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EDITORIAL

Perspectives towards antibiotic resistance: from molecules to population

Joon-Hee Lee*

Department of Pharmacy, College of Pharmacy, Pusan National University, Busan 46241, Republic of Korea

For a long time, antibiotics have been 'magical weapons' to combat pathogenic microbes. The success of antibiotics is now greatly threatened by resistance to antibiotics and many scientists have already talked about the coming of the post-antibiotic era. This special issue is prepared to understand recent research findings and new concepts about antibiotic resistance. Above all, this special issue explores mechanisms for the generation, selection, and spread of antibiotic resistance, and gives insight into what to target to prevent the development of antibiotic resistance. Just as antibiotics came from the concept of "magic bullet", a breakthrough will come from a new concept based on a profound understanding of antibiotic resistance.

Keywords: antibiotic resistance, tripartite efflux pump, adaptive resistance, herd resistance, persister

Introduction

Until the beginning of the 20th century, the life expectancy of human at birth was about 47 years even in the industrialized world and a variety of infectious diseases were a cause of high mortality (Adedeji, 2016). As a way to cope with these infectious diseases, Paul Ehrlich (1854–1915), a German scientist who developed a synthetic arsenic drug, arsphenamine (salvarsan) to treat syphilis, speculated about a "magic bullet" that could destroy a pathogen without harming the host (Funke *et al.*, 2016). The concept of the "magic bullet" became a reality with the discovery of penicillin by Alexander Fleming (1881–1955), which is one of the greatest triumphs of science so far. Unfortunately, mankind is at a serious risk of taking away the trophy of this great triumph and the antibiotic medication have now reached a crisis point due to the resistance problem.

Actually, the antibiotic resistance was already cautioned by the first discoverer of antibiotics, A. Fleming. He predicted the high public demand and abuse of antibiotics, and eventual generation of resistance in early 1945 (Spellberg and Gilbert, 2014). Penicillin was discovered in 1928, and a penicillinase was identified in 1940 before the therapeutic use of penicillin (Abraham and Chain, 1988; Davies and Davies, 2010). The spread of penicillin resistance was already documented by 1942, when penicillin was only used for a short period of time in clinic, and during the next few years, the infections caused by penicillin-resistant *Staphylococcus aureus* rapidly rose, spreading quickly from hospitals to communities (Lobanovska and Pilla, 2017). MRSA (methicillin-resistant *S. aureus*) is now a common term we hear on a daily basis and even the general public is familiar with this term. Unfortunately, resistance seems to appear for almost all antibiotics developed so far. Many scientists are warning of post-antibiotic era, which means no longer use of antibiotics in therapy and returning to the pre-antibiotic age.

How can we solve the problem of antibiotic resistance? Just as antibiotics came from the concept of "magic bullet", a breakthrough will come from a new concept based on a profound understanding of antibiotic resistance. This special issue is designed to assist in this profound understanding and covers the latest advances in our knowledge of antibiotic resistance. Above all, this special issue explores mechanisms for the generation, selection, and spread of antibiotic resistance, and gives insight into what to target to prevent the resistance.

Molecular mechanisms of antibiotic resistance and their origins

Mechanisms and cellular molecules responsible for antibiotic resistance have been elucidated and well documented in many review papers (Davies and Davies, 2010; Blair *et al.*, 2015). Enzymatic destruction or inactivation of the antibiotics is the first known antibiotic resistance mechanism, and penicillinases or β-lactamases that selectively hydrolyze β-lactam ring of penicillin and penicillin-related antibiotics are a good example for this mechanism. Alteration of the drugs' target sites is also an important mechanism. Minor modification at these sites can neutralize the effects of antibiotics without significantly affecting the cellular function. Prevention of penetration to the target site within the microbial cells is also important resistance mechanism. This mechanism is the most important reason why Gram-negative bacteria are generally more resistant to antibiotics than Gram-positive bacteria. Otherwise, the resistance may occur by rapid efflux of anti-

*For correspondence. E-mail: joonhee@pusan.ac.kr; Tel.: +82-51-510-2821; Fax: +82-51-513-6754

biotics.

