.2,2.6)
Cefotaxime	8	99 (16,320)	130 (81,210)	1.8 (1.2,2.7)
Cefsulodin	2	150 (17,330)	130 (77,200)	1.8 (1.2,2.7)
Ceftazidime	2	66 (17,340)	130 (69,210)	1.8 (1.2,2.5)
Ceftizoxime	3	270 (14,290)	130 (74,210)	1.8 (1.2,2.7)
Ceftriaxone	6	36 (19,370)	120 (81,200)	1.8 (1.2,2.7)
Moxalactam	4	76 (17,340)	130 (80,220)	1.8 (1.2,2.7)
Fluoroquinolones				
Ciprofloxacin	13	22 (0.64,100)	39 (3.6,140)	1.9 (1.1,12)
Gatifloxacin	2	8.0 (1.0,90)	35 (5.5,150)	4.0 (0.99,10)
Gemifloxacin	2	3.3 (0.68,120)	29 (6.7,140)	6.1 (1.1,11)
Levofloxacin	7	16 (0.64,92)	49 (4.8,150)	3.3 (1.1,11)
Moxifloxacin	12	2.9 (0.39,89)	25 (4.7,150)	7.7 (1.0,11)
Nalidixic acid	3	64 (0.61,75)	84 (5.3,140)	2.0 (1.1,10)
Nemonoxacin	2	1.7 (0.88,110)	12 (4.1,150)	4.6 (1.0,8.7)
Trovafloxacin	2	5.8 (0.82,66)	41 (4.9,160)	5.6 (1.1,9.1)
Glycopeptides				
Teicoplanin	4	0.16 (0.069,1.5)	0.19 (0.060,3.8)	1.5 (1.0,2.9)
Vancomycin	23	0.68 (0.067,1.5)	1.3 (0.075,4.3)	1.8 (1.1,2.6)

Table 2b. Estimated antibiotic MIC_{50}, MIC_{90}, and MIC_{90}:MIC_{50} ratio for *C. difficile* bacteria for antibiotics with at least 2 observations (N=66 antibiotics)

