19. Lefebvre, B. *et al*. (2018) Ceftriaxone-resistant *Neisseria gonorrhoeae*, Canada, 2017. *Emerg. Infect. Dis.* **24**, 381–383. doi:10.3201/eid2402.171756

20. European Centre for Disease Prevention and Control (2018) Rapid risk assessment: extensively drug-resistant (XDR) *Neisseria gonorrhoeae* in the United Kingdom and Australia. https://ecdc.europa.eu/sites/portal/files/documents/RRA-Gonorrhoea%2C%20Antimicrobial%20resistance-United%20Kingdom%2C%20Australia.pdf (accessed 7 March 2019).

21. Whiley, D.M. *et al*. (2018) Genetic characterisation of *Neisseria gonorrhoeae* resistant to both ceftriaxone and azithromycin, Australia 2018. *Lancet Infect. Dis.* **18**, 717–718. doi:10.1016/S1473-3099(18)30340-2

22. Handsfield, H.H. *et al*. (1992) Evaluation of new anti-infective drugs for the treatment of uncomplicated gonorrhea in adults and adolescents. *Clin. Infect. Dis.* **15**, S123–S130. doi:10.1093/clind/15.Supplement_1.S123

23. Whiley, D.M. *et al*. (2017) Molecular antimicrobial resistance surveillance for *Neisseria gonorrhoeae*, Northern Territory, Australia. *Emerg. Infect. Dis.* **23**, 1478–1485. doi:10.3201/eid2309.170427

Biography

Dr David Speers is an Infectious Diseases Physician at Sir Charles Gairdner Hospital, Head of Microbiology, QEII Network at PathWest Laboratory Medicine WA and a Clinical Associate Professor for the Department of Medicine and Pharmacology, University of WA. He is a fellow of the Royal Australasian College of Physicians, the Royal College of Pathologists of Australasia, and the Australasian College of Tropical Medicine.

The rise and rise of antimicrobial resistance in Gram-negative bacteria



Adam Stewart, Hugh Wright and Krispin Hajkowicz

Infectious Diseases, Royal Brisbane and Women's Hospital, Brisbane, Qld, Australia
Tel: 0427 858 102, Email: Krispin.Hajkowicz@health.qld.gov.au

Antimicrobial resistance is a major threat to the delivery of effective care and already causes 700 000 excess deaths per year worldwide. International consensus on action to combat antimicrobial resistance was reached in 2015. Australia is implementing a national strategy. The clinical consequences of antimicrobial resistance are seen most acutely in multi-drug resistant Gram-negative bacterial infections, where they cause increased mortality and morbidity and threaten the delivery of once routine medical care. The solution to antimicrobial resistance is complex and multifaceted. Antimicrobial stewardship, that is optimising the use of the antibiotics we currently have, is the most rapidly deployable mitigation. Several novel antibiotics with activity against a range of drug-resistant bacteria are now available clinically, leading to hope that innovative solutions will reduce the impact of resistance. It is critical that these new drugs are protected from inappropriate use.

Critical to the survival of microorganisms on Earth is their ability to evade destruction by mutation of key effector genes, leading to adaptation and selective evolution. Antimicrobial resistance (AMR) emerged billions of years ago in nature, unrelated to human use of antimicrobials[1]. Most human pathogenic bacteria develop AMR to antibiotics within a few years of their first use. For example, the Florey group demonstrated penicillinases able to hydrolyse penicillin in *Staphylococcus aureus* in 1940, before clinical use in humans[2]. Penicillin resistance leading to clinical treatment failure was described in 1942[3]. The selection of antimicrobial resistance is inevitable whether or not antimicrobial use is appropriate, but interventions that impede this process can delay the onset of AMR

as a public health problem. In some cases, AMR gene mutations lead to a 'fitness cost' to the organism so that, with time, they are less able to compete with non-resistant, 'wild-type' organisms and reversion to susceptibility occurs.