In this special issue, all of these mechanisms will not be covered, but Jo *et al*., will introduce a recent remarkable progress in knowledge about a type of efflux system, tripartite efflux pump systems (Jo *et al*., 2019). Tripartite efflux pumps and the type I secretion system of Gram-negative bacteria are large protein complexes in cell envelope that function to expel toxic substances or transport toxins. In their review, Jo *et al*. describe the current assembly models with a historical view to date, emphasizing the common assembly mechanism for diverse tripartite pumps and type I secretion systems.

Where and how did these resistance mechanisms originate? Recently, remarkable insight into this intriguing question have been given by Gerard D. Wright. He suggested new term "resistome" that is the complete set of all the antibiotic resistance genes and their precursors in the entire ecosystem (Wright, 2007). In his suggestion, the resistome includes various types of genes: resistance genes found in pathogenic or antibiotic-producing bacteria, cryptic (or silent) resistance genes, and precursor genes (Wright, 2007). These gene groups are not independent, and some are overlapped. In natural environment, the resistance genes are usually found in antibiotic-producing microorganisms, regardless of their pathogenicity, because they should have their own protection mechanisms to protect themselves from the antibiotics. These resistance genes are a natural source for the antibiotic resistance of pathogenic microbes, and provide pathogens with the potential to be selected in human antibiotic medication. Cryptic resistance genes are just embedded in microbial chromosomes with too low or no expression levels, so it is not obvious whether they really confer resistance, or not. Precursor genes encode proteins with potential resistance activity, so possibly evolve into effective resistance genes. Cryptic and precursor genes may evolve to a full resistance gene given the appropriate modification and selection; they are large reservoir for the antibiotic resistance.

How does the resistance spread?

When we say that antibiotic resistance is spreading, we need to understand what exactly is spreading, in order to precisely target the things that is responsible for resistance. Antibiotic resistance is often divided into intrinsic and acquired resistance: bacteria can be intrinsically resistant to certain antibiotics but can also acquire the resistance from surroundings. Since the resistance mechanisms mentioned above are mediated by cellular molecules and based on genetic modification, resistance can be transferred between bacteria by the acquisition of genetic elements containing resistance genes, so called, horizontal gene transfer. However, since not all of these genetic elements are deliverable, some must have been to themselves from the beginning, and this represents intrinsic resistance, so to speak, proto-resistance.

When antibiotic resistance are spreading, in the case of intrinsic resistance, the resistant strains are selected, but in the case of acquired resistance, the genetic elements involved in the resistance are actually selected. This is a small difference, but worth mentioning. The Frederick Griffith's experiment in 1928, which first discovered bacterial transformation, showed that genetic elements could be transferred from dead cells (Griffith, 1928). Therefore, acquired resistance can be transmitted in microbial population via contact with dead bacteria (or their debris containing genetic element) or contact between different species, and spread through repeated processes of transmission and selection. If the selective pressure is withdrawn, the resistance gene may disappear, but the strain can exist susceptible to antibiotics. However, the spreading of intrinsic resistance is a result of the dominance of the resistant strain, and there is no exchange of genetic elements. Therefore, it is the resistance gene that is selected in the spread of the acquired resistance, and the one that is selected in the spread of the intrinsic resistance is the resistant strain itself. In any case, selection is a very important process in the spread of antibiotic resistance, and this is why the most important factor in antibiotic resistance problem has been recognized as misuse or overuse of antibiotics.

In this special issue, Kwan Soo Ko introduces the international spread of clinically important antibiotic resistant pathogens (Ko, 2019). Since antibiotic resistant pathogens can spread nationally, internationally, and sometimes globally, this review shows us the urgency of global monitoring and international cooperation to prevent the emergence and spread of antibiotic resistance.

