Nanodelivery strategies for the treatment of multidrug-resistant bacterial infections

Jiang, L., Lin, J., Taggart, C., Bengoechea, J., & Scott, C. (2018). Nanodelivery strategies for the treatment of multidrug-resistant bacterial infections. *Journal of Interdisciplinary Nanomedicine*, 1-11. https://doi.org/10.1002/jin2.48

Published in:
Journal of Interdisciplinary Nanomedicine

Document Version:
Publisher's PDF, also known as Version of record

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2018 the authors. This is an open access article published under a Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution and reproduction in any medium, provided the author and source are cited.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.
REVIEW

Nanodelivery strategies for the treatment of multidrug-resistant bacterial infections

Lai Jiang,1 Jia Lin,3 Clifford C. Taggart,1 José A. Bengoechea1 & Christopher J. Scott2*

1 Centre for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen’s University Belfast, Belfast, UK
2 Centre for Cancer Research and Cell Biology, School of Medicine, Dentistry and Biomedical Sciences, Queen’s University Belfast, Belfast, UK
3 School of Pharmacy, Queen’s University Belfast, Belfast, UK

Keywords
Antibiotics, ESKAPE, infection, intracellular, nanoparticles.

Correspondence
Christopher J. Scott, Centre for Cancer Research and Cell Biology, School of Medicine, Dentistry and Biomedical Sciences, Queen’s University Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK.
Tel: +44(0)2890327473
Email: c.scott@qub.ac.uk

FUNDING INFORMATION
Royal Society Industrial Fellowship; Engineering and Physical Sciences Research Council (EP/M027473/1); Biotechnology and Biological Sciences Research Council (BB/P006078/1)

Received: 17 May 2018;
Revised: 27 June 2018;
Accepted: 10 July 2018

Journal of Interdisciplinary Nanomedicine, 2018; 0(0), doi: 10.1002/jin2.48

Introduction
Since the first discovery and development of antibiotics, successful treatment of bacterial infectious diseases was achieved in a rapid timescale. However, along with the broad-spread use of antibiotics, the persistence of bacterial infections caused by multidrug-resistant (MDR) bacteria now poses a significant public health challenges worldwide.
Nanodelivery of antibiotics

In 2008, the Infectious Diseases Society of America proposed the acronym ESKAPE to standardize a group of deadly bacterial pathogens with rapidly growing MDR properties, including Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species (Rice, 2008). These ESKAPE pathogens were able to “escape” from the biocidal action of currently marketed antibiotic drugs, causing increasingly serious life-threatening nosocomial infections, also known as hospital-acquired infections (HAIs) (Boucher et al., 2009; Slavcovicci et al., 2015).

The English National Point Prevalence Survey (Health Protection Agency 2012) identified that 6.4% of inpatients in 2011 had HAIs in the UK. The Centers for Disease Control and Prevention estimated that over 2 million infections and about 23,000 deaths per year worldwide were caused by antibiotic-resistant ESKAPE pathogens (Najafi et al., 2016). In recent years, the HAIs problem has caused great concern all over the world, but with the collaboration of the Infectious Diseases Society of America, the European Centre for Disease Prevention and Control (ECDC), and the World Health Organization (WHO), pharmaceutical industries were encouraged to reinvestigate their research with a focus on novel antimicrobial combinations to treat infections caused by ESKAPE pathogens (Boucher et al., 2009; European Commission, 2011; WHO, 2015).

However, despite these ongoing efforts and some notable successes, our therapeutic options for ESKAPE pathogens, such as vancomycin-resistant E. faecium, fluoroquinolone-resistant P. aeruginosa, and carbapenem-resistant Klebsiella species, are extremely limited. Therefore, further understanding of resistance mechanisms in these pathogens may lead to innovative strategies for the development of new antimicrobial options.

The Issue of Current Therapy

Antibiotic resistance in ESKAPE pathogens

Enterococcus faecium

Enterococcus species are Gram-positive facultative anaerobes, in which E. faecium is the most clinically relevant implicated in HAIs (Ciftci et al., 2009; Silva et al., 2011). These E. faecium infections account for approximately 40% of all enterococcal infections currently (Gao et al., 2018). And recently, increasing resistance of E. faecium to beta-lactam antibiotics has been reported in health-care facilities (Chen et al., 2015). Among them, the vancomycin-resistant E. faecium isolate has emerged in North America during the late 1980s, with an alarming increase in resistance of 61% by 2002 (Cetinkaya et al., 2000). In the UK and Ireland, it was reported by the British Society and Antimicrobial Chemotherapy that the incidence of vancomycin-resistant E. faecium had risen from approximately 20% to over 30% in the period 2001 to 2006 (Brown et al., 2008). Nine different types of vancomycin resistance genes in Enterococci (Van-A to E, G, L, M, and N) have been reported, in which Van-A was the most prevalent worldwide, showing the highest resistance to all glycopeptides (Cattoir et al., 2013; Protonotariou et al., 2010).

Staphylococcus aureus

Staphylococcus aureus is a Gram-positive coccal bacterium, which is present in around 20% of healthy individuals (Kluytmans et al., 1997; Zeeuwen et al., 2018). Because of the excessive use of beta-lactam antibiotics, Staphylococcus species possess a method of gene transfer to produce beta-lactamase positive isolates, resulting in 65-85% staphylococcal clinical isolates being resistant to penicillin G (Santajit et al., 2016). Meticillin-resistant S. aureus (MRSA) is defined as a strain of S. aureus that had developed resistance to beta-lactam antibiotics, including all penicillin, cephalosporins, and carbapenems. Currently, MRSA accounts for about 25% of S. aureus isolates, and in some regions, the prevalence is greater than 50% (ECDC, 2011). According to the Centers for Disease Control and Prevention, there are 80,000 cases and 11,000 deaths due to invasive MRSA strains each year (Najafi et al., 2016). In the clinic, glycopeptide antibiotics such as vancomycin were always used as the first choice treatment for MRSA infections. However, since the first reported resistant strains in Japan in the mid-1990s, these MDR strains have now emerged all over the world (Chambers et al., 2009).

