Speaking for Myself

The suicide bombers of modern clinical medicine

KRUSHAN NIRMIT YAJNIK

'If I have seen further than others, it is by standing upon the shoulders of giants.'

—Sir Isaac Newton

Around ten thousand people may visit a popular mall daily; many regularly. With the popularity come numerous minor threats that are dealt with not only by the security guards outside the mall, but also those present inside the mall. Selectively located and isolated, these threats are nullified even before the owner of the mall knows about them.

One fine day, following some inadequate security measures outside the mall, a group of suicide bombers entered one such 'popular mall'. All of them went in different directions and blended with the crowd, making it difficult for the security to isolate and identify them. They were all carrying bombs within their bodies. They had no guns, but they could not be shot down either. An announcement was made saying the doors of the mall would remain closed till certain situations were resolved.

If the anti-terrorist squad (ATS) is called in, they would nullify those threats, but numerous people surrounding those terrorists would also be killed. I believe that the ATS should be made to wait outside the mall so that they may be on guard and if the need arises, they could enter the mall through a safe but alternative route of entry.

For the terrorists within the mall, the trained internal security guards could screen all the visitors and find these terrorists and beat them up and not shoot at them and prevent any further harm to the innocent people within the mall. The screening may take a long time, and there would be some wrongly identified innocents, but they would definitely be a lot less damage than what the ATS would effect.

This scenario is one way of looking at our immune reactions. The mall is our vascular system, the security guards inside are the innate immune system, ATS would be the antibiotics and the terrorists would be the Gram-negative bacteria (GNB) that have endotoxin in their cell wall. Endotoxin, i.e. lipopolysaccharide (LPS), is a type of pathogen-associated molecule that can stimulate a pro-inflammatory host response via toll-like receptors (TLRs), or C-type lectin receptors. The host response, in extreme cases, presents as septicaemia.[1] It is potentially fatal, but only when present in large quantities, unlike exotoxins (such as botulinum or tetanus toxin), which could be fatal even in small amounts.

Endotoxin is released by the lysis of these bacteria.[2] Shenep *et al*.[3] evaluated the blood levels of endotoxin in rabbits with *Escherichia coli* septicaemia, before and after the administration of one of the following antibiotics: gentamicin and moxalactam being bactericidal,[4] whereas chloramphenicol is bacteriostatic.[5] They found that the levels of free endotoxin rose rapidly following treatment with either gentamicin or moxalactam, but not with chloramphenicol.

As a corollary to 'the mall' analogy, we may allow the immune system within to deal with GNB, rather than calling in the ATS (antibiotics). Endotoxin can activate macrophages, mast cells and the coagulation and complement pathways.[6] The LPS of killed bacteria can be taken up by many cells, such as macrophages,[7] and the reticuloendothelial system (RES).[8] LPS binds to TLR4-receptors and therefore becomes sequestered in an immune cell. The immune response they evoke is the basis for the pathogenesis of endotoxic shock. Arredondo and Kampschmidt[8] reported that LPS from GNB produced considerable increase in the phagocytic activity of the RES within 2 hours of injection in rats. They also stated that this increase in the phagocytic activity failed to provide any additional protection against infection by *Salmonella typhimurium.*

As there is no currently available rapid diagnostic test to differentiate between Gram-negative and Gram-positive sepsis, antibiotic use in septicaemia will continue. Further, not all GNBs have endotoxins, and not all GPBs produce exotoxins. Thus, there would be many false-positives and false-negatives, e.g. diarrhoeagenic *E. coli*,[9] *Shigella*,[9] *Vibrio cholerae*[9] and *Pseudomonas*[9,10] are all GNBs that produce exotoxins.

Antibodies against LPS are commercially available and are used as intravenous immunoglobulins (IVIg). These antibodies are effective in clinical use, as suggested by Schmidt *et al*.[11] in a study designed to evaluate the role of IVIg for infection prophylaxis, which concluded that patients who received IVIg had significantly fewer infections compared to those who received no prophylactic treatment. Baker *et al*.[12] found that in premature infants weighing between 500 and 1750 g at birth, prophylactic IVIg administration significantly reduced the risk of first nosocomial infection. Another study[13] found that the incidence of Gram-negative pneumonia in patients receiving standard immunoglobulin (400 mg/kg body weight) was significantly lower (p=0.02) than those receiving placebo (25% albumin, 8 ml/kg). However, core-LPS hyperimmune globulin was not found to be effective in preventing either Gram-negative infections or ensuing complications.