Adaptive resistance

In addition to intrinsic and acquired antibiotic resistance, another important concept, "adaptive resistance" has recently become an issue. So far, the acquisition of resistance genes and selection of resistant strains have been recognized as the most important factors for the generation and spread of the antibiotic resistance. However, adaptive resistance is an induced resistance to one or more antibiotics by a specific signal or environmental condition, and this adaptive resistance is conditional, unstable, transient, and not vertically inherited: this increase of resistance generally reverts to original state upon removal of the signal or condition (Fernandez *et al.*, 2011). Adaptive resistance is a complicated type of microbial resistance, but it is apparent that with adaptive resistance, microbes can save the cost for genetic modification and more rapidly respond to antibiotic challenge. Among several suggested mechanisms for adaptive resistance, many microbiologists pay attention to the biofilm formation of microbes that is often noted for notorious antibiotic resistance mechanism. Biofilm is a complex community of microorganisms and an example of bacterial group behavior. Biofilm is usually considered a sessile mode of life derived from the attached growth of microbes to surfaces, and most biofilms are embedded in self-produced extracellular matrix composed of extracellular polymeric substances (EPSs), such as polysaccharides, extracellular DNAs (eDNA), and proteins (Kim and Lee, 2016). Because of the extreme resistance of biofilms, most persistent chronic infections are believed to be associated with biofilms and biofilm is now a main target in the development of antimicrobial agents (Li and Lee, 2017).

Sometimes, the regulation of the genes involved in environmental stress responses provides antibiotic resistance for microbes (Marrakchi *et al.*, 2014; Kim *et al.*, 2018b). This is

also a kind of adaptive resistance. In this special issue, Kim *et al*. introduces recent advance in knowledge about the relation between the bacterial stress response and antibiotic resistance (Kim *et al*., 2019). In particular, Kim *et al*. focuses on the oxidative stress response: although the oxidative stress spontaneously generates during oxygenic respiration and metabolism, the oxidative stress response is not essential for normal growth. Instead, this response is critical to survive the oxidative stress conditions and despite some controversy, a number of antibiotics have been claimed to kill bacteria by causing oxidative stress (Kohanski *et al*., 2007; Keren *et al*., 2013), suggesting a possible link between oxidative stress response and antibiotic resistance. In their review, Kim *et al*. suggest the ROS-inspired antibacterial strategies as well as a core concept of the bacterial response against the oxidative stress.

Herd resistance

Over the past decade, new and challenging concepts including adaptive resistance have been raised about antibiotic resistance mechanism: the formation of biofilm or cell aggregative structure, persisters, increasing heterogeneity by high mutation rates or insurance effect, and kin selection and altruistic cell death (Boles *et al*., 2004; Bayles, 2007; Thomas *et al*., 2008; Lee *et al*., 2010; Jung *et al*., 2015). Some of which are related to adaptive resistance, but the key to these new concepts is antibiotic resistance at the microbial community level. The main concern in these concepts is not the survival of individual cells but the survival of the microbial community, and researchers in this field always look at antibiotic resistance in herd of microbes. Basically, this community-based resistance mechanism shows that a small number of resistant strain can provide protection to other non-resistant cells, enhancing the survival capacity of the overall population. Perhaps this resistance at the community level can be said to be a "herd resistance" of pathogenic microbes to respond to the "herd immunity" that is mentioned in human community. Herd immunity (also called herd effect, community immunity, or population immunity) is an indirect protection mechanism in which people who do not have immunity are also protected if the majority of the population has immunity to certain diseases. The major difference between human herd immunity and herd resistance of microbes is that, in the case of human, a majority of resistant individuals protect a minority of non-resistant individuals, whereas in the case of microbes, small portion of resistant cells survive and restore the whole populations.