Antibiotic	**N**	**MIC_{50} Estimate (95%CI)**	**MIC_{90} Estimate (95%CI)**	**MIC_{90}:MIC_{50} Estimate (95%CI)**
Imidazoles				
Metronidazole	27	0.33 (0.24,0.46)	0.66 (0.15,3.2)	2.0 (1.7,2.4)
Lincosamides				
Clindamycin	25	8.2 (1.6,210)	110 (43,290)	11 (5.4,23)
Lincomycin	3	40 (1.4,390)	140 (29,720)	11 (2.2,62)
Macrolides				
Clarithromycin	2	4.5 (0.69,34)	46 (5.0,410)	7.6 (1.0,130)
Erythromycin	10	4.5 (1.4,19)	46 (12,150)	11 (1.8,55)
Penicillins				
Broad Spectrum				
Amoxicillin/Clavulanate	5	0.46 (0.20,110)	0.95 (0.58,270)	2.0 (0.87,7.0)
Ampicillin	11	1.0 (0.19,88)	2.9 (0.66,310)	2.7 (0.83,7.6)
Ampicillin/Sulbactam	2	2.2 (0.20,110)	3.6 (0.54,250)	1.9 (0.83,6.4)
Carbenicillin	4	14 (0.16,130)	28 (0.48,190)	2.1 (0.88,6.8)
Mezlocillin	2	3.0 (0.15,110)	15 (0.53,320)	3.8 (0.80,7.8)
Piperacillin	10	5.7 (0.16,180)	13 (0.53,370)	2.4 (0.89,8.1)
Piperacillin/Tazobactam	10	4.8 (0.20,140)	7.5 (0.41,300)	1.7 (0.80,7.8)
Ticarcillin	2	14 (0.17,110)	37 (0.56,280)	2.7 (0.90,7.0)
Narrow Spectrum				
Benzylpenicillin	2	1.4 (0.089,24)	3.5 (0.57,38)	2.8 (1.4,6.3)
Meticillin	2	8.9 (0.088,31)	16 (0.77,30)	2.8 (1.5,6.1)
Penicillin	12	1.2 (0.088,25)	3.4 (0.71,35)	2.8 (1.9,4.2)
Rifamycins				
Rifampicin	7	0.057 (0.0080,0.14)	3.9 (0.33,54)	92 (7.4,980)
Rifaximin	3	0.026 (0.0070,0.14)	3.9 (0.23,73)	92 (4.4,2500)
Tetracyclines				
Minocycline	3	0.40 (0.013,4.1)	3.8 (0.0073,410)	8.1 (0.32,140)
Tetracycline	10	0.74 (0.027,4.7)	26 (0.0075,530)	25 (0.72,98)
Tigecycline	6	0.082 (0.024,4.1)	0.11 (0.0059,490)	2.7 (0.53,130)
Other				
Aztreonam	2	400 (0.044,1900)	420 (0.12,2000)	1.3 (0.65,6.0)
Bacitracin	3	73 (0.051,2200)	150 (0.079,4300)	2.0 (0.63,6.8)
Chloramphenicol	7	3.4 (0.023,1100)	24 (0.068,2000)	5.9 (0.50,5.8)
Cycloserine	2	120 (0.052,1100)	120 (0.089,2600)	1.3 (0.67,5.3)
Daptomycin	4	0.90 (0.049,820)	1.5 (0.078,2300)	1.7 (0.61,6.3)
Fidaxomicin	3	0.18 (0.070,2200)	0.29 (0.046,2400)	1.7 (0.62,5.2)
Fusidic acid	7	0.68 (0.036,1900)	1.1 (0.045,2400)	1.7 (0.69,6.3)
Linezolid	4	1.3 (0.046,940)	3.0 (0.079,2200)	2.2 (0.60,5.4)
Sulfamethoxazole	2	120 (0.064,1200)	170 (0.064,3300)	1.6 (0.71,5.5)
Trimethoprim	2	120 (0.043,1300)	170 (0.064,2100)	1.6 (0.63,4.6)
Trimethoprim/sulfamethoxazole	2	29 (0.040,2100)	59 (0.048,1800)	2.0 (0.63,5.1)

Figure 1. Flow chart of studies screened and included.

