however, questions remain unanswered. If virulence blockers are used only in combination with antibiotics, as in the current study,[1] the theoretical advantage of avoiding selection of antibiotic resistance genes cannot be harnessed. Furthermore, the species-dependent or even strain-dependent activity of antibody-derived toxin inhibitors is a large obstacle to overcome when it comes to empirical treatment, and therefore requires rapid in-depth pathogen diagnostics. However, pathogen diagnostics is also evolving, as the first studies using next-generation sequencing to detect pathogens directly in clinical samples are underway.[13]

Most of the virulence blockers under development are antibodies or antigen-binding fragments, reflecting von Behring and Kitasato's approach. Very few virulence blockers have been described to date that do not resemble this principle, and, to our knowledge, the present study is the first completed clinical trial. Therefore, CAL02 represents a milestone, since it is the first non-antibody-based virulence blocker that has successfully overcome this hurdle of a clinical study and it appears to inactivate a broad range of secreted toxins, making it potentially suitable for adjunctive empirical treatment.

At last, we might be one step closer to precisely targeting pathogens without inflicting selective pressure and damaging the microbiome. Nevertheless, compared with the rapid translation of novel principles into clinical practice 120 years ago, clinical development processes in the present day are highly regulated and have many pitfalls. We keep our fingers crossed that CAL02 will not get lost on its way to the patients who need it.

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We declare no competing interests

1 Laterre P-F, Colin G, Dequin P-F, et al. CAL02, a novel antitoxin liposomal agent, in severe pneumococcal pneumonia: a first-in-human, double-blind, placebo-controlled, randomised trial. *Lancet Infect Dis* 2019; published online May 2. http://dx.doi.org/10.1016/S1473-3099(18)30805-3.
2 Azeredo da Silveira S, Perez A. Liposomes as novel anti-infectives targeting bacterial virulence factors? *Expert Rev Anti Infect Ther* 2015; **13:** 531–33.
3 Xiao H, Siddiqui J, Remick DG. Mechanisms of mortality in early and late sepsis. *Infect Immun* 2006; **74:** 5227–35.
4 Daviaud F, Grimaldi D, Dechartres A, et al. Timing and causes of death in septic shock. *Ann Intensive Care* 2015; **5:** 16.
5 Friesecke S, Stecher SS, Gross S, Felix SB, Nierhaus A. Extracorporeal cytokine elimination as rescue therapy in refractory septic shock: a prospective single-center study. *J Artif Organs* 2017; **20:** 252–59.
6 Gruda MC, Ruggeberg K-G, O'Sullivan P, et al. Broad adsorption of sepsis-related PAMP and DAMP molecules, mycotoxins, and cytokines from whole blood using CytoSorb® sorbent porous polymer beads. *PLoS ONE* 2018; **13:** e0191676.
7 Welte T, Dellinger RP, Ebelt H, et al. Efficacy and safety of trimodulin, a novel polyclonal antibody preparation, in patients with severe community-acquired pneumonia: a randomized, placebo-controlled, double-blind, multicenter, phase II trial (CIGMA study). *Intensive Care Med* 2018; **44:** 438–48.
8 Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance—the need for global solutions. *Lancet Infect Dis* 2013; **13:** 1057–98.
9 Sommer MOA, Church GM, Dantas G. The human microbiome harbors a diverse reservoir of antibiotic resistance genes. *Virulence* 2010; **1:** 299–303.
10 Brandt C, Makarewicz O, Fischer T, et al. The bigger picture: the history of antibiotics and antimicrobial resistance displayed by scientometric data. *Int J Antimicrob Agents* 2014; **44:** 424–30.
11 Enke U. 125 Jahre Diphtherieheilserum: „Das Behring'sche Gold". *Deutsches Ärzteblatt* 2015; **112:** 2088–90.
12 Dickey SW, Cheung GYC, Otto M. Different drugs for bad bugs: antivirulence strategies in the age of antibiotic resistance. *Nat Rev Drug Discov* 2017; **16:** 457–71.
13 Brenner T, Decker SO, Grumaz S, et al. Next-generation sequencing diagnostics of bacteremia in sepsis (Next GeneSiS-Trial): study protocol of a prospective, observational, noninterventional, multicenter, clinical trial. *Medicine (Baltimore)* 2018; **97:** e9868.