However, the role of the non-resistant majority is also important in microbial herd resistance: the majority continuously generates the resistant minority and increases heterogeneity of the population to help survival of the minority. Some mechanisms to explain the occurrence of this type of antibiotic resistance have been suggested: altruistic autolysis in microbial community (Bayles, 2007), autolysis-dependent extracellular DNA release for biofilm development (Thomas *et al*., 2008), indole-based population-wide resistance and altruism (Lee *et al*., 2010), autolysis-based survival of *Helicobacter pylori* in acidic environment (Krishnamurthy *et al*., 1998), and persister cell formation (Wood *et al*., 2013).

Most results about this type of resistance have obtained from the studies on biofilms. In fact, our knowledge of antibiotic resistance in biofilms is not yet sufficient and the antibiotic resistance in biofilm seems a composite result of several mechanisms. The form of biofilm structure itself has been reported to influence antibiotic resistance (Kim *et al*., 2018a). Recently, persister cells become key topics in the studies on biofilms and community based-antibiotic resistance. At the beginning of understanding of persister cells, this special issue provides a remarkable review by Wood *et al*. about the formation and resuscitation of persister cells (Wood *et al*., 2019).

I would note that Kim Lewis has made important comments about antibiotic resistance of the biofilm (Lewis, 2001). Conventionally, antibiotic resistance means an ability of microbes to grow in the presence of an elevated level of the antibiotic drug. However, when we say that the biofilm is resistant to antibiotics, it emphasizes that the microorganisms in the biofilm can survive more persistently than better growth in the presence of antibiotics (Lewis, 2001). In this case, the term "resistance" may be replaced by the more precise term "tolerance" or "persistence" (Bayles, 2007; Brauner *et al*., 2016). Antibiotic tolerance seems fundamentally different from resistance, but our knowledge about this is still limited (Bayles, 2007).

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References

Abraham, E.P. and Chain, E. 1988. An enzyme from bacteria able to destroy penicillin (Reprinted from Nature, Vol 146, P. 837, 1940). *Rev. Infect. Dis.* **10**, 677–678.

Adedeji, W.A. 2016. The treasure called antibiotics. *Ann. Ib. Postgrad. Med.* **14**, 56–57.

Bayles, K.W. 2007. The biological role of death and lysis in biofilm development. *Nat. Rev. Microbiol.* **5**, 721–726.

Blair, J.M., Webber, M.A., Baylay, A.J., Ogbolu, D.O., and Piddock, L.J. 2015. Molecular mechanisms of antibiotic resistance. *Nat. Rev. Microbiol.* **13**, 42–51.

Boles, B.R., Thoendel, M., and Singh, P.K. 2004. Self-generated diversity produces "insurance effects" in biofilm communities. *Proc. Natl. Acad. Sci. USA* **101**, 16630–16635.

Brauner, A., Fridman, O., Gefen, O., and Balaban, N.Q. 2016. Distinguishing between resistance, tolerance and persistence to antibiotic treatment. *Nat. Rev. Microbiol.* **14**, 320–330.

Davies, J. and Davies, D. 2010. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* **74**, 417–433.

Fernandez, L., Breidenstein, E.B., and Hancock, R.E. 2011. Creeping baselines and adaptive resistance to antibiotics. *Drug Resist. Updat.* **14**, 1–21.

Funke, B.R., Tortora, G.J., and Case, C.L. 2016. Microbiology (5MB/6MB): Adapted from microbiology: an introduction. Pearson Education south Asia Pte Limited.

Griffith, F. 1928. The significance of pneumococcal types. *J. Hyg.* **27**, 113–159.

