Where are we with monoclonal antibodies for multidrug-resistant infections?

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Widespread antibiotic resistance threatens the continued efficacy of antimicrobial therapy based on small-molecule antibiotics. Infections caused by multidrug-resistant Gram-negative bacteria are particularly worrisome owing to the lack of antimicrobials retaining sufficient activity against these microorganisms. Despite the explosion in monoclonal antibody therapies that have been developed for oncologic and rheumatic indications, only three antibacterial monoclonal antibodies have been approved for clinical use. In the present review, the therapeutic potential of this drug class for treating multidrug-resistant infections is discussed, and considerations for the development of antibacterial monoclonal antibodies are presented. Finally, the state of development of monoclonal antibody therapies for some of the most problematic multidrug-resistant Gram-negative infections is summarized.

Introduction

Over the past decades, antimicrobial resistance has emerged as an important public health problem owing to the global dissemination of bacterial strains from different species with resistance to multiple antibiotic classes. A report from the Review on Antimicrobial Resistance, a study commissioned by the British government in 2014 to assess the global impact of antibiotic resistance, estimated that in 2050 antimicrobial resistance would result in 10 million deaths per year if current trends continue [1]. The results of this report are cause for concern given that the current number of global deaths due to antimicrobial resistance is ~700 000 and the current number of deaths due to all cancers is ~8.2 million [1]. In this context, it is clear that antimicrobial stewardship programs that optimize the use of existing antibiotics and the development of novel antibiotic classes should be promoted. However, the increased prevalence of multidrug-resistant infections and the paucity of novel antibiotics that have entered clinical use over the past four decades could warrant the development of non-antibiotic-based approaches for the treatment and prevention of certain resistant infections.

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Monoclonal antibodies have been used as therapeutics for >30 years since the approval of the first monoclonal antibody: Orthoclone, an anti-CD3 antibody used for preventing rejection in kidney transplant recipients [2]. Since then, >70 therapeutic monoclonal antibodies have been approved in Europe and the USA. Further evidence of the increased interest in this drug class is that a recent search of the clinicaltrials.gov registry for trials evaluating monoclonal antibodies revealed almost 900 trials that were either active or currently recruiting patients [3]. The majority of monoclonal antibodies approved or in clinical stages of development are for the treatment of rheumatic diseases and different types of cancers. By contrast, only three monoclonal antibodies have been approved for bacterial diseases. Raxibacumab and obiltoxaximab were approved for the prophylaxis or treatment of inhalational anthrax [4,5]; and in 2017 bezlotoxumab, an antibody directed against the *Clostridium difficile* toxin B, was approved for the prevention of recurrent infections in high-risk patients [6]. The large difference between the numerous antibodies approved or in development for rheumatic and oncologic indications and the small number of monoclonal antibodies for bacterial infections is surprising given that it has long been know that immune response and specifically antibodies induced either through natural infection or immunization can provide protection against

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https://doi.org/10.1016/j.drudis.2019.03.002

bacterial infection. Although multiple factors contribute to the paucity of monoclonal antibodies for bacterial diseases, the most likely might be the success of small-molecule-based antibiotic therapy over the preceding century, which has obviated the need for developing alternative approaches. In the current context of widespread antimicrobial resistance, monoclonal-antibody-based approaches could provide a viable alternative for the treatment of certain multidrug-resistant infections. This could be especially true for certain infections caused by multidrug-resistant Gram-negative infections for which traditional antibiotics no longer have sufficient activity. In the present review, potential benefits and disadvantages of monoclonal-antibody-based therapies for multidrug-resistant infections are outlined and considerations for promoting the development of these therapies are discussed. In addition, salient examples of monoclonal antibodies that are currently being developed for these problematic infections are presented.