Klebsiella pneumoniae

As a member of the Enterobacteriaceae family, K. pneumoniae, a Gram-negative bacterium, frequently causes lower respiratory tract infection and catheter-associated urinary tract infection (Navon-Venezia et al., 2017). Because of the presence of extended-spectrum beta-lactamas (ESBLs), K. pneumoniae is not only simply resistant to penicillin and ampicillin but
also increasingly multidrug resistance to cephalosporin and ceftazidime (Petrosillo et al., 2013). Historically, carbapenems were typically used to treat only the most difficult MDR infections caused by Gram-negative bacteria (Bush, 2010). However, in recent years, carbapenem-resistant K. pneumonia, harbouring the carbapenemase gene on resident plasmids, were reported to have become a significant obstacle to treatment by clinicians in many areas of the world and were associated with high rates of mortality (Bratu et al., 2005; Naas et al., 2005; Nordmann et al., 2009; Queenan et al., 2007; Wei et al., 2007). There are currently three types of K. pneumoniae producing carbapenemases (KPCs): KPC-1, KPC-2, and KPC-3 (Yigit et al., 2001; Monteiro et al., 2009; Humphries et al., 2015). Currently, carbapenem-resistant K. pneumonia with multidrug resistance only can be reduced and not completely eradicated; therefore, effective treatments are urgently needed to tackle this pathogen.

**Acinetobacter baumannii**

*Acinetobacter baumannii* is a non-fermentative Gram-negative opportunistic pathogen, with the ability to cause HAIs, particularly respiratory tract, urinary tract, and wound infections (Abbo et al., 2005; Al Mobarak et al., 2014; Fournier, 2006), the latter becoming increasingly prevalent in war areas, such as Iraq (Howard et al., 2012). In the period of January 2002 to August 2004, *A. baumannii* bloodstream infections were detected in 85 soldiers in Iraq and Afghanistan; among them, 35% of the strains were susceptible to only one type of antibiotic, and worryingly, 4% were resistant to all antibiotics (Montefour et al., 2008). This has created pressure on treatment options especially in those novel isolates with carbapenem resistance. In addition, imipenem metallo-beta-lactamases and oxacillinase serine beta-lactamases, both of which are carbapenemases, have been discovered in *A. baumannii* isolates (Queenan et al., 2007; Vila et al., 2007). These strains show resistance not only to imipenem and colistin but also to traditional antimicrobial compounds, such as aminoglycosides, fluoroquinolones, and third generation of cephalosporins (Boucher et al., 2009; Fournier et al., 2006).

**Pseudomonas aeruginosa**

*Pseudomonas aeruginosa* is a Gram-negative rod-shaped facultative anaerobe, an opportunistic pathogen with a mortality rate of 40-60% (Najafi et al., 2016). *P. aeruginosa* is found in patients suffering the genetic condition cystic fibrosis (CF) (Mir et al., 2011). In CF patients, it has been found that *P. aeruginosa* survive in biofilms, thus creating an antibiotic-resistant shield to treatment in the thick mucus of the CF lung. Recent reports documented that MDR *P. aeruginosa* was not only resistant to carbapenems, aminoglycosides, and quinolones, but also the polymyxins (Hirsch et al., 2011). A common MDR feature of *P. aeruginosa* is the combination of chromosomal AmpC production and upregulation of an efflux pump, which induce high-level carbapenem resistance (Livermore, 2002). Additionally, *P. aeruginosa* can also produce ESBLs and other resistant enzymes such as KPC, imipenem metallo-beta-lactamases, and Verona integrin-encoded metallo-beta-lactamases (Bush et al., 1995; Livermore, 2002). The emergence of carbapenem resistance and MDR isolates represents a barrier to successful antibiotic therapies for the treatment of this microbe.

**Enterobacter species**

The genus *Enterobacter* is an important Gram-negative nosocomial pathogen, causing an increasing number of serious HAIs, such as bloodstream infection and lower respiratory infection, resulting in MDR mediated by plasmid-encoded ESBLs and carbapenemases (Verona integrin-encoded metallo-beta-lactamases, KPC, and oxacillinase serine beta-lactamases) (Castanheira et al., 2011). Other than colistin and tigecycline, these MDR *Enterobacter* species have developed resistance to almost all current available antimicrobials (Deshpande et al., 2006; Pfaffer et al., 2006).

**Intracellular pathogens represent reservoirs of infection**

In addition to the various mechanisms to develop resistance towards antimicrobial agents, recent studies have now shown another mechanism by which ESKAPE pathogens exhibit antibiotic resistance through the ability to survive intracellularly in host cells. Persistent bacterial infections caused by intracellular bacteria continue to impose significant challenges worldwide (Proctor et al., 2006). In order to maintain their infection cycle, certain pathogen species are able to localize inside host cells, creating a niche to reproduce and spread without damage to the host cells, leading to severe and latent infections (Imbuluzqueta et al., 2010; Silva et al., 2013; Hand et al., 2003).

Usually, phagocytes recognize and eliminate bacteria by phagocytosis. However, some bacteria can survive through various escape mechanisms. Recent research
demonstrated that *K. pneumoniae* is engulfed by alveolar macrophages and resides inside the cell in a phagosome, called a Klebsiella-contained vacuole (Cano et al., 2015). Whilst inside these vacuoles, *K. pneumoniae* promotes activation of Akt to arrest phagosome maturation and avoid fusion into lysosomes, which would otherwise result in destruction. Another ESKAPE pathogen, *S. aureus*, used to be considered as an extracellular bacterium; however, accumulating evidence suggests that *S. aureus* can invade and survive in either professional or nonprofessional phagocytes, including keratinocytes, endothelial cells, epithelial cells, fibroblast, and osteoblasts (Lacoma et al., 2017; Garzoni et al., 2009; Hanses et al., 2011; Reott et al., 2008). The adhesion of *S. aureus* to the host cell surface results in cytoskeletal rearrangement to allow *S. aureus* to move into cells. Once inside a cell, *S. aureus* can persist and even replicate within the acidic phagolysosome (Brouillette et al., 2003; Edwards et al., 2011). Moreover, other studies suggested another mechanism that some other bacteria, including *S. aureus*, can use to escape from the phagocytic vacuole to the cytosol (Fraunholz et al., 2012).

Furthermore, some other pathogens also exhibited similar mechanisms to maintain their life in host cells. For example, *Coxiella burnetii* infects mononuclear phagocytes, acquiring late endosomal-early lysosomal markers, and resides inside the acidic vacuoles (Howe et al., 2010). *Mycobacterium tuberculosis* was reported recently that it can create its own vacuole to prevent phagosome-lysosome fusion (Weiss et al., 2015) and also has the ability to escape into cytosol by permeabilizing the phagosome membranes (Jamwal et al., 2016).