AMR is recognised as one of the major threats to the health and wellbeing of humans on a global scale. It is estimated that 700 000 people now die every year as a consequence of AMR. This may result in an estimated 0.06–3.1% decline in world Gross Domestic Product by 2050 if current trends continue[4]. In 2015, the General Assembly of the United Nations endorsed a declaration to take broad, coordinated international action to address the root causes of antimicrobial resistance across human and animal health and agriculture.

In Australia, the importance of AMR is recognised in the First National Antimicrobial Resistance Strategy 2015–2019, in which seven objectives to reduce AMR are established[5]. When released in 2015, the Australian Strategy was a world leader. A second strategy is due in 2020.

In this review, we choose to focus on AMR in Gram-negative bacteria such as including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Escherichia coli* and other Enterobacteriales as we feel this is the most urgent aspect of the AMR crisis. High risk infections including pneumonia, bloodstream infection, urinary tract and intra-abdominal sepsis are often caused by Gram-negative bacteria. Gram-negatives are a common cause of rapidly progressive septic shock, leading to death in as little as 12 hours from the onset of illness, particularly in immunocompromised patients. Currently, treatment of Gram-negative infection is 'empirical' in the first 48 hours, that is, the selection of treatment is based on knowledge of the likely organisms and resistance spectrum based on population data and often therefore requires use of broad-spectrum therapy until culture and susceptibility results become available.

Common resistance mechanisms among Gram-negative organisms include: beta-lactamase production leading to lysis of beta-lactam antibiotics; target-enzyme mutations; porin mutations; and drug efflux pumps[6]. Multiple, simultaneously acquired resistance mechanisms are readily transmitted by plasmids between organisms and across different species, leading to multiple-drug resistant (MDR) organisms. Beta-lactamase production is clinically divided into those that hydrolyse third-generation cephalosporins, known as extended spectrum beta-lactamases (ESBLs), and those that hydrolyse and carbapenems such as meropenem, known as carbapenemases[7]. ESBLs are now found in 15–25% of Enterobacteriales responsible for hospital acquired infections in the United States and Europe, with rates much higher reported in Asia and South America[8]. Australia has observed a relatively low frequency caused by MDR-pathogens with ceftriaxone resistance in *E. coli* and *Klebsiella pneumoniae* seen in 3.2% and 3.6% of community isolates, respectively[9]. The proportion in hospital-acquired infection is likely to be higher.

Clinical outcomes are worse in individuals infected with MDR-Gram-negative bacteria. In the critically ill population with ESBL bloodstream infection, 30-day mortality ranges 7–23.3%[10,11]. Individuals with carbapenemase-producing *Klebsiella pneumoniae* infection have a mortality of 33–50%[12]. Similar adverse outcomes are described with *P. aeruginosa* and other Enterobacteriales[13,14]. Contributors to worse outcomes in this patient group include delay in effective antibiotic therapy, increased antimicrobial toxicity and presence of significant comorbidities. Rectal colonisation with ESBL-producing Enterobacteriales is associated with increased risk of hospital acquired pneumonia, bacteraemia and urinary tract infection with the same organism[15]. A recent study identified multi-drug resistant Gram-negative colonisation as an independent predictor of death in the ICU at 28 days, although other studies contradict this finding[11,16]. Colonised patients have both longer hospital and intensive care unit stays, as well as experience an increased exposure to broad-spectrum antibiotics, further selecting AMR[11].

AMR in Gram-negatives significantly impairs healthcare delivery, particularly in vulnerable patient groups. Haematopoietic stem cell transplant recipients with a carbapenem-resistant *K. pneumoniae* infection had a mortality rate of 64% in one Italian study[17]. In areas with a high prevalence of carbapenem-resistant Enterobacteriales (CRE), *Pseudomonas aeruginosa* (CRP) and *Acinetobacter baumannii* (CRAB), such as India and the Middle East, delivery of much healthcare currently seen as routine is under threat. This includes solid organ and haematopoietic stem cell transplantation; cancer chemotherapy; surgery involving implantation of prosthetic material and intensive care. A multinational cohort study showed that patients with ESBL-Enterobacteriaecae colonisation prior to colorectal surgery had an increased risk of deep surgical site infection[18]. Gram-negative multi-drug resistance is associated with increased inpatient costs in patients with bloodstream infection[19].