Therapeutic potential of monoclonal antibodies

There are multiple characteristics of monoclonal antibodies that could make them well suited for the treatment of antibiotic-resistant infections, particularly infections caused by multidrug-resistant Gram-negative species. First, antibacterial monoclonal antibodies typically target surface-exposed antigens or secreted toxins that are not targets of currently used antimicrobials. For this reason, the efficacy of monoclonal antibodies is unlikely to be affected by existing resistance mechanisms. By contrast, different antibiotics that act on the same bacterial targets can be affected by cross resistance, which occurs when the development of resistance to one antibiotic decreases susceptibility to a different antibiotic. Second, monoclonal antibodies are thought to have minimal effect on non-target bacterial species, such as those that make up the normal bacterial flora. This is in stark contrast to antibiotics, especially broad-spectrum antibiotics, which have been shown to have significant impact on the composition of the bacteria residing in the host in humans and animals [7–9]. In light of numerous studies over the previous decade indicating that the human microbiome plays a part in regulating multiple physiologic processes and diseases, such as neurologic, cardiovascular and respiratory disease [10], antimicrobial therapeutics with a very narrow spectrum of activity might be desirable for certain infections. A final consideration is that monoclonal antibodies could also contribute to reducing the emergence of antimicrobial resistance. The use of antibacterial monoclonal antibodies has potential to reduce the need for prescribing traditional small-molecule antibiotics, which could result in less selective pressure for the emergence of resistant strains. Studies demonstrating that reducing antibiotic prescribing correlates with decreased resistance rates support this idea [11–13]. Additionally, as mentioned above, monoclonal antibodies do not affect non-target bacteria present within the host. Monoclonal-antibody-based therapy would thus not produce selective pressure on the normal human flora, which might be of importance in light of studies demonstrating that this flora can serve as a reservoir for genetic determinants of resistance [14–16].

Although there are potential advantages associated with the use of antibacterial monoclonal antibodies, there are important limitations. Owing to the high specificity of monoclonal antibodies for their target pathogen, the bacterial species producing infection should ideally be identified before initiation of therapy. This aspect is especially relevant in serious infections in hospitalized patients, often produced by multidrug-resistant Gram-negative microorganisms. Upon clinical suspicion of infection these patients typically receive empiric antimicrobial therapy before the results of microbiologic testing are available. Empiric therapy is often based on broad-spectrum antibiotics that provide coverage against the multiple bacterial species that produce infection in these settings [17,18]. For these reasons, the use of monoclonal-antibody-based therapies in these settings would be facilitated by the development and implementation of diagnostics that permit rapid species identification directly from clinical samples. Numerous techniques are currently being developed for rapidly identifying bacterial pathogens and their antimicrobial susceptibility profiles [19,20]. An additional limitation of monoclonal antibodies is that they are typically delivered intravenously. Although this might not be problematic in hospitalized patients, who probably represent the most important target population for monoclonal antibodies against highly resistant Gram-negative infections, intravenous therapies are not ideal in most outpatient settings. A final aspect is that the efficacy of monoclonal antibodies is dependent upon the expression and conservation of the corresponding epitope. Although there is very little clinical experience with antibacterial monoclonal antibodies, the possibility exists that they could select for escape mutants in which mutations alter the sequence of the targeted epitope or the expression of the target antigen. Considerations for antigen selection that could reduce the possibility of selecting for these escape mutants are discussed below.

Mechanisms of action

Antibodies can have a therapeutic effect on bacterial infections through different mechanisms. Antibody binding to its cognate antigen on the bacterial cell surface induces a conformational change in the antibody Fc region that facilitates binding of the C1 complement component. This results in the initiation of the complement cascade, which produces bacterial cell lysis through the formation of the membrane attack complex (Fig. 1a). Binding of antibodies to surface-exposed antigens can also promote opsonophagocytolysis of the bacteria via interaction between the Fc region and its cognate receptor on phagocytic cells (Fig. 1b). Finally, antibodies against bacterial exotoxins can neutralize the activity of these molecules either by inhibiting binding to their cellular targets or by blocking multimerization with other toxin subunits (Fig. 1c). These toxin-targeted antibodies might not have significant antibacterial activity against the infecting pathogen. However, toxin neutralization presumably affords the host sufficient time to mount an adequate immune response against the bacteria. It is interesting to note that all three of the antibacterial monoclonal antibodies that have been approved to date act via exotoxin neutralization. The two monoclonal antibody products approved for treatment of inhalation anthrax: raxibacumab and obiltoxaximab, act via binding to the *Bacillus anthracis* toxin subunit protective antigen, preventing its interaction with its cognate cellular receptor. This inhibits the intracellular entry and toxicity of the other *B. anthracis* toxin subunits: edema factor and lethal factor [4,5]. Bezlotoxumab, for preventing the recurrence of *C. difficile* infection, has a similar mechanism of action in