As a consequence, professional phagocytes are not only unable to eradicate intracellular pathogens but provide a reservoir of latent infection that represents a significant barrier to successful treatment with antibiotics.

**Treatments for ESKAPE Pathogens**

**Nano-based drug delivery system**

Macrophages are responsible for removing foreign pathogens or particles in the blood and tissue via phagocytotic pathways (Desjardins et al., 2003; Muppidi et al., 2011). However, phagocytes harbouring ESKAPE pathogens, such as *S. aureus* and *K. pneumoniae*, act as “Trojan Horse” to allow these intracellular bacteria to establish secondary infection foci, resulting in recurrent systemic infections (Tan et al., 2013). However, these same phagocytosis pathways could also be co-utilised to allow antibiotics to be delivered to macrophages. Theoretically, nano-carriers containing antibiotics are able to passively accumulate in infection foci via recognition and uptake by phagocytes. Upon phagocytosis of particulate drugs, the antibiotic payload has the potential to be delivered into the infected cells, therefore enhancing penetration and release of antibiotics inside the infected cells (Briones et al., 2008; Bakker-Woudenberg et al., 2004; Pinto-Alphandary et al., 2000; Schifflers et al., 2001).

Furthermore, biofilm-associated antimicrobial resistance associated with ESKAPE pathogens like *P. aeruginosa* and *K. pneumoniae* is also resistant to antibiotics (Rasamiravaka et al., 2014; Vuotto et al., 2017). Nano-carriers could act as a protective coat, shielding against interactions and minimizing inactivation of drug by biofilm compartments and resident enzymes. In this scenario, nano-based drug delivery systems (DDSs) are a promising way to treat either intracellular and biofilm-forming ESKAPE pathogen infections. Diverse nano-formulations, such as liposomes and polymeric nanoparticles (NPs), have been developed and employed for the delivery of antibiotics to difficult-to-treat bacteria (Table 1).

**Liposomes**

Liposomes, first explored in the 1960s, represent the most developed DDS platform (Deamer, 2010; Düzgün et al., 2005). They are spherical vesicles composed of phospholipid bilayers surrounding an aqueous core. Within the structure, liposomes are capable of carrying hydrophilic drugs like aminoglycosides in their core or embed hydrophobic drugs like beta-lactams into the bilayers (Druulis-Kawa et al., 2010; Jones, 1995). Various studies have suggested that liposomal formulation could significantly enhance the antimicrobial effect of antibiotics against intracellular ESKAPE pathogens. For example, Pumerantz et al. (2011) demonstrated that vancomycin-loaded liposomes composed of cholesterol and 1,2-distearoyl-sn-glycero-3-phosphocholine a significant intracellular reduction in MRSA colony forming units compared with free drug. Additionally, liposome formulations have also been shown to exhibit enhanced therapeutic effects towards biofilm-forming *P. aeruginosa*. As reported by Alhajlan et al. (2013), the biofilm-forming strains of *P. aeruginosa* were more susceptible to clarithromycin-encapsulated liposome than the free drug. However, some drawbacks were
revealed in the development of antibiotic-loaded liposomes, such as instability of the vesicles and low drug encapsulation. Therefore, polymeric NPs have been developed as alternative nano-formulation platforms to improve stability and drug loading.

### Polymeric nanoparticles

Polymeric NPs are prepared from natural or synthetic polymers with a size between 10 and 1000 nm. They can be a polymeric matrix with homogenous drug distribution or nano-capsules with drug entrapped in a

---

#### Table 1. Nano-based drug delivery systems for ESKAPE pathogens.

| Nanoparticles platform | Loaded antibiotics | Target pathogen(s) | Outcomes | Reference |
|------------------------|--------------------|--------------------|----------|-----------|
| Pyochelin-based PEGylated liposomes | Cefepime, imipenem, and ceftazidime | *P. aeruginosa* (MDRPa) | Killed MDRPa within infected HaCaT keratinocytes without any cytotoxic effects at four times MIC concentrations after 72 h | Pushparaj Selvadoss et al. (2017) |
| DPPC, cholesterol liposomes | Ciprofloxacin | *P. aeruginosa* | The ciprofloxacin concentration required to achieve similar biofilm inhibition was 125-fold lower compared with free ciprofloxacin | Bandara et al. (2016) |
| DA liposomes | Chloramphenicol | *S. aureus* (MRSA) | An inhibition zone about twofold higher, compared with free drug, was achieved by DA liposomes. DA liposomes augmented antibacterial activity on keratinocyte-infected MRSA | Hsu et al. (2017) |
| Liposome, made by phosphatidylcholine, cholesterol, Tween 80, and stearylamine | Amikacin | *K. pneumoniae* | The liposome was able to deliver entrapped phage inside macrophages, caused 94.6% killing of intracellular *K. pneumoniae* and showed synergistic activity to eradicate mature biofilm of *K. pneumoniae* | Singla et al. (2016) |
| PLGA nanoparticles | Amikacin | *P. aeruginosa* | Particles penetrated through the entire biofilm thickness, more effective than free drug in biofilm eradication | Sabaiefard et al. (2017) |
| Mesoporous silica nanoparticles | Gentamicin | *S. aureus* | The inflammation-related gene expression in infected preosteoblast or macrophage was downregulated significantly after treatment by the antibiotic loaded nanoparticles | Yang et al. (2018) |
| PpZEV-NPs, made by PEG-PLGA, Eudragit E100, and chitosan derivative CSNPs | Vancomycin | *S. aureus* (MRSA) | PpZEV-NPs showed better antimicrobial activity than free vancomycin against intracellular MRSA | Pei et al. (2017) |
| | Cefazolin | *K. pneumoniae*, *P. aeruginosa* | Excellent antimicrobial potential of cefazolin-loaded CSNPs was demonstrated against multidrug-resistant *K. pneumoniae* and *P. aeruginosa* | Jamil et al. (2015) |