The response to AMR is complex, requiring improved clinical practice, education of healthcare workers and the community, targeted clinical and translational research, enhanced surveillance, rapid diagnosis, and novel antimicrobials. The response must adopt a 'One Health' approach including human and veterinary medicine, agricultural and environmental management.

Sadly, the Australian community has an addiction to antibiotics, using more antibiotics per head of population than almost any

other country[20]. Antimicrobial stewardship (AMS) – ensuring the quality use of the antimicrobials to prevent inappropriate use whilst optimising outcomes of infection – is the most rapidly deployable response to slow the further emergence of AMR. Mandated by hospital accreditation, AMS is much more developed and effective in hospital settings in Australia, compared with community settings where most antibiotic use occurs. Interventions that include both persuasive educational and restrictive governance components are the most successful. AMS interventions need to be multidisciplinary including specific medical, nursing and pharmacy components. Patient and community engagement including mass-marketing campaigns are essential.

Most Australian microbiology laboratories have developed extensive capabilities in the timely identification of resistant Gram-negatives, although detection of some resistance mechanisms is technically difficult. However, national standardised reporting and notification of AMR is not established, impeding surveillance efforts and rapid public health response to emerging AMR threats in Australia.

The development of new antimicrobials to combat AMR has languished until recently. In the USA, the GAIN (Generating Antibiotic Incentives Now) Act and the European Union's Innovative Medicines Initiative (IMI) ND4BB (New Drugs for Bad Bugs) provide financial incentives and streamlined processes encouraging pharmaceutical companies to develop antibiotics, leading to the release of two new antibiotics in the past five years and over 30 compounds in active development[21].

Several promising novel agents are now available or in late-stage development for MDR-Gram-negative organisms. Ceftolozane/tazobactam is a novel cephalosporin combined with a beta-lactamase inhibitor, notable for its potent activity against MDR-*P. aeruginosa*[22]. Ceftazidime/avibactam is a combination agent comprising a third-generation cephalosporin with the novel beta-lactamase inhibitor avibactam, which protects ceftazidime from hydrolysis from many serine beta-lactamases. Notably ceftazidime/avibactam has potent activity against CPE-producing KPC carbapenemases, but not metallo-beta lactamases[23]. These agents are available and in use in Australia. Multiple other beta lactam/beta lactamase inhibitor combinations are in development.

Cefiderocol is a novel siderophore cephalosporin with a broad spectrum of activity against Gram-negative organisms. A side chain uses the organisms' iron transport system to be transported intracellularly[24]. Cefiderocol has been shown to be active against ESBL-producing organisms including those containing metallo-beta lactamases. It is also potent against MDR *Acinetobacter baumanii*, *P. aeruginosa* and *Stenotrophomonas maltophilia*. A study examining cefiderocol in the setting of serious infections caused by carbapenem-resistant organisms is underway[25].

A novel aminoglycoside, plazomicin is active *in vitro* against CPE and ESBL-Enterobacteriales isolates. In a randomised controlled trial comparing plazomicin with meropenem for the treatment of cUTI, plazomicin showed non-inferiority for clinical and microbiological cure[26]. A study examining the use of plazomicin for serious infections caused by carbapenem resistant Enterobacteriales was unfortunately terminated early due to difficulties with recruitment[27].

Recent progress in drug development will provide clinicians with additional options for treating infections caused by multi-drug resistant Gram-negative organisms. However, reports of clinical failure and the development of antimicrobial resistance have already been reported to the new agents[28]. Optimal AMS is essential to preserve the effectiveness of novel antibiotics.

AMR in Gram-negatives is an urgent, important threat to global health. Co-ordinated global action on the clinical, governance and research is underway but requires markedly increased investment. Recently, several exciting novel agents with activity against MDR Gram-negatives have emerged. There is hope that human innovation will limit the consequences of this potentially catastrophic problem.

Conflicts of interest

Dr Wright is an investigator on a clinical trial sponsored by Shionogi Inc. Dr Stewart and Dr Hajkowicz declare no conflicts of interest.

Acknowledgements

This research did not receive any specific funding.