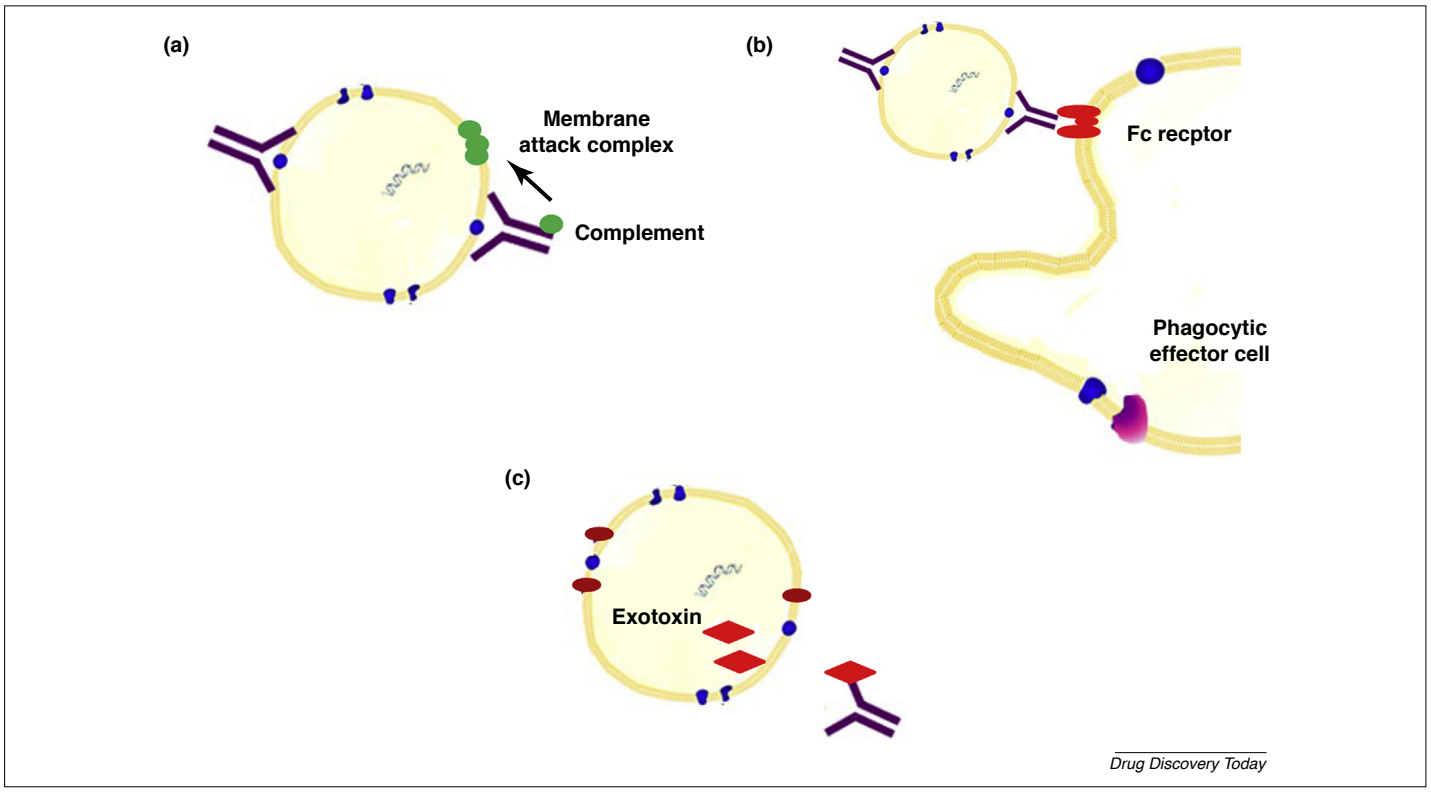

FIGURE 1

Mechanisms of action of antibacterial monoclonal antibodies. Monoclonal antibodies can have a therapeutic effect through **(a)** promoting complement-mediated cell lysis, **(b)** facilitating opsonophagocytolysis of bacteria by effector cells and **(c)** neutralizing the activity of disease-producing toxins.

which antibody binding prevents interaction between the toxin and its cellular receptor on intestinal mucosal cells [6]. Clearly, approaches based on neutralization of exotoxins are possible only when the disease-related pathology of the infecting bacteria is produced by the activity of these toxins. Notably, for many of the Gram-negative species producing multidrug-resistant infections, disease pathology is not primarily caused by exotoxin production.