The last problem could be even more challenging. However, it would not be too far-fetched to assume that if the species can be identified, then there would be some opposing antibody and neutralizing antitoxins bound to these antibodies could be used. However, they would all have to be different and specific to the microbe. Some progress was made on the rapid diagnosis of infectious diseases nearly a decade ago with technologies such as loop-mediated isothermal amplification gene amplification technique[14] and nano/microfluidics.[15]

Pramukhswami Medical College and Shree Krishna Hospital, Karamsad, Gujarat, India; A-21 Balaji Bungalows, Opp. Ramakaka Temple, Chhani, Vadodara 391740, Gujarat, India; *knyajnik@yahoo.co.in*

GNBs are reportedly more resistant to antibiotics than GPBs. Vaara[16] showed that >90% of antibiotics of natural origin lacked activity against *E. coli*, though they were bactericidal to GPB. Nearly 20% of *Klebsiella pneumoniae* and 30% of *Enterobacter* spp. infections in intensive care units in the USA are resistant to third-generation cephalosporins, due to the production of extended-spectrum β-lactamases (ESBLs).[17] ESBL-producing *E. coli* and *K. pneumoniae* are now not only common in healthcare settings, but also in the community. These enzymes are located in the periplasmid region; therefore, the antibiotic is destroyed soon after its entry into the bacterium. Carbapenem resistance, although rare, is now increasing. Certain mutants of *Pseudomonas aeruginosa* are less susceptible to a wide range of antibiotics, including β-lactams, fluoroquinolones, chloramphenicol and trimethoprim. The multiple antibiotic resistance (Mar) locus on *E. coli* and *Salmonella* confers antibiotic resistance, by upregulating the efflux of certain antibiotics.[18] It also downregulates influx via the outer membrane protein F.[18] Strains that constitutively express Mar protein are resistant to tetracyclines and chloramphenicol.[19] The role of the misuse of biocides (disinfectants, antiseptics and preservatives) in the emergence of such mutants has been extensively debated. Manzoor *et al*.[20] showed gluteraldehyde-resistant *Mycobacterium chelonae* strains from endoscope washer disinfectors and endoscope rinse water.

In India, Chitnis *et al*.[21] found that the combination of amoxycillin with clavulanic acid faced resistance from 72% to 79.5% of the isolates, suggesting the presence of ESBLs. Gupta *et al*.[22] found increasing resistance among GNBs against meropenem and imipenem. They also suggested a strict adherence to the concept of 'reserve drugs' to minimize the misuse of antimicrobial agents (AMAs).

Antibiotic resistance can have serious implications. Akimitsu *et al*.[23] suggested a link between community-acquired methicillin-resistant *Staphylococcus aureus* and the use of antibacterial products. A gene, called the *New Delhi Metallo β-lactamase* (NDM)-1 gene, was isolated from a patient in Sweden in 2008. It confers antibiotic resistance to *E. coli* and *K. pneumoniae*, and can resist all β-lactams, except aztreonam (i.e. a broad-spectrum β-lactamase and carbapenamase). NDM was described in a patient who had been admitted to a hospital in New Delhi in 2007, with urinary tract infection caused by MDR *K. pneumoniae*. According to Nordmann *et al*.,[24] many factors contribute to the selection and spread of NDM-1, some of them being self-administered AMAs, overcrowding and poor hygienic and sanitary conditions. In a study by Kumarasamy *et al*.[25] to find the prevalence of NDM-1 in MDR *Entero-bacteriaceae* in India, Pakistan and the UK, NDM-1 was isolated in 44 samples from Chennai, 26 from Haryana and 37 from the UK. It was most commonly isolated from *E. coli* and *K. pneumoniae*.

The comparison of management of patients based solely on vital stabilization and without use of AMAs with those in whom AMAs are used would be aided by matrix-assisted laser desorption/ionisation-time of flight (MALDI/ToF). According to Shannon *et al*.,[26] MALDI-ToF MS has a faster turnaround time as well as a lower cost than 16s rRNA gene polymerase chain reaction (PCR) plus sequencing for anaerobes. Limited MALDI-ToF-MS-based identification of Gram-negative anaerobes is also possible by brief outgrowths on blood broth subcultures. Other tests such as PCR-restriction fragment length polymorphism can also be done to identify the species. However, these tests have a long way to go before they become routine tests in patients with septicaemia. Moreover, it would be an ethical dilemma whether or not to give AMAs to such a moribund patient, let alone explaining to the relatives the basis of not giving AMAs.

Therefore, in the future, either we may run out of pharmacological stock or we could possibly have made a few more drugs and bought ourselves a few years of time. However, that is what it will be: borrowed time. And, at some point or the other, we may need to turn our attention towards non-AMA management of microbes. However, the question that we would need to answer before that time comes would be: could this actually be a blessing in disguise?