A step further in a vaccine for *Escherichia coli*

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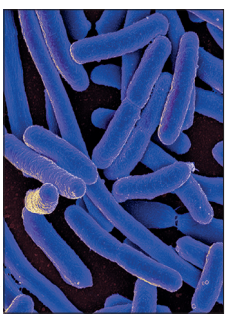
See **Articles** page 631

Extra-intestinal pathogenic *Escherichia coli* (ExPEC) are responsible for almost as many severe infections as pneumococci or meningococci. Estimates of *E coli* bacteraemia and sepsis in the health-care system in the USA suggest that more than 85 000 deaths are due to *E coli* sepsis.[1] Additionally, multidrug resistance is an increasing problem, especially in *E coli*, and even more pronounced in severe infections, such as urosepsis.[2] Alternative anti-infective strategies, such as a specific vaccine against ExPEC, would definitively increase the options for the treatment of invasive *E coli* disease and could be considered a great clinical advantage and contribute to decreasing antibiotic selection pressure.

Serum resistance is an important virulence property of bacteria in invasive infections, because blood serum is otherwise highly toxic to most bacteria. ExPEC strains are characterised by a high resistance to serum.[3] Serum resistance is due to resistance to the complement system and is affected by the length of the O-antigen and its type.[4] Although the O-antigen represents a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major mechanism of ExPEC serum resistance, other factors, including the capsule, also contribute to this phenotype.[5] About ten O-serotypes account for 90% of meningitis isolates and more than 60% of bacteraemia isolates.[6] Therefore, development of a vaccine against O-antigens from *E coli* O-serotypes is pragmatic.

In a phase 2 dose finding study by Robert Frenck and colleagues[7] reported in this issue, a single dose of a four-valent vaccine, containing the O1A, O2, O6A, and O25B antigens in five different content compositions, was administered to 843 healthy volunteers. Safety, reactogenicity, immunogenicity, and antibody functionality were evaluated by assessment of opsonophagocytic killing.

The vaccine was generally considered to be safe, with only one serious adverse event, (trigeminal neuralgia) reported, which was considered unrelated to the vaccine.

The study also attempted to investigate functional immunogenicity by assessment of opsonophagocytic killing, which is especially important, because there is no extensive human experience with the immunogenicity in ExPEC vaccines. Opsonophagocytic killing is a functional assay suitable for vaccine evaluation used in other vaccine developments. Although not described in detail by the authors, the performance of the assay seemed to be assessed with human serum.[8] Showing the functionality in serum is important, because up to 24% of patients with urosepsis were found to express inhibitory antibodies capable of preventing serum-mediated killing of their infecting isolate.[5] Instead of protecting against infection, the antibodies in these patients allowed otherwise serum-sensitive ExPEC strains to cause sepsis.[5] Opsonophagocytic killing results showed that, at day 15, 70% of vaccinees had titres of 100 or more, and 55% maintained these titres until day 360, and they correlated well with ELISA measurement of IgG levels, indicating robust immunogenicity.

The results described in this study seem to be promising and it is a further proof of concept that a vaccine in humans might be effective against *E coli* infections. To determine in which clinical situations such an *E coli* vaccine could be useful, well designed clinical studies in different infections need to be done. Until then it can only be speculated that such a vaccine might be effective in invasive *E coli* infections. Given the experience with antibody-coated bacteria in urinary tract infections, production of serum antibodies is relevant in invasive urinary tract infections but might not be relevant in immune defense of cystitis. A study[9] from 1974 showed that antibody-coated bacteria were observed in urine specimens from patients with pyelonephritis but not in urine from patients with cystitis. Most of the patients with antibody-coated bacteria in the urine also had high serum antibody titres against their own infecting bacteria, which suggests a role for the adaptive immune response in invasive urinary tract infections. A vaccine targeting the main infecting *E coli* clones might, therefore, be successful in preventing invasive *E coli* disease, as has been shown in an earlier phase 1b trial in healthy women with a history of recurrent urinary tract infection.[10] Further well designed clinical studies are needed to establish the clinical value of vaccination for *E coli* in the different categories of urinary tract infection, such as recurrent, uncomplicated, pyelonephritis, and urosepsis.