CSNPs, chitosan nanoparticles; DA, deoxycholic acid; DPPC, dipalmitoylphosphatidylcholine; *K. pneumoniae*, Klebsiella pneumoniae; MDRPa, multidrug-resistant *P. aeruginosa*; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; PLGA, poly(lactide-co-glycolide); *P. aeruginosa*, Pseudomonas aeruginosa; *S. aureus*, Staphylococcus aureus. 
polymeric shell (Kreuter, 1991; Soppimath et al., 2001). Because of the development of advanced nanotechnologies and new polymers, NPs are currently the subject of extensive research as passive macrophage targeting agents, showing an ideal way to target macrophages to deliver therapeutic effects (Bains et al., 2016; Spence et al., 2015). Chitosan, a representative of commonly investigated natural polymers, has been widely used for mucoadhesive drug delivery and gene transfection (Kim et al., 2014). Our group previously reported that tobramycin-loaded NPs, made of chitosan, and then functionalized with dornase alfa, demonstrated enhanced antibacterial effects on P. aeruginosa, DNA degradation, and improved NP penetration of CF sputum (Deacon et al., 2015). O-carboxymethyl chitosan NPs loaded with tetracycline were reported by Maya et al. (2012), where the NPs were sixfold more effective in killing intracellular S. aureus compared with tetracycline alone in HEK-293 and differentiated THP-1 macrophage cells proving it to be an efficient nanomedicine to treat intracellular S. aureus infections. Another report illustrated that gentamicin-loaded chitosan/fucoidan NPs were developed to provide multiple antimicrobial capabilities against K. pneumoniae, representing an improvement in antimicrobial efficacy (Huang et al., 2016). Synthetic polymers have been used for targeted delivery of antibiotics to macrophages for the treatment of intracellular ESKAPE infections. Among them, poly(lactide-co-glycolide) (PLGA) is a nontoxic, biodegradable, and bio-compatible copolymer (Rajeev, 2000). Our previous work demonstrated that gentamicin-loaded PLGA NPs were able to significantly eradicate biofilm-forming P. aeruginosa, improving the antimicrobial effects of gentamicin (Abdelghany et al., 2012). Recently, our group also reported that using an emulsification-solvent evaporation method, PLGA NPs containing gentamicin could effectively eliminate intracellular K. pneumoniae in a bacteria and macrophage coculture model (Jiang et al., 2018). Another study has shown that the effective delivery of encapsulated antibiotics (gentamicin and nafcillin) into cells augmented their therapeutic activity against intraphagosomal S. aureus (Imbuluzqueta et al., 2011, 2012; Pillai et al., 2008).

Furthermore, nano-formulated DDSs are also used for other intracellular bacteria, such as M. tuberculosis (Dollnellan et al., 2017). For example, PLGA NPs have been previously explored to deliver isoniazid into M. tuberculosis infected murine bone marrow-derived macrophages (Faria et al., 2012).

**Nano-formulation of antimicrobial treatments**

The emergence of MDR ESKAPE pathogens has driven the urgent need for novel alternatives to antibiotics and has made researchers consider nano materials themselves as antibacterial agents. Silver has been known for its antibacterial activity since the ancient Egyptians and Greek periods, but it was only recently discovered that silver ion (Ag⁺) has broad-spectrum antimicrobial activity (Klassen, 2000). With the development of nanotechnology, it is now possible to use silver NPs against infections caused by ESKAPE pathogens. For instance, Bankalgi et al. (2016) demonstrated that phenolics-coated silver NPs showed strong antibacterial effects against Gram-negative P. aeruginosa and Enterobacter aerogenes. Furthermore, Kedziora et al. (2016) demonstrated that silver nanoform complexed with amorphous TiO₂ exhibits antimicrobial efficacy against S. aureus and K. pneumoniae. Although silver NPs show potential in antimicrobial applications, some adverse events associated with the use of these NPs, such as reactive oxygen species production, could damage the host cell. As an alternative, gold (AuNPs), which possess low cytotoxicity, have also been investigated as antimicrobial agents. It was reported that AuNPs functionalized with ampicillin elicited effective broad-spectrum bactericidal activity against the ESKAPE pathogens, P. aeruginosa and E. aerogenes (Brown et al., 2012). Additionally, Zhao et al. (2013) reported that pyrimidinethiol-modified AuNPs demonstrated synergistic antimicrobial effects against MDR E. faecium, MDR P. aeruginosa, MRSA, MDR K. pneumoniae, and M. baumannii. Thus, the application of AuNPs seems to hold much potential for the treatment of MDR ESKAPE infectious diseases, but further developments such as manufacturability and pharmacokinetics must be addressed.

Some studies demonstrated other NP formulations that were capable of eliminating ESKAPE pathogens. For example, Friedman et al. (2011) have described a nitric oxide-releasing NP with efficacy against not only MRSA and A. baumannii but also all the clinical isolates of Streptococcus pyogenes, Enterococcus faecalis, K. pneumoniae, and P. aeruginosa. Similarly, another report demonstrated that nitric oxide-releasing silica NPs could be utilized as novel antibacterial agents against intracellular P. aeruginosa in L929 mouse fibroblasts (Hetrick et al., 2008). Wu et al. (2013) reported magnetic reduced graphene oxide NPs functionalized with glutaraldehyde, which provided rapid and effective killing of up to 99% of S. aureus. Additionally,
Jones et al. (2008) demonstrated that ZnO NPs have a wide range of antibacterial effects against a number of microorganisms, having significantly higher antibacterial effects on *S. aureus* than other metal NPs.

**Antibody drug conjugation treatments**

Antibody drug conjugates developed as “magic bullets” have been successfully developed for cancer treatment, consisting of monoclonal antibodies specifically targeted to antigen-expressing tumour cells with cytotoxic drug payloads (Casi et al., 2012; Wang et al., 2015). This approach has now been exploited for treatment of infectious diseases - antibody antibiotic conjugates (AACs). In AACs, an antibiotic is conjugated to an antibody to target the bacteria of interest. Recently, scientists at Genentech have developed an AAC called THIOMAB™ antibiotic conjugate aimed at the treatment of intracellular MRSA. This AAC consists of an antibody that binds the surface of *S. aureus* (wall-teichoic acids and pathogen-specific polyanionic glycopolymers) and carries the potent rifalogue antibiotic (Lehar et al., 2015). Once the bacterium/AAC complex is internalized by host cells, the host-resident proteases release the antibiotic payload, so that it can act directly at the site of intracellular infection (Fig. 1). These researchers have demonstrated that this AAC could significantly eradicate intracellular *S. aureus* infections with a superior therapeutic effect than vancomycin. This therapeutic (DSTA-46375) is currently in phase I clinical trials. AACs have the potential to modify a broad-spectrum antibiotic into a pathogen-specific antibiotic as a result of the antibody used, therefore minimizing side effects such as ototoxicity and nephrotoxicity. However, AACs are complex molecules, so further developments in design and manufacturing are required for application against difficult-to-treat ESKAPE bacterial infections.