Conflicts of interest. Nil

REFERENCES

1 Russo TA, Johnson JR. Diseases caused by Gram-negative enteric bacilli. In: Jameson JL, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J. *Harrison's principles of internal medicine*. 20th ed. USA:McGraw-Hill Education; 2018:1147.
2 Sastry AS, Bhat KS. Microbial pathogenicity. *Essentials of medical microbiology*. Ch. 8. New Delhi:Jaypee Brothers; 2016:91.
3 Shenep JL, Barton RP, Mogan KA. Role of antibiotic class in the rate of liberation of endotoxin during therapy for experimental Gram-negative bacterial sepsis. *J Infect Dis* 1985;**151:**1012–18.
4 Tripathi KD. Aminoglycoside antibiotics. *Essentials of medical pharmacology*. Ch. 53. 7th ed. New Delhi:Jaypee Brothers; 2013:743.
5 Tripathi KD. Tetracyclines and chloramphenicol. *Essentials of medical pharmacology*. Ch. 52. 7th ed. New Delhi: Jaypee Brothers; 2013:739.
6 Roos KL, Tyler KL. Brain abscess and empyema. In: Jameson JL, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J. *Harrison's principles of internal medicine*. 20th ed. USA:McGraw-Hill Education; 2018:1015.
7 Cohn ZA. The fate of bacteria within phagocytic cells 3. Destruction of an *Escherichia coli* agglutinogen within polymorphonuclear leucocytes and macrophages. *J Exp Med* 1964;**120:**869–83.
8 Arredondo MI, Kampschmidt RF. Effect of endotoxins on phagocytic activity of the reticuloendothelial system of the rat. *Proc Soc Exp Biol Med* 1963;**112:**78–81.
9 Sastry AS, Bhat SK. Microbial pathogenicity. *Essentials of medical microbiology*. Ch. 8. New Delhi:Jaypee Brothers; 2016:93.
10 Ramphal R. Infections due to *Pseudomonas*, *Burkholderia*, and *Stenotrophomonas* species. In: Jameson JL, Fauci AS, Kasper DL, Hauser SL, Longo DL, Loscalzo J. *Harrison's principles of internal medicine*. 20th ed. USA:McGraw-Hill Education; 2018:1168.
11 Schmidt RE, Hartlapp JH, Niese D, Illiger HJ, Stroehmann I. Reduction of infection frequency by intravenous gammaglobulins during intensive induction therapy for small cell carcinoma of the lung. *Infection* 1984;**12:**167–70.
12 Baker CJ, Melish ME, Hall RT, Casto DT, Vasan U, Givner LB, *et al*. Intravenous immune globulin for the prevention of nosocomial infection in low-birth-weight neonates. The multicenter group for the study of immune globulin in neonates. *N Engl J Med* 1992;**327:**213–19.
13 Intravenous Immunoglobulin Collaborative Study Group, Cometta A, Baumgartner JD, Lee ML, Hanique G, Glauser MP, *et al*. Prophylactic intravenous administration of standard immune globulin as compared with core-lipopolysaccharide immune globulin in patients at high risk of postsurgical infection. *N Engl J Med* 1992;**327:** 234–40.
14 Mori Y, Notomi T. Loop-mediated isothermal amplification (LAMP): A rapid, accurate, and cost-effective diagnostic method for infectious diseases. *J Infect Chemother* 2009;**15:**62–9.
15 Lee WG, Kim YG, Chung BG, Demirci U, Khademhosseini A. Nano/microfluidics for diagnosis of infectious diseases in developing countries. *Adv Drug Deliv Rev* 2010;**62:**449–57.
16 Vaara M. Antibiotic-supersusceptible mutants of *Escherichia coli* and *Salmonella typhimurium*. *Antimicrob Agents Chemother* 1993;**37:**2255–60.
17 Paterson DL. Resistance in Gram-negative bacteria: *Enterobacteriaceae*. *Am J Infect Control* 2006;**34:**S20–8.
18 Randall LP, Woodward MJ. The multiple antibiotic resistance (mar) locus and its significance. *Res Vet Sci* 2002;**72:**87–93.
19 McMurry LM, Oethinger M, Levy SB. Overexpression of MarA, SoxS, or AcrAB produces resistance to triclosan in laboratory and clinical strains of *Escherichia coli*. *FEMS Microbiol Lett* 1998;**166:**305–9.
20 Manzoor SE, Lambert PA, Griffiths PA, Gill MJ, Fraise AP. Reduced glutaraldehyde susceptibility in *Mycobacterium chelonae* associated with altered cell wall polysaccharides. *J Antimicrob Chemother* 1999;**43:**759–65.
21 Chitnis SV, Chitnis V, Sharma N, Chitnis DS. Current status of drug resistance among gram-negative bacilli isolated from admitted cases in a tertiary care centre. *J Assoc Physicians India* 2003;**51:**28–32.
22 Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A, *et al*. Emerging

resistance to carbapenems in a tertiary care hospital in North India. *Indian J Med Res* 2006;**124:**95–8.
23 Akimitsu N, Hamamoto H, Inoue R, Shoji M, Akamine A, Takemori K, *et al.* Increase in resistance of methicillin-resistant *Staphylococcus aureus* to beta-lactams caused by mutations conferring resistance to benzalkonium chloride, a disinfectant widely used in hospitals. *Antimicrob Agents Chemother* 1999;**43:**3042–3.
24 Nordmann P, Poirel L, Toleman MA, Walsh TR. Does broad-spectrum beta-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by gram-negative bacteria? *J Antimicrob Chemother* 2011;**66:**689–92.
25 Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, *et al.* Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010;**10:**597–602.
26 Shannon S, Kronemann D, Patel R, Schuetz AN. Routine use of MALDI-TOF MS for anaerobic bacterial identification in clinical microbiology. *Anaerobe* 2018;**54:**191–6.