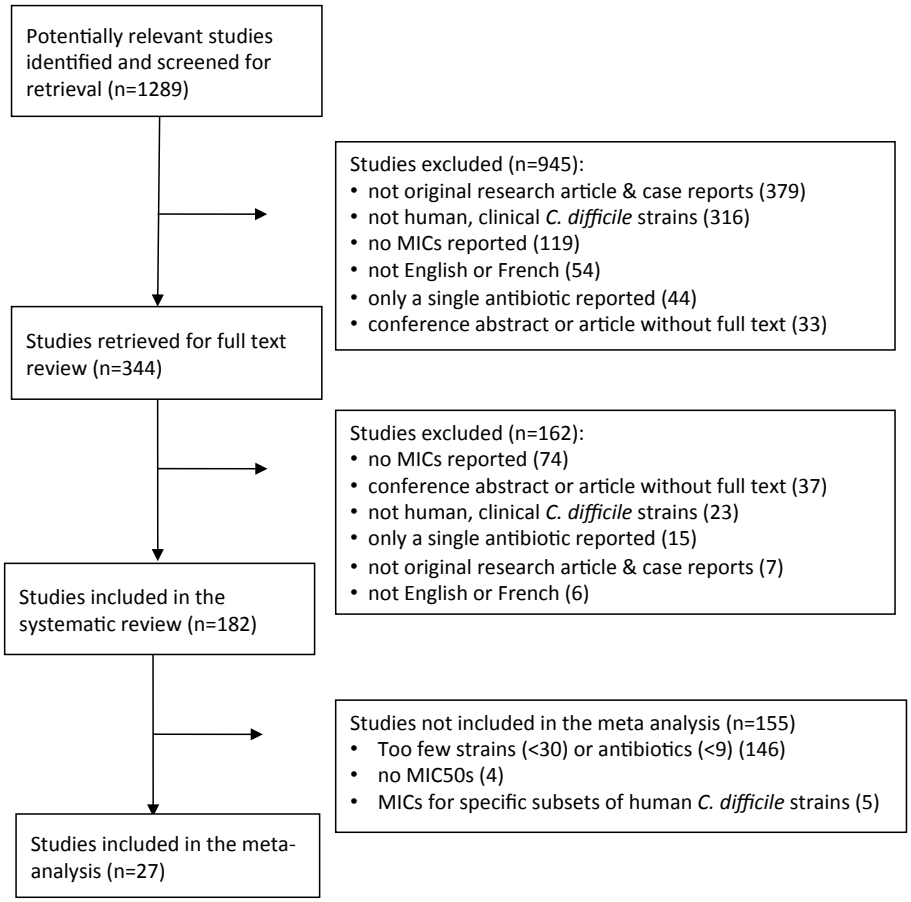
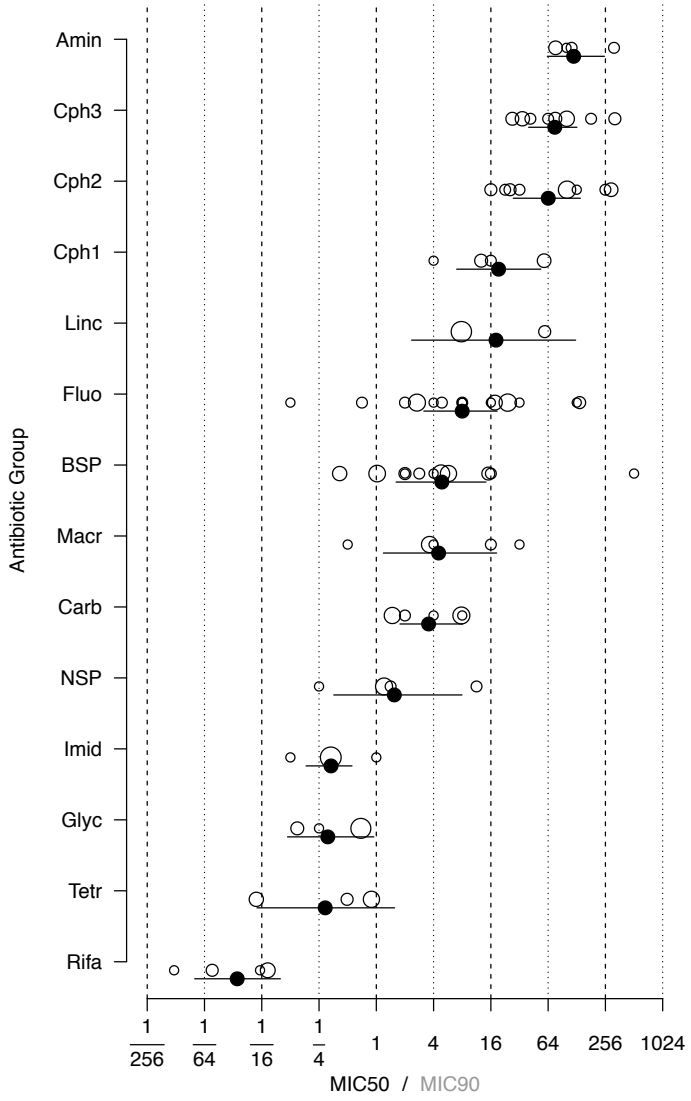


Figure 2. Mean MIC_{50} for antibiotics (open circles, size represents number of estimates) and antibiotic classes (N=15, closed circles, whiskers represent 95% confidence intervals).



Amin: aminoglycosides; BSP: broad spectrum penicillins; Carb: carbapenems; Cph1: 1st generation cephalosporins; Cph2: 2nd generation cephalosporins: Cph3, 3rd generation cephalosporins; Fluo: fluoroquinolones; Glyc: glycosamides; Imid: imidazoles; Linc: lincosamides; Macr: macrolides; NSP: narrow spectrum penicillins; Rifa: rifamycins; Tetra: tetracyclines.