Communication is key: do bacteria use a universal 'language' to spread resistance?

"Understanding (how) bacterial cells communicate ... would ultimately reduce the window of therapeutic failure in treating bacterial infections."

Omar M El-Halfawy^{1,2} & Miguel A Valvano^{*1,3}

¹Center for Human Immunology & Department of Microbiology & Immunology, University of Western Ontario, London, ON, N6A 5C1, Canada

²Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Alexandria University, Egypt

³Centre for Infection & Immunity, Queen's University, Belfast, BT9 7AE, UK

*Author for correspondence: Tel.: +44 28 9097 2878 ■ Fax: +44 28 9097 2671 ■ m.valvano@qub.ac.uk

The relentless increase in antibiotic resistance poses serious problems to treat microbial infections. It is more common for patients to suffer therapeutic failures upon antibiotic treatment, leading to greater morbidity and mortality, as well as higher economic and social burden on healthcare systems. It did not take long after the discovery of the first antibiotics early in the 20th century for antibiotic-resistant bacteria to be detected. Bacterial resistance to antibiotics was reported before the mid-1940s, marking the start of an endless race between introducing novel antimicrobials and the continuing emergence of resistance.

For more than 50 years, research focused on the transfer of genetic determinants encoding antibiotic resistance markers. Such efforts led to tremendous advances in the understanding of the mobilization of genetic elements among different bacteria and revealed the mechanism of stable acquirement of new phenotypes by bacteria. Nevertheless, this does not fully explain therapeutic failures by antibiotics and cases of transient increase in resistance to antibiotics by certain bacteria. The therapeutic outcome of antibiotic treatment does not necessarily correlate with the expectations based on *in vitro* susceptibility testing performed on individual clinical isolates [1].

Several small molecules modulate the bacterial response to antibiotics [2]. Some of these molecules are produced and secreted from bacteria themselves. These are not enzymes that degrade antibiotics such as β -lactamases or proteases, but rather molecules modulating antibiotic resistance through signaling pathways not involving acyl-homoserine lactones. Such molecules were not recognized as communicators of resistance

between different bacterial cells until recently. Here, we would like to highlight the contribution of small molecule-mediated intercellular communication of antibiotic resistance, in particular involving putrescine, indole and ammonia. The polymicrobial nature of many infections makes the possibility of crosstalk between the different bacterial species coexisting in the same community more likely [3]. Hence, molecules sensed and utilized by a wider range of bacterial species that communicate resistance across different bacterial species can be highly relevant clinically and could provide more conceivable targets for drug design.

Polyamines

Polyamines are small organic molecules derived from certain amino acids. The commonly found natural polyamines are the diamines putrescine and cadaverine, the triamine spermidine and the tetramine spermine. They are found in almost all living organisms [4]. Polyamines are produced by nearly all bacteria, with rare exceptions such as *Staphylococcus aureus* strains to which some polyamines, namely spermidine and spermine, are toxic as they lack polyamine-detoxifying enzymes [5]. The most common bacterial polyamines are putrescine and spermidine [6]. While polyamines play roles in bacterial growth and other physiological processes [6], they also contribute to the bacterial responses to antibiotics demonstrated by the response of polyamine-deprived mutants and bacteria exposed to exogenous polyamines and antibiotics [2].

Recently, putrescine released from bacteria was shown to directly communicate antibiotic resistance between different bacterial cells within the same or different species [7].

Keywords

■ ammonia ■ antibiotic resistance ■ chemical communication ■ indole ■ polyamines ■ putrescine

Future
Medicine part of

Editorial
Future Microbiology

Putrescine released in excess from a highly resistant subset of the population of the extremely antibiotic-resistant environmental bacterium *Burkholderia cenocepacia* protected less resistant *B. cenocepacia* cells from the action of the antimicrobial peptide polymyxin B [7]. Given that polyamines are being produced and utilized by most of the bacterial species, it could be speculated that the excessive secretion of putrescine can communicate resistance to antibiotic-sensitive cells from species other than *B. cenocepacia*. Interestingly, this notion was supported by the protection of *Pseudomonas aeruginosa* PAO1 from the action of polymyxin B by putrescine released from *B. cenocepacia* in direct coculture [7]. Putrescine secretion was stimulated by the exposure to polymyxin B as a result of the overexpression of the ornithine decarboxylase enzyme, supporting its role in response to the antibiotic. Putrescine could be involved in similar communication of resistance against other antibiotics, especially bactericidal antibiotics such as the fluoroquinolone norfloxacin, the β -lactam ceftazidime and rifampicin [7].