Identifying targets for monoclonal antibody development

In the absence of disease-defining exotoxins, monoclonal antibodies for the treatment of multidrug-resistant Gram-negative species are likely to be targeted to antigens exposed on the surface of bacterial cells, such as surface polysaccharides and outer membrane proteins. Characteristics of potential surface antigens that would make them desirable include: (i) antigens that are present in most circulating strains within a bacterial species; (ii) antigens that are highly conserved between circulating strains, especially the surface-exposed epitopes that will be involved in antibody binding; and (iii) antigens that are highly expressed during human infection or colonization. It might also be desirable to target epitopes that have essential roles in bacterial pathogenesis or survival during infection, such as nutrient acquisition or mucosal cell binding. Bacteria might be less able to tolerate the down-regulation of expression of these antigens or mutations in these epitopes (for protein antigens), thus potentially reducing the probability of selecting for escape mutants that are no longer recognized by the monoclonal antibody.

The identification of protein antigens that meet these criteria has been facilitated by the development of genomic, transcriptomic and proteomic techniques over the past two decades (Table 1). Comparative genomics and the availability of hundreds to thousands of sequenced genomes for many bacterial species can be used to identify antigens that are highly conserved at the amino acid level between strains within a species. In addition, the estimation of bacterial core genomes – the collection of genes present in all strains within a species – could facilitate the identification of genes that encode functions that are essential for bacterial survival. Notably, studies describing core genomes have been reported for *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [21–23]. Whole-genome mutagenesis techniques, such as insertion sequencing (INseq) and transposon sequencing (Tn-seq), rely on next-generation sequencing to determine the relative abundance of mutants after exposure of mutant libraries to defined experimental conditions [24]. A decrease in the abundance of mutants in a gene indicates that the gene probably encodes a function that participates in bacterial fitness or survival under the condition tested. Data obtained from studies using these techniques can facilitate the identification of antigens that participate in functions that are essential for bacterial survival during infections. INseq has been used to identify genes from *A. baumannii* and *P. aeruginosa* that contribute to bacterial pathogenesis in experimental models of infections [25,26]. Transcriptomic and quantitative proteomic techniques can identify protein antigens that are highly expressed during infection in animal models or *in vitro* conditions that mimic human infection. Proteomic techniques, such as

TABLE 1

Examples of omics technologies that can be used for identifying bacterial antigens for the development of monoclonal antibodies

Technology	Examples	Use in identifying antigens
Comparative genomics	Identification of core genomes	Identification of highly conserved antigens Identification of antigens present in all strains within a species (core genome)
Whole-genome mutagenesis	INseq, Tn-seq	Identification of antigens essential for survival or pathogenesis
Transcriptomics	DNA microarrays, RNA-seq	Identification of antigens highly expressed during infection
Proteomics	iTRAQ, surface shaving	Identification of antigens highly expressed during infection Identification of antigens on the bacterial cell surface

isobaric tags for relative and absolute quantification (iTRAQ), might be particularly useful because they can simultaneously identify proteins present in outer membrane fractions and their relative expression levels [27]. A technique termed 'surface shaving' can also provide valuable information for the development of monoclonal antibodies. This approach employs mass spectrometry to identify peptides that are liberated from the bacterial cell surface after treatment of whole bacteria with trypsin [28]. Because this approach identifies peptides on the cell surface that are accessible for cleavage by trypsin, they could also be accessible for neutralization by antibodies. In addition, this approach identifies short peptides that could be used for defining the epitopes within an antigen that should be targeted by neutralizing monoclonal antibodies.

Monoclonal antibodies against multidrug-resistant Gram-negative infections

There are currently multiple monoclonal-antibody-based therapies in development for the treatment of multidrug-resistant infections. Although most of these antibodies are still in preclinical development, a few have been evaluated in early-stage clinical trials. A summary of recent developments in therapeutic monoclonal antibodies that have been reported for some of the most worrisome antibiotic-resistant infections caused by multidrug-resistant Gram-negative microorganisms is presented below.