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1 Poolman JT, Wacker M. Extraintestinal pathogenic Escherichia coli, a common human pathogen: challenges for vaccine development and progress in the field. *J Infect Dis* 2016; **213:** 6–13.
2 Tandoğdu Z, Bartoletti R, Cai T, et al. Antimicrobial resistance in urosepsis: outcomes from the multinational, multicenter global prevalence of infections in urology (GPIU) study 2003–2013. *World J Urol* 2016; **34:** 1193–200.
3 Huja S, Oren Y, Biran D, et al. Fur is the master regulator of the extraintestinal pathogenic Escherichia coli response to serum. *mBio* 2014; **5:** e01460-14.
4 Reeves P. Role of O-antigen variation in the immune response. *Trends Microbiol* 1995; **3:** 381–86.
5 Coggon CF, Jiang A, Goh KGK, Henderson IR, Schembri MA, Wells TJ. A novel method of serum resistance by Escherichia coli that causes urosepsis. *mBio* 2018; **9:** e00920-18.
6 Cross A, Artenstein A, Que J, et al. Safety and immunogenicity of a polyvalent Escherichia coli vaccine in human volunteers. *J Infect Dis* 1994; **170:** 834–40.
7 Frenck Jr RW, Ervin J, Chu L, et al. Safety and immunogenicity of a vaccine for extra-intestinal pathogenic *Escherichia coli* (ESTELLA): a phase 2 randomised controlled trial. *Lancet Inf Dis* 2019; published online May 9. http://dx.doi.org/10.1016/S1473-3099(18)30803-X.

8 Abbanat D, Davies TA, Amsler K, et al. Development and qualification of an opsonophagocytic killing assay to assess immunogenicity of a bioconjugated Escherichia coli vaccine. *Clin Vaccine Immunol* 2017; **24:** e00123-17.
9 Thomas V, Shelokov A, Forland M. Antibody-coated bacteria in the urine and the site of urinary-tract infection. *N Engl J Med* 1974; **290:** 588–90.
10 Huttner A, Hatz C, van den Dobbelsteen G, et al. Safety, immunogenicity, and preliminary clinical efficacy of a vaccine against extraintestinal pathogenic *Escherichia coli* in women with a history of recurrent urinary tract infection: a randomised, single-blind, placebo-controlled phase 1b trial. *Lancet Infect Dis* 2017; **17:** 528–37.

Retrospective versus real-time Ebola virus sequencing

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See **Articles** page 641

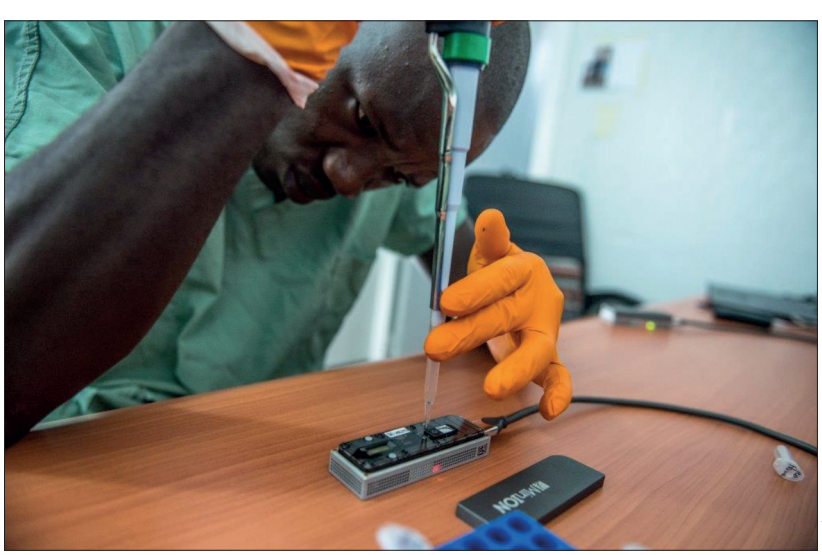
In *The Lancet Infectious Diseases*, Placide Mbala-Kingebeni and colleagues[1] report the effect of retrospective viral genome analysis of the 2018 Équateur Province Ebola virus disease (EVD) outbreak in the Democratic Republic of the Congo. They describe the outputs of an international response, working with local scientists, using in-country, whole-genome sequencing. The 2018 Democratic Republic of the Congo EVD outbreak was the ninth recorded in the country. 54 cases were reported with 33 fatalities, resulting in a case fatality rate of approximately 60%, which is in line with that of the much larger 2013–16 west African outbreak.

Mbala-Kingebeni and colleagues describe how they sequenced 16 Ebola virus (EBOV) genomes to uncover the presence of a novel strain that they named "Tumba". The genome evaluation was done with an Illumina iSeq100 (Illumina Technologies, San Diego, CA, USA), although an initial short read of an early diagnostic sample was done with the highly field-adaptable Oxford Nanopore Technologies' MinION device. Importantly, the sequencing and analysis