**Perspective**

Given the increasing prevalence of antibiotic resistance, the treatment of ESKAPE pathogens is becoming increasingly more challenging. Intracellular ESKAPE pathogens represent a group of bacteria that are particularly difficult to treat as a result of their intracellular residual location. Pathogen-harbouring phagocytes effectively shield the intracellular bacteria from antibiotics, resulting in difficulties in eradicating the infection as well as limitations in clinical treatment options. Antibiotic-loaded DDS may represent an exciting approach for the treatment for intracellular bacteria in the future using existing and novel antibiotics. With the developments of DDS, intracellular accumulation of these poorly cell-permeable drugs has
been circumvented, leading to an enhancement of antimicrobial activities.

A key concept in the strategy of employing nanotechnological delivery systems for ESKAPE intracellular infections is that the pathogens and the NPs tend to accumulate in the same cell - the professional phagocytes of the reticuloendothelial system. It may be possible in the future to explore more "active" targeting strategies, exemplified by the current interest in AACs.

In conclusion, through reformulation of existing antimicrobials, it may be possible to extend the useful life span of these drugs and their ability to treat dangerous intracellular infections.

Funding Information
This work was funded in part through Biotechnology and Biological Sciences Research Council Award BB/P006078/1 and Engineering and Physical Sciences Research Council Award EP/M027473/1 and a Royal Society Industrial Fellowship to C. J. Scott.

Conflict of Interest
None declared.

REFERENCES
Abbo, A., Navon-Venezia, S., Hammer-Muntz, O., Krichali, T., Siegman-Igra, Y., and Carmeli, Y. 2005. Multidrug-resistant Acinetobacter baumannii. Emerg. Infect. Dis. 11(1):22-29.

Abdelghany, S. M., Quinn, D. J., Ingram, R. J., Gilmore, B. F., Donnelly, R. F., Taggart, C. C., and Scott, C. J. 2012. Gentamicin-loaded nanoparticles show improved antimicrobial effects towards Pseudomonas aeruginosa infection. Int. J. Nanomedicine 7:4053-4063.

Al Mobarak, M. F. et al. 2014. Antimicrobial resistance patterns among Acinetobacter baumannii isolated from King Abdulaziz Hospital, Jeddah, Saudi Arabia, four-year surveillance study (2010-2013). Egyptian Journal of Medical Microbiology. 23(4):53-60.

Alhaljani, M., Alhariri, M., and Omri, A. 2013. Efficacy and safety of liposomal clarithromycin and its effect on Pseudomonas aeruginosa virulence factors. Antimicrob. Agents Chemother. 57(6):2694-2704.

Bains, B. K., Greene, M. K., McGirr, L. M., Dorman, J., Farrow, S. N., and Scott, C. J. 2016. Encapsulation of the p38 MAPK inhibitor GSK 678361A in nanoparticles for inflammatory-based disease states. Journal of Interdisciplinary Nanomedicine. 1(3):85-92.

Bakker-Woudenberg, I. A. J. M. et al. 2004. Long-circulating sterically stabilized liposomes in the treatment of infections. Methods Enzymol. 391:228-260.

Bandara, H. M. H. N., Herpin, M. J., Kolacny, D., Jr., Harb, A., Romanovicz, D., and Smyth, H. D. C. 2016. Incorporation of farnesol significantly increases the efficacy of liposomal ciprofloxacin against Pseudomonas aeruginosa biofilms in vitro. Mol. Pharm. 13(8):2760-2770.

Bankaiga, S. C., Londonkar, R. L., Madire, V., and Tukapa, N. K. A. 2016. Biosynthesis, characterization and antibacterial effect of phenolics-coated silver nanoparticles using Cassia javanica L. Journal of Cluster Science. 27(4):1485-1497.

Boucher, H. W., Talbot, G. H., Bradley, J. S., Edwards, J. E., Gilbert, D., Rice, L. B., Scheld, M., Spellberg, B., and Bartlett, J. 2009. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin. Infect. Dis. 48(1):1-12.

Bratu, S., Mooty, M., Nichani, S., Landman, D., Gullans, C., Pettinato, B., Karumudi, U., Tolaney, P., and Quale, J. 2005. Emergence of KPC-possessing Klebsiella pneumoniae in Brooklyn, New York: epidemiology and recommendations for detection. Antimicrob. Agents Chemother. 49(7):3018-3020.

Briones, E., Isabel Colino, C., and Lanoa, J. M. 2008. Delivery systems to increase the selectivity of antibiotics in phagocytic cells. J. Control. Release 125(3):210-227.

Brouillette, E. et al. 2003. In vivo and in vitro demonstration that Staphylococcus aureus is an intracellular pathogen in the presence or absence of fibronectin-binding proteins. Microb. Pathog. 35(4):159-168.

Brown, A. N., Smith, K., Samuels, T. A., Lu, J., Obare, S. O., and Scott, M. E. 2012. Nanoparticles functionalized with ampicillin destroy multiple-antibiotic-resistant isolates of Pseudomonas aeruginosa and Enterobacter aerogenes and methicillin-resistant Staphylococcus aureus. Appl. Environ. Microbiol. 78(8):2768-2774.

Brown, D. F. J. et al. 2008. Non-susceptibility trends among Enterococci and non-pneumococcal streptococci from bacteremias in the UK and Ireland, 2001-06. Journal of Antimicrobial Chemotherapy 62(2):i175-i185.

Bush, K. 2010. Alarming b-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Curr. Opin. Microbiol. 13(5):558-564.

Bush, K. et al. 1995. A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. 39(6):1211-1233.

Cano, V., March, C., Insua, J. L., Agullo, N., Llobet, E., Moranta, D., Regueiro, V., Brennan, G. P., Millán-Lou, M. I., Martin, C., Carmendia, J., and Bengoechea, J. A. 2015. Klebsiella pneumoniae survives within macrophages by avoiding delivery to lysosomes. Cell. Microbiol. 17(11):1537-1560.