P. aeruginosa

Multidrug-resistant *P. aeruginosa* causes infections in the healthcare setting, typically ventilator-associated pneumonia, bloodstream infections and burn-site infections in critically ill patients [29]. It also frequently colonizes and produces infection in individuals with cystic fibrosis. Multidrug resistance is common in *P. aeruginosa* isolates, and the emergence of strains with resistance to first-line carabapenem therapy has severely complicated the clinical managements of infections produced by this microorganism [29]. The therapeutic potential of multiple monoclonal antibodies against *P. aeruginosa* surface antigens has been tested in preclinical models. Human and mouse monoclonal antibodies against flagellar antigens have been shown to provide protection in different rodent models of infection [30–35]. However, one study indicated that protection using antisera against recombinant flagellin type A was only observed when homologous strains were used in challenge experiments, whereas survival was significantly reduced if heterologous strains were employed [36]. These findings underscore the importance of targeting highly conserved antigens and epitopes for antibody-based therapies. Two fully human antibodies against the exopolysaccharide alginate were able to promote opsonophagocytolysis of *P. aeruginosa in vitro* and provided protection against mucoid and non-mucoid strains in a mouse model of pneumonia [37]. Finally, monoclonal antibodies against the *P. aeruginosa* pilin structural protein were able to inhibit pilus-mediated adherence to respiratory epithelial cells, and provided partial protection in a mouse infection model [38].

Three *P. aeruginosa* monoclonal antibodies have been evaluated in early-stage clinical trials. Panobacumab is a fully human antilipopolysaccharide IgM directed against the O-polysaccharide that was developed for the treatment of O11 serotype *P. aeruginosa* infections. In preclinical studies, panobacumab was able to bind serotype O11 *P. aeruginosa* strains with high avidity and reduce lung bacterial load, reduce inflammatory markers and facilitate recruitment of neutrophils to the lung in a mouse model of pneumonia [39,40]. Treatment with panobacumab also demonstrated additive effects in combination with imipenem in a neutropenic mouse model of *P. aeruginosa* pneumonia [41]. A dose-escalation study evaluating the pharmacokinetics of panobacumab in healthy volunteers receiving a single administration of the antibody demonstrated a serum half-life of between 70 and 95 h with no reported serious adverse events [42]. Panobacumab was evaluated in a Phase IIa trial in 17 patients developing nosocomial pneumonia caused by serotype O11 *P. aeruginosa* [43]. A *post hoc* analysis of the study indicated that infection was resolved in 85% of the 13 patients receiving the full three-dose course compared with 64% in the control group, and the time to clinical resolution of the treatment group was 8 days, versus 18.5 days in the control group [44].

PcrV is a protein subunit of the injectisome apparatus of the *P. aeruginosa* type III secretion system. During infection, the type III secretion system directly injects toxins into the cellular cytoplasm [45]. A murine monoclonal antibody against the C terminus of PcrV, mAb166, protected against infection in a murine model [46], and showed synergistic activity in combination with different antibiotics [47]. KB001 is a PEGylated monoclonal antibody fragment against PcrV, which was developed based on mAb166. The pharmacokinetics and pharmacodynamics of a single dose of KB001 were evaluated in 27 cystic fibrosis patients with chronic *P. aeruginosa* infections demonstrating a serum half-life of 11.9 days [48]. The study showed no difference in *P. aeruginosa* density, symptoms or lung function testing results between treatment and placebo groups. However, patients receiving 10 mg/kg of KB001 had significantly decreased neutrophil counts in sputum. In a separate clinical trial that included 169 cystic fibrosis patients, KB001 was well-tolerated and was associated with a small improvement in lung function testing results. However, there was no difference between treatment

and placebo groups regarding the time that elapsed before requiring antibiotic therapy [49]. Finally, a clinical trial evaluating the safety of KB001 in 39 patients receiving mechanical ventilation that were colonized but not infected with *P. aeruginosa* demonstrated that treatment was well tolerated in these patients [50]. Importantly, patients receiving either 3 mg/kg or 10 mg/kg KB001 were less likely to develop *P. aeruginosa* pneumonia (33% and 31%, respectively) compared with the control group (60%).