Jo, I., Kim, J.S., Xu, Y., Hyun, J., Lee, K., and Ha, N.C. 2019. Recent

paradigm shift in the assembly of bacterial tripartite efflux pumps and the type I secretion system. *J. Microbiol.* **57**, 185–194.
Jung, Y.G., Choi, J., Kim, S.K., Lee, J.H., and Kwon, S. 2015. Embedded biofilm, a new biofilm model based on the embedded growth of bacteria. *Appl. Environ. Microbiol.* **81**, 211–219.
Keren, I., Wu, Y.X., Inocencio, J., Mulcahy, L.R., and Lewis, K. 2013. Killing by bactericidal antibiotics does not depend on reactive oxygen species. *Science* **339**, 1213–1216.
Kim, S.K. and Lee, J.H. 2016. Biofilm dispersion in *Pseudomonas aeruginosa*. *J. Microbiol.* **54**, 71–85.
Kim, S.K., Li, X.H., Hwang, H.J., and Lee, J.H. 2018a. Antibiofilm effect of biofilm-dispersing agents on clinical isolates of *Pseudomonas aeruginosa* with various biofilm structures. *J. Microbiol.* **56**, 902–909.
Kim, S.K., Park, S.J., Li, X.H., Choi, Y.S., Im, D.S., and Lee, J.H. 2018b. Bacterial ornithine lipid, a surrogate membrane lipid under phosphate-limiting conditions, plays important roles in bacterial persistence and interaction with host. *Environ. Microbiol.* **20**, 3992–4008.
Kim, S.Y., Park, C., Jang, H.J., Kim, B., Bae, H.W., Chung, I.Y., Kim, E.S., and Cho, Y.H. 2019. Antibacterial strategies inspired by the oxidative stress and response networks. *J. Microbiol.* **57**, 203–212.
Ko, K.S. 2019. Antibiotic-resistant clones in Gram-negative pathogens: presence of global clones in Korea. *J. Microbiol.* **57**, 195–202.
Kohanski, M.A., Dwyer, D.J., Hayete, B., Lawrence, C.A., and Collins, J.J. 2007. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* **130**, 797–810.
Krishnamurthy, P., Parlow, M., Zitzer, J.B., Vakil, N.B., Mobley, H.L.T., Levy, M., Phadnis, S.H., and Dunn, B.E. 1998. *Helicobacter pylori* containing only cytoplasmic urease is susceptible to acid. *Infect. Immun.* **66**, 5060–5066.
Lee, H.H., Molla, M.N., Cantor, C.R., and Collins, J.J. 2010. Bacterial charity work leads to population-wide resistance. *Nature* **467**, 82–85.
Lewis, K. 2001. Riddle of biofilm resistance. *Antimicrob. Agents Chemother.* **45**, 999–1007.
Li, X.H. and Lee, J.H. 2017. Antibiofilm agents: A new perspective for antimicrobial strategy. *J. Microbiol.* **55**, 753–766.
Lobanovska, M. and Pilla, G. 2017. Penicillin's discovery and antibiotic resistance: lessons for the future? *Yale J. Biol. Med.* **90**, 135–145.
Marrakchi, M., Liu, X.B., and Andreescu, S. 2014. Oxidative stress and antibiotic resistance in bacterial pathogens: state of the art, methodologies, and future trends, pp. 483–498. *In* Woods, A.G. and Darie, C.C. (eds.), Advancements of mass spectrometry in biomedical research, Springer, Cham, Switzerland.
Spellberg, B. and Gilbert, D.N. 2014. The future of antibiotics and resistance: a tribute to a career of leadership by John Bartlett. *Clin. Infect. Dis.* **59 Suppl 2**, S71–75.
Thomas, V.C., Thurlow, L.R., Boyle, D., and Hancock, L.E. 2008. Regulation of autolysis-dependent extracellular DNA release by *Enterococcus faecalis* extracellular proteases influences biofilm development. *J. Bacteriol.* **190**, 5690–5698.
Wood, T.K., Knabel, S.J., and Kwan, B.W. 2013. Bacterial persister cell formation and dormancy. *Appl. Environ. Microbiol.* **79**, 7116–7121.
Wood, T.K., Song, S., and Yamasaki, R. 2019. Ribosome dependence of persister cell formation and resuscitation. *J. Microbiol.* **57**, 213–219.
Wright, G.D. 2007. The antibiotic resistome: the nexus of chemical and genetic diversity. *Nat. Rev. Microbiol.* **5**, 175–186.