Indole

Indole is an aromatic heterocyclic organic compound derived from the amino acid tryptophan in a reaction mediated by the *tnaA* tryptophanase. It is produced by approximately 85 species of Gram-positive and Gram-negative bacteria [8]. Lee *et al.* revealed that few antibiotic-resistant mutant cells that arise in an *Escherichia coli* population in response to norfloxacin or gentamicin improved the overall response of the bacterial population towards the antibiotics in part due to indole production [9]. Indole production was not induced in the more resistant mutant cells by the antibiotics, but rather its level was unchanged regardless of antibiotic exposure, as opposed to wild-type bacteria in which indole production was suppressed in response to the antibiotics [9]. The improved response to antibiotics mediated by indole was attributed to the stimulation of certain drug efflux pumps and oxidative stress protective mechanisms.

Whether or not indole is a universal signal mediating the communication of antibiotic resistance between different bacteria has not been explored. Given that only few bacterial species are indole-positive from a total of more than 7000 bacterial species [10], it may be speculated that indole would not be a common signal mediating antibiotic resistance. Nevertheless, recent reports have shown that exogenous indole might influence antibiotic resistance in indole-negative

bacteria by inducing the expression of multidrug efflux pumps in *Salmonella enterica* serovar *Typhimurium* [11], whereas it increased resistance of *P. aeruginosa* PAO1 to tetracycline, gentamicin and ampicillin by repressing genes encoding the *mexGHI-opmD* multidrug efflux pump [12]. Future studies are required to elucidate the role of indole in interspecies communication of antibiotic resistance.

Volatile signals

Bacteria produce diverse volatile compounds; however, the ecological role of many of these compounds remains unknown [13]. Volatile-mediated transfer of antibiotic resistance to ampicillin in *E. coli* was first reported in 2002; however, the nature of the airborne signal was unknown [14]. A recent study showed that exposure to gaseous ammonia, a catabolic product of L-aspartate, released from stationary phase *E. coli* K12 cultures alters the antibiotic resistance profile of several Gram-negative and Gram-positive bacteria [15]. Ammonia increased resistance to tetracycline in *E. coli* BL21, *P. aeruginosa* Lm1, *Bacillus subtilis* and *S. aureus* Xen36, whereas it increased sensitivity to the aminoglycoside kanamycin. These effects resulted from an ammonia-dependent increase in polyamine levels; however, whether or not the ammonia release was induced in response to antibiotics was not determined [15]. Interestingly, volatiles emitted from the tested Gram-positive and Gram-negative bacteria increased resistance of *E. coli* to tetracycline. However, volatiles emitted from *E. coli* did not alter resistance to ticarcillin, chloramphenicol, ofloxacin and vancomycin [15].

“...future research is required to elucidate the molecular basis of the chemical communication of antibiotic resistance, the chemical signals involved, and its role in the spread of resistance among bacteria...”

The filtered supernatant of an overnight culture of a highly resistant subpopulation of *B. cenocepacia* in polymyxin B communicated higher-level resistance to physically distant less polymyxin-resistant mutant of *B. cenocepacia* and three different *E. coli* strains. This volatile-dependant protection was proposed to be attributed to the overexpression of putrescine in response to polymyxin B [7], owing to the volatile nature of polyamines [16].

Conclusion & future perspective

Understanding the language by which bacterial cells communicate resistance against antibiotics to other bacterial cells from the same or different species would significantly pave the way to design new therapeutics that would reduce antibiotic resistance in individual bacteria and block the communication of such resistance among different bacteria. This would ultimately reduce the window of therapeutic failure in treating bacterial infections. Indeed, the inhibitors would better serve this goal if they target a more universal signal rather than a molecule specific to a certain group of bacteria. Putrescine and other polyamines appear to be a plausible target for such inhibitors given their universal nature and their newly established role in the communication of antibiotic resistance. For example, pharmacological inhibition of ornithine decarboxylase, which converts ornithine into putrescine, reduced resistance of *B. cenocepacia* to polymyxin B [7]. This encourages future development

of similar inhibitors against putrescine and other small molecules communicating antibiotic resistance that can be safe for human use as antibiotic adjuvants. On the other hand, future research is required to elucidate the molecular basis of the chemical communication of antibiotic resistance, the chemical signals involved and its role in the spread of resistance among bacteria leading to therapeutic failure of antimicrobial therapy.