References

1. Van Goethem, M.W. *et al.* (2018) A reservoir of 'historical' antibiotic resistance genes in remote pristine Antarctic soils. *Microbiome* **6**, 40. doi:10.1186/s40168-018-0424-5
2. Abraham, E.P. and Chain, E. (1988) An enzyme from bacteria able to destroy penicillin. *Rev. Infect. Dis.* **10**, 677–678.
3. Rammelkamp, T. (1942) Resistance of *Staphylococcus aureus* to the action of penicillin. *Exp. Biol. Med.* **51**, 386–389. doi:10.3181/00379727-51-13986
4. O'Neill, J. (2016). Tackling drug-resistant infections globally: final report and recommendations. The Review on Antimicrobial Resistance. HM Government of the United Kingdom, London.
5. Commonwealth of Australia (2015) Responding to the Threat of Antimicrobial Resistance. Australia's First National Antimicrobial Resistance Strategy 2015–2019. Canberra: Commonwealth of Australia.
6. Peleg, A.Y. and Hooper, D.C. (2010) Hospital-acquired infections due to Gram-negative bacteria. *N. Engl. J. Med.* **362**, 1804–1813. doi:10.1056/NEJMra0904124

7. Harris, P. *et al*. (2015) Facing the challenge of multidrug-resistant gram-negative bacilli in Australia. *Med. J. Aust.* **202**, 243–247. doi:10.5694/mja14.01257
8. World Health Organization (2014) Antimicrobial resistance – global report on surveillance. https://apps.who.int/iris/bitstream/handle/10665/112642/9789241564748_eng.pdf;jsessionid=519244EE0EEF520027CE4098504150B4?sequence=1
9. Rogers, B.A. *et al*. (2014) Predictors of use of infection control precautions for multiresistant Gram-negative bacilli in Australian hospitals: analysis of a national survey. *Am. J. Infect. Control* **42**, 963–969. doi:10.1016/j.ajic.2014.05.035
10. Corcione, S. *et al*. (2018) Epidemiology and risk factors for mortality in bloodstream infection by CP-Kp, ESBL-E, Candida and CDI: a single center retrospective study. *Eur. J. Intern. Med.* **48**, 44–49. doi:10.1016/j.ejim.2017.10.015
11. Emmanuel Martinez, A. *et al*. (2019) ESBL-colonization at ICU admission: impact on subsequent infection, carbapenem-consumption, and outcome. *Infect. Control Hosp. Epidemiol.* doi:10.1017/ice.2019.5
12. Xu, L. (2017) Systematic review and meta-analysis of mortality of patients infected with carbapenem-resistant *Klebsiella pneumoniae*. *Ann. Clin. Microbiol. Antimicrob.* **16**, 18. doi:10.1186/s12941-017-0191-3
13. Zhang, Y. *et al*. (2016) Mortality attributable to carbapenem-resistant *Pseudomonas aeruginosa* bacteremia: a meta-analysis of cohort studies *Emerg. Microbes Infect.* **5**, e27. doi:10.1038/emi.2016.22
14. Chiotos, K. *et al*. (2018) Increased 30-day mortality associated with carbapenem-resistant Enterobacteriaceae in children *Open Forum Infect. Dis.* **5**, ofy222-doi:10.1093/ofid/ofy222
15. Biehl, L.M. *et al*. (2016) Colonization and infection with extended spectrum beta-lactamase producing Enterobacteriaceae in high-risk patients – review of the literature from a clinical perspective. *Crit. Rev. Microbiol.* **42**, 1–16. doi:10.3109/1040841X.2013.875515
16. Barbier, F. *et al*. (2016) Colonization and infection with extended-spectrum beta-lactamase-producing Enterobacteriaceae in ICU patients: what impact on outcomes and carbapenem exposure? *J. Antimicrob. Chemother.* **71**, 1088–1097. doi:10.1093/jac/dkv423
17. Girmenia, C. *et al*. (2015) Infections by carbapenem-resistant *Klebsiella pneumoniae* in SCT recipients: a nationwide retrospective survey from Italy *Bone Marrow Transplant.* **50**, 282–288. doi:10.1038/bmt.2014.231
18. Dubinsky-Pertzov, B. *et al*. (2018) Carriage of extended-spectrum beta-lactamase-producing Enterobacteriaceae and the risk of surgical site infection after colorectal surgery: a prospective cohort study. *Clin. Infect. Dis.* ciy768