Casi, G., and Neri, D. 2012. Antibody-drug conjugates: basic concepts, examples and future perspectives. J. Control. Release 161(2):422-428.

Castanheira, M., Deshpande, L. M., Mathai, D., Bell, J. M., Jones, R. N., and Mendes, R. E. 2011. Early dissemination of NDM-1 and OXA-181-producing Enterobacteriaceae in India: reports from the SENTRY Antimicrobial Surveillance Program, 2006-2007. Antimicrob. Agents Chemother. 55(3):1274-1278.

Cattoir, V., and Leclercq, R. 2013. Twenty-five years of shared life with vancomycin-resistant Enterococci: is it time to divorce? Journal of Antimicrobial Chemotherapy. 68(4):731-740.

Cetinkaya, Y., Fark, P., and Mayhall, C. G. 2000. Vancomycin-resistant Enterococci. Clin. Microbiol. Rev. 13(4):686-707.

Chambers, H. F., and DeLeo, F. R. 2009. Waves of resistance: Staphylococcus aureus in the antibiotic era. Nat. Rev. Microbiol. 7(9):629-641.

Chen, C., Sun, J., Guo, Y., Lin, D., Guo, Q., Hu, F., Zhu, D., Xu, X., and Wang, M. 2015. High prevalence of vanM in vancomycin-resistant Enterococcus faecium isolates from Shanghai, China. Antimicrob. Agents Chemother. 59(12):7795-7798.

Ciftci, A. et al. 2009. Slime production and antibiotic resistance of Enterococcus faecalis isolated from arthritides in chickens. Journal of Veterinary Medical Science. 71(6):849-853.

Deacon, J., Abdelghany, S. M., Quinn, D. J., Schmid, D., Megaw, J., Donnelly, R. F., Jones, D. S., Kissenpenning, A., Elborn, J. S., Gilmore, B. F., Taggart, C. C., and Scott, M. E. 2011. Antiinfective efficacy of tobramycin polymeric nanoparticles for Pseudomonas aeruginosa infections in cystic fibrosis: formulation, characterisation and functionalisation with dornase alfa (DNase). J. Control. Release 198:55-61.
Deamer, D. W. 2010. From “banghasomes” to liposomes: a memoir of Alec Bangham, 1921–2010. The PASEB Journal: official publication of the PASEB. American Society for Experimental Biology 24(5):1308-1310.

Deshpande, L. M., Jones, R. N., Fritsche, T. R., and Sader, H. S. 2006. Occurrence and characterization of carbapenemase-producing Enterobacteriaceae: report from the SENTRY Antimicrobial Surveillance Program (2000–2004). Microb. Drug Resist. 12(4):223–230.

Desjardins, M., and Griffiths, G. 2003. Phagocytosis: latex leads the way. Curr. Opin. Cell Biol. 15(4):498-503.

Donnellan, S., Stone, V., Johnston, H., Giardiello, M., Owen, A., Rannard, S., Aljayyousi, G., Swift, B., Tran, L., Watkins, C., and Stevenson, K. 2017. Intracellular delivery of nano-formulated antituberculosis drugs enhances bacterial activity. Journal of Interdisciplinary Nanomedicine. 2(3):146-156.

Droits-Kawa, Z., and Dorotkiewicz-Jach, A. 2010. Liposomes as delivery systems for antibiotics. Int. J. Pharm. 387(1-2):187-198.

Düzgün, N., and Gregoriadis, G. 2005. Introduction: the origins of liposomes: Alec Bangham at Babraham. Methods Enzymol. 391:1-3.

ECDC. European Centre for Disease Prevention and Control 2011. Annual epidemiological report 2011. Reporting on 2009 surveillance data and 2010 epidemiological data. https://ecdc.europa.eu/en/publications-data/annual-epidemiological-report-2011-2009-data.

Düzgün, N., and Gregoriadis, G. 2005. Introduction: the origins of liposomes: Alec Bangham at Babraham. Methods Enzymol. 391:1-3.

Faria, T. J. et al. 2010. Staphylococcus aureus keratinocyte invasion is dependent upon multiple high-affinity fibronectin-binding repeats within FnBPA. PLoS ONE. 6(4:e18899).

European Commission. Brussels, 15.11.2011. Communication from the Commission to the European Parliament and the Council. Action plan against the rising threats from antimicrobial resistance. https://eur-lex.europa.eu/en/legislation-text/uri=CELEX%3A52011DC0748

Faria, T. J. et al. 2012. An isoniazid analogue promotes Mycobacterium tuberculosis-nanoparticle interactions and enhances bacterial killing by macrophages. Antimicrob. Agents and Chemotherapy. 56(5):2259-2267.

Fourrier, P. E. et al. 2006. The epidemiology and control of Acinetobacter baumannii in health care facilities. Healthcare Epidemiology. 42:692-699.

Fourrier, P. E., Vallenet, D., Barbe, V., Audic, S., Ogata, H., Guiton, H., Rojot, F., Mangeot, S., Abergel, C., Nordmann, P., Weissenbach, J., Raoult, D., and Claverie, J. M. 2006. Comparative genomics of multidrug resistance in Acinetobacter baumannii. PLoS Genet. 2(1):e7.

Frahnhözl, M. et al. 2012. Intracellular Staphylococcus aureus: live-in and let die. Front. Cell. Infect. Microbiol. 2:1-10.

Friedman, A. et al. 2011. Impact of multidrug-resistant Pseudomonas aeruginosa infection on patient outcomes. Expert Opin. Pharmacoecon. Outcomes Res. 10(4):441-451.

Howard, A., O’Donoghue, M., Feeney, A., and Sleator, R. D. 2012. Acinetobacter baumannii: an emerging opportunistic pathogen. Virulence 3(3):243-250.

Humphries, R. M., Yang, S., Hemarajata, P., Ward, K. W., Hindler, J. A., Miller, S. A., and Gregson, A. 2015. First report of cefazidime-avibactam resistance in a KPC-3 expressing Klebsiella pneumoniae isolate. Antimicrobial Agents and Chemotherapy. 59(10):6605-6607.