Financial & competing interests disclosure

Research in the authors' laboratory is funded by grants from Cystic Fibrosis Canada and a Marie Curie Career Integration Grant, project 618095. OM El-Halfawy was supported by an Ontario Graduate Scholarship. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

- Smith AL, Fiel SB, Mayer-Hamblett N, Ramsey B, Burns JL. Susceptibility testing of *Pseudomonas aeruginosa* isolates and clinical response to parenteral antibiotic administration: lack of association in cystic fibrosis. *Chest* 123(5), 1495–1502 (2003).
- El-Halfawy OM, Valvano MA. Non-genetic mechanisms communicating antibiotic resistance: rethinking strategies for antimicrobial drug design. *Expert Opin. Drug Discov.* 7(10), 923–933 (2012).
- Peters BM, Jabra-Rizk MA, O'May GA, Costerton JW, Shirliff ME. Polymicrobial interactions: impact on pathogenesis and human disease. *Clin. Microbiol. Rev.* 25(1), 193–213 (2012).
- Tabor CW, Tabor H. Polyamines. *Annu. Rev. Biochem.* 53, 749–790 (1984).
- Joshi GS, Spontak JS, Klapper DG, Richardson AR. Arginine catabolic mobile element encoded speG abrogates the unique hypersensitivity of *Staphylococcus aureus* to exogenous polyamines. *Mol. Microbiol.* 82(1), 9–20 (2011).
- Wortham BW, Patel CN, Oliveira MA. Polyamines in bacteria: pleiotropic effects yet specific mechanisms. *Adv. Exp. Med. Biol.* 603, 106–115 (2007).
- El-Halfawy OM, Valvano MA. Chemical communication of antibiotic resistance by a highly resistant subpopulation of bacterial cells. *PLoS ONE* 8(7), e68874 (2013).
- Lee JH, Lee J. Indole as an intercellular signal in microbial communities. *FEMS Microbiol. Rev.* 34(4), 426–444 (2010).
- Lee HH, Molla MN, Cantor CR, Collins JJ. Bacterial charity work leads to population-wide resistance. *Nature* 467(7311), 82–85 (2010).
- Achtman M, Wagner M. Microbial diversity and the genetic nature of microbial species. *Nat. Rev. Microbiol.* 6(6), 431–440 (2008).
- Nikaido E, Shirotsuka I, Yamaguchi A, Nishino K. Regulation of the AcrAB multidrug efflux pump in *Salmonella enterica* serovar *Typhimurium* in response to indole and paraquat. *Microbiology* 157(Pt 3), 648–655 (2011).
- Lee J, Attila C, Cirillo SL, Cirillo JD, Wood TK. Indole and 7-hydroxyindole diminish *Pseudomonas aeruginosa* virulence. *Microb. Biotechnol.* 2(1), 75–90 (2009).
- Schulz S, Dickschat JS. Bacterial volatiles: the smell of small organisms. *Nat. Prod. Rep.* 24(4), 814–842 (2007).
- Heal RD, Parsons AT. Novel intercellular communication system in *Escherichia coli* that confers antibiotic resistance between physically separated populations. *J. Appl. Microbiol.* 92(6), 1116–1122 (2002).
- Bernier SP, Letoffe S, Delepierre M, Ghigo JM. Biogenic ammonia modifies antibiotic resistance at a distance in physically separated bacteria. *Mol. Microbiol.* 81(3), 705–716 (2011).
- Ross BM, Babay S, Ladouceur C. The use of selected ion flow tube mass spectrometry to detect and quantify polyamines in headspace gas and oral air. *Rapid Commun. Mass Spectrom.* 23(24), 3973–3982 (2009).