MEDI3902 is an engineered bispecific antibody that binds to PcrV and to Psl, an exopolysaccharide that pays a part in biofilm formation and cellular adhesion in *P. aeruginosa* [51]. MEDI3902 was shown to facilitate opsonophagocytoloysis and inhibit cellular adhesion *in vitro* [52], and provided protection against infection in murine models of *P. aeruginosa* infection [53]. In a Phase I dose-escalation study, safety and pharmacokinetics of a single administration of MEDI3902 were evaluated in 56 healthy adults [54]. No serious treatment-associated adverse events were reported, although antidrug antibodies were detected in one patient receiving the highest dose (3000 mg). *In vitro* opsonophagocytolytic activity with patient samples correlated with serum MEDI3902 concentrations.

A. baumannii

A. baumannii produces ventilator-associated pneumonia and bloodstream infections in hospitalized patients [55]. Owing to the widespread dissemination of multidrug-resistant strains, very few treatment options remain for some infections caused by this microorganism. Compared with *P. aeruginosa*, much less work has been done regarding the development of therapeutic monoclonal antibodies for *A. baumannii*, with all candidates currently in preclinical stages of development. Mouse monoclonal antibodies against two unidentified iron-regulated outer-membrane proteins were bactericidal and able to opsonize *A. baumannii in vitro*, and blocked siderophore-mediated iron uptake [56]. In a separate study, the mouse anti-K1 capsular polysaccharide monoclonal antibody13D6 was able to opsonize K1-positive strains and reduce post-infection bacterial loads in a rat soft tissue challenge model [57]. Finally, a mouse monoclonal antibody targeting a capsular carbohydrate on the bacterial surface improved survival in a mouse model of sepsis caused by a multidrug-resistant strain of *A. baumannii* [58]. Notably, a recent study indicated that the capsular polysaccharide of *A. baumannii* could inhibit the interaction between monoclonal antibodies and target bacteria [59].

K. pneumoniae

K. pneumoniae is a leading cause of multidrug-resistant hospital acquired infections. The increasing prevalence of infections caused by strains encoding extended-spectrum β-lactamases and carbapenemases, together with the emergence of hypervirulent strains, has complicated the treatment of *K. pneumoniae* infections [60]. Similar to *A. baumannii*, the monoclonal antibodies that have been developed to date for *K. pneumoniae* infections are in the preclinical stages of development. Two mouse monoclonal antibodies against the capsular polysaccharide of the hypervirulent ST258 strain were shown to inhibit biofilm formation and facilitate complement and neutrophil recruitment [61]. Additionally, these antibodies reduced bacterial dissemination after intratracheal infection with *K. pneumoniae*. In a separate study, monoclonal antibodies against the capsular polysaccharide provided protection in murine sepsis and pulmonary infection models [62]. Human monoclonal antibodies against the O-antigen of lipopolysaccharide were protective in mouse models of infection, and showed synergistic activity with meropenem [63]. Additionally, low doses of a human antibody against the lipopolysaccharide O-antigen protected rabbits from infection with the ST258 strain [64]. Finally, monoclonal antibodies targeting MrkA, a major protein in the type III fimbriae complex, inhibited biofilm formation and provided protection in murine pulmonary infection models [65].

Concluding remarks

In the context of widespread antibiotic resistance and few new antibiotics for the treatment of multidrug-resistant Gram-negative infections in the development pipeline, monoclonal-antibody-based therapy could represent a promising approach for reducing the clinical and economic impact of these infections. Multiple studies have employed omics technologies in the identification of high-value antigens that could potentially be used for monoclonal antibody development; however, only a handful of antibodies against these antigens has been evaluated in preclinical studies. Early-stage clinical trials with antibody candidates for *P. aeruginosa* have provided promising results; however, larger trials are needed to evaluate the therapeutic potential of this drug class for multidrug-resistant infections. In summary, although important progress has been made in the development of monoclonal antibodies for multidrug-resistant infections, significant development is still required to realize the potential of these promising therapeutics.

Conflict of interest statement

MJM is a founding partner and shareholder of Vaxdyn, a biotechnology company developing vaccines and antibody-based therapies for bacterial infections.

Acknowledgments

This work was supported by Plan Nacional de I + D + i 2013–2016 and Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Economía, Industria y Competitividad, Spanish Network for Research in Infectious Diseases (REIPI RD16/0016) – cofinanced by European Development Regional Fund 'A way to achieve Europe', Operative program Intelligent Growth 2014–2020, and a Grant from the Instituto de Salud Carlos IIII (PI14/1530) awarded to MJM.

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