Hutson, J. Y. 2017. Anti-MRSA malleable liposomes carrying chloramphenicol for ameliorating hair follicle targeting. Int. J. Nanomedicine. 12:8227-8238.

Hu, Y. et al. 2016. Biphase release of gentamicin from chitosan fucoidan nanoparticles. Carbohydr. Polym. 138:114-122.

Imbuluzqueta, E. et al. 2010. Drug delivery systems for potential treatment of intracellular bacterial infections. Front. Biosci. 15:397-417.

Jamil, B. et al. 2016. Cefazolin-loaded chitosan nanoparticles to cure multi drug resistant Gram-negative pathogens. Carbohydr. Polym. 136:682-691.

Jamal, S. V., Meihotra, P., Singh, A., Siddiqui, Z., Basu, A., and Rao, K. V. S. 2016. Mycobacterial escape from macrophage phagosomes to the cytoplasm represents an alternate adaptation mechanism. Nature Scientific Reports. 6:23089.

Jones, M. N. 1995. Systems and their characterisation. Adv. Colloid Interface Sci. 54:93-128.

Kedziora, A. et al. 2016. Silver nanoflours as a therapeutic agent for killing Escherichia coli and certain ESKAPE pathogens. Curr. Microbiol. 73(1):139-147.

Kim, J. K., Kim, H. J., Chung, J. Y., Lee, J. H., Young, S. B., and Kim, Y. H. 2014. Natural and synthetic biomaterials for controlled drug delivery. Adv. Mater. Chem. 4(10):60-68.

Klassen, H. J. 2000. A historical review of the use of silver in the treatment of burns. Burns 26:117-130.

Kluytmans, J., van Belkum, A., and Verbrugh, H. 1997. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clin. Microbiol. Rev. 10(3):505-520.

Kreuter, J. 1991. Nanoparticle-based drug delivery systems. J. Control. Release. 16:155-167.
Nanodelivery of antibiotics

Lehar, S. M. et al. 2015. Novel antibiotic-antibiotic conjugate eliminates intracellular S. aureus. Nature 524:173-177. doi:10.1038/nature14697

Livermore, D. M. 2002. Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: our worst nightmare? Clin. Infect. Dis. 34(5):634-640.

Maya, S., Indulekha, S., Sukhithasri, V., Smitha, K. T., Nair, S. V., Jayakumar, R., and Biswas, R. 2012. Efficacy of tetracycline encapsulated O-carboxymethyl chitosan nanoparticles against intracellular infections of Staphylococcus aureus. Int. J. Biol. Macromol. 51(4):392-399.

Mir, T. A., Ashraf, M., Ahmed, K., Chowdhary, J., Rehana, B., and Ahmed, J. 2011. Clinical profile, diagnostic delay, and genetic make-up of cystic fibrosis in Kashmir, India. Lung India: official organ of Indian Chest Society. 28(2):97-100.

Montefour, K., Frieden, J., Hurst, S., Helmich, C., Headley, D., Martin, M., and Boyle, D. A. 2008. An emerging multidrug-resistant pathogen in critical care. Crit. Care Nurse 28(1):15-25.

Monteiro, J., Santos, A. F., Asensi, M. D., Peirano, G., and Gales, A. C. 2009. First report of KPC-2-producing Klebsiella pneumoniae strains in Brazil. Antimicrob. Agents Chemother. 53(1):333-334.

Muppidi, K., Wang, J., Betageri, G., and Pumerantz, A. S. 2011. PEGylated liposome encapsulation increases the lung tissue concentration of vancomycin. Antimicrob. Agents Chemother. 55(10):4537-4542.

Naas, T., Nordmann, P., Vedel, G., and Poyart, C. 2005. Plasmid-mediated carbapenem-hydrolysing beta-lactamase KPC in a Klebsiella pneumoniae isolate from France. Antimicrob. Agents Chemother. 49(10):4423-4424.

Najafi, A., et al. 2016. There is no escape from the ESKEAP pathogens. http://emerypharma.com/blog/eskeap-pathogens-explained/

Navon-Venezia, S., Kondratyeva, K., and Carattoli, A. 2017. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS Microbiol. Rev. 41:252-275.

Nordmann, P., Cuzon, G., and Naas, T. 2009. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect. Dis. 9(4):228-236.

Pei, Y., Mohamed, M. F., Seleem, M. N., and Yeo, Y. 2017. Particle engineering for intracellular delivery of vancomycin to methicillin-resistant Staphylococcus aureus (MRSA)-infected epithelial cells. J. Control. Release. 267:133-143.

Petrossino, N., Giannella, M., Lewis, R., and Viale, P. 2013. Treatment of carbapenem-resistant Klebsiella pneumoniae: the state of the art. Expert Reviews. 11(2):159-177.

Pflaier, M. A., Sader, H. S., Fritsche, T. R., and Jones, R. N. 2006. Fimbrial activity of ceftepime tested against ceftazidime-resistant Gram-negative clinical strains from North American Hospitals: report from the SENTRY Antimicrobial Surveillance Program (1998-2004). Diagn. Microbiol. Infect. Dis. 56(1):63-68.

Pillai, R. R., Somayaji, S. N., Rabinovich, M., Hudson, M. C., and Consales, K. E. 2008. Nafcillin-loaded PLGA nanoparticles for treatment of osteomyelitis. Biomed. Mater. 3(3):7.

Pinto-Alphandary, H., Andremont, A., and Couverture, P. 2000. Targeted delivery of antibiotics using liposomes and nanoparticles: research and applications. Int. J. Antimicrob. Agents 13(3):155-168.

Proctor, R. A., von Eiff, C., Kahl, B. C., Becker, K., McNamara, P., Herrmann, M., and Peters, G. 2006. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. Nat. Rev. Microbiol. 4(4):295-305.

Protonotaridis, E., Dimitroulia, E., Pournaras, S., Ptitiriga, V., Sofianou, D., and Tsakris, A. 2010. Trends in antimicrobial resistance of clinical isolates of Enterococcus faecalis and Enterococcus faecium in Greece between 2002 and 2007. Journal of Hospital Infection. 75(3):225-227.

Pumerantz, A., et al. 2011. Preparation of liposomal vancomycin and intracellular killing of metillin-resistant Staphylococcus aureus (MRSA). Int. J. Antimicrob. Agents 37(2):140-144.