19. Thaden, J.T. *et al*. (2017) Increased costs associated with bloodstream infections caused by multidrug-resistant Gram-negative bacteria are due primarily to patients with hospital-acquired infections. *Antimicrob. Agents Chemother.* **61**, e01709-16. doi:10.1128/AAC.01709-16
20. Australian Commission on Safety and Quality in Health Care (ACQSHC) (2016). AURA 2016 – First Australian report on antimicrobial use and resistance in human health. Sydney: ACSQHC. https://www.safetyandquality.gov.au/publications/aura-2016-first-australian-report-on-antimicroibal-use-and-resistance-in-human-health/ (accessed 20 March 2019).
21. Bush, K. and Page, M.G. (2017) What we may expect from novel antibacterial agents in the pipeline with respect to resistance and pharmacodynamic principles. *J. Pharmacokinet. Pharmacodyn.* **44**, 113–132. doi:10.1007/s10928-017-9506-4
22. Zhanel, G.G. *et al*. (2014) Ceftolozane/tazobactam: a novel cephalosporin/b-lactamase inhibitor combination with activity against multidrug-resistant Gram-negative bacilli. *Drugs* **74**, 31–51. doi:10.1007/s40265-013-0168-2
23. Li, H. *et al*. (2015) *In vitro* susceptibility of characterized β-lactamase-producing strains tested with avibactam combinations. *Antimicrob. Agents Chemother.* **59**, 1789–1793. doi:10.1128/AAC.04191-14
24. Möllmann, U. *et al*. (2009) Siderophores as drug delivery agents: application of the 'Trojan Horse' strategy. *Biometals* **22**, 615–624. doi:10.1007/s10534-009-9219-2
25. ClinicalTrials.gov (2016) Study of S-649266 or best available therapy for the treatment of severe infections caused by carbapenem-resistant Gram-negative pathogens (CREDIBLE e CR). https://clinicaltrials.gov/ct2/show/NCT02714595 (accessed March 2019).
26. Wagenlehner, F.M.E. *et al*. (2019) Once-daily plazomicin for complicated urinary tract infections. *N. Engl. J. Med.* **380**, 729–740. doi:10.1056/NEJMoa1801467
27. McKinnell, J.A. *et al*. (2019) Plazomicin for infections caused by carbapenem-resistant Enterobacteriaceae. *N. Engl. J. Med.* **380**, 791–793. doi:10.1056/NEJMc1807634
28. Shields, R.K. *et al*. (2016) Clinical outcomes, drug toxicity, and emergence of ceftazidime-avibactam resistance among patients treated for carbapenem-resistant Enterobacteriaceae infections. *Clin. Infect. Dis.* **63**, 1615–1618. doi:10.1093/cid/ciw636

Biographies

Adam Stewart is an Infectious Diseases Advanced Trainee at the Royal Brisbane and Women's Hospital. He is a Medical Research Fellow at University of Queensland, Centre for Clinical Research (UQCCR) and is currently undertaking a Doctor of Philosophy (PhD) in the field of resistant Gram-negative bloodstream infections and clinical trials. He is also the winner of a Queensland Health Junior Doctor Research Fellowship and is a lecturer at University of Queensland, School of Medicine.

Dr Hugh Wright is an infectious diseases physician at the Royal Brisbane and Women's Hospital and a lecturer at the University of Queensland. He is currently undertaking a PhD examining new treatment options for severe infections caused by multi-resistant Gram-negative bacteria.

Krispin Hajkowicz is the Director of Infectious Diseases and the Infection Management and Prevention Service at Royal Brisbane and Women's Hospital and a Senior Lecturer in the University of Queensland School of Clinical Medicine. He was also the founding Director of the Queensland Statewide Antimicrobial Stewardship Program and has published widely in the international literature in antimicrobial resistance and stewardship.