Pushparaj, S. P. et al. 2017. Novel pyocin-based PEGylated liposomes for enhanced delivery of antibiotics against resistant clinical isolates of Pseudomonas aeruginosa. Artificial Cells, Nanomedicine, and Biotechnology:1-11.

Queenan, A. M., and Bush, K. 2007. Carbapenemases: the versatile β-lactamases. Clin. Microbiol. Rev. 20(3):440-458.

Rajeev, A. J. 2000. The manufacturing techniques of various drugs loaded biodegradable poly (lactide-co-glycolide) PLGA devices. Biomaterials 21:2475-2490.

Rasamiravaka, T. et al. 2014. The formation of biofilms by Pseudomonas aeruginosa: a review of the natural and synthetic compounds interfering with control mechanisms. Biomed. Res. Int. 2015:1-17.

Reott, M. A. et al. 2008. TRAIL expression is induced in both osteoblasts containing intracellular Staphylococcus aureus and uninfected osteoblasts in infected cultures. FEMS Microbiol. Lett. 278(2):185-192.

Rice, L. B. 2008. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKEAPE in sight. Infect Dis 197(8):1079-1081.

Sabaeifard, P., Abdi-Ali, A., Gamazo, C., Irache, J. M., and Soudi, M. R. 2017. Improved effect of amikacin-loaded poly (D,L-lactide-co-glycolide) nanoparticles against planktonic and biofilm cells of Pseudomonas aeruginosa. J. Med. Microbiol. 66(2):137-148.

Santajit, S., and Indrawattana, N. 2016. Mechanisms of antimicrobial resistance in ESKEAPE pathogens. Biomed. Res. Int. 2016:1-8.

Schiffelers, R. M., Storm, G., and Bakker-Woudenberg, I. A. J. M. 2001. Host factors influencing the preferential localization of sterically stabilized liposomes in Klebsiella pneumoniae infected rat lung tissue. Pharm. Res. 18(6):780-787.

Silva, M. T., and Silva Pestana, N. T. 2013. The in vivo extracellular life of facultative intracellular bacterial parasites: role in pathogenesis. Immunobiology 218(3):325-337.

Silva, V. et al. 2011. Commensal gut bacteria: distribution of Enterococcus species and prevalence of Escherichia coli phylogenetic groups in animals and humans in Portugal. Ann. Microbiol. 62(2):449-459.

Singla, S. et al. 2016. Encapsulation of bacteriophage in liposome accentuates its entry in to macrophage and shields it from neutralizing antibodies. PLoS ONE. 11(4):1-16.

Slavcovici, A. et al. 2015. Antimicrobial resistance of ESKEAPE-pathogens in culture-positive pneumonia. Farmacia 63(2):201-205.

Soppimuth, K. S., Aminabahvi, T. M., Kulkarni, A. R., and Rudzinski, W. E. 2001. Biodegradable polymeric nanoparticles as drug delivery devices. J. Control. Release. 70(1-2):1-20.

Spence, S., 2015. Targeting Siglecs with a sialic acid-decorated nanoparticle abrogates inflammation. Science Translational Medicine. 7(303):303ra140.

Tan, N. C. W., Foreman, A., Jardeleza, C., Douglas, R., Vreugde, S., and Wormald, P. J. 2013. Intracellular Staphylococcus aureus: the Trojan horse of recalcitrant chronic rhinosinusitis? International Forum of Allergy and Rhinology. 3(4):261-266.

Vila, J. et al. 2007. Porins, efflux pumps and multidrug resistance in Acinetobacter baumannii. Journal of Antimicrobial Chemotherapy. 59(6):1210-1215.

Vuotto, C. et al. 2017. Biofilm formation and antibiotic resistance in Klebsiella pneumoniae urinary strains. J. Appl. Microbiol. 123(4):1003-1018.
Wang, R. E., Liu, T., Wang, Y., Cao, Y., du, J., Luo, X.,
Deshmukh, V., Kim, C. H., Lawson, B. R., Tremblay, M.
S., Young, T. S., Kazane, S. A., Wang, F., and Schultz, P.
G. 2015. An immunosuppressive antibody–drug conjuga-
gate. J. Am. Chem. Soc. 137(9):3229–3232.
Wei, Z. Q., du, X. X., Yu, Y. S., Shen, P., Chen, Y. G., and Li,
L. J. 2007. Plasmid-mediated KPC-2 in a Klebsiella
pneumoniae isolate from China. Antimicrob. Agents
Chemother. 51(2):763–765.
Weiss, G., and Schaible, U.E. 2015. Macrophage defense
mechanisms against intracellular bacteria. Immunol
Rev 264(1): 182–302.
WHO. World Health Organization 2015. Antibiotic resis-
tance: multi-country public awareness survey. http://
www.who.int/drugresistance/documents/
baselinesurveyen2015/en/
Wu, M. C., Deokar, A. R., Liao, J. H., Shih, P. Y., and Ling,
Y. C. 2013. Graphene-based photothermal agent for
rapid and effective killing of bacteria. ACS Nano
7(2):1281-1290.
Yang, S., Han, X., Yang, Y., Qiao, H., Yu, Z., Liu, Y., Wang,
J., and Tang, T. 2018. Bacteria-targeting nanoparticles
with microenvironment-responsive antibiotic release to
eliminate intracellular Staphylococcus aureus and
associated infection. Applied Materials & Interfaces.
10:14299-14311.
Yigit, H., Queenan, A. M., Anderson, G. J., Domenech-
Sanchez, A., Biddle, J. W., Steward, C. D., Alberti, S.,
Bush, K., and Tenover, F. C. 2001. Novel carbapenem-
hydrolyzing beta-lactamase, KPC-1, from a
carbapenem-resistant strain of Klebsiella pneumoniae.
Antimicrob. Agents Chemother. 45(4):1151–1161.
Zeeuwen, P. et al. 2018. Gram-positive anaerobe cocci are
underrepresented in the micro-biome of filaggrin-
deficient human skin. Journal of Allergy and Clinical
Immunology. 139(4):1368–1371.
Zhao, Y., Chen, Z., Chen, Y., Xu, J., Li, J., and Jiang, X.
2013. Synergy of non-antibiotic drugs and pyrimidinethiol on gold nanoparticles against superbugs. J. Am.
Chem. Soc. 135(35):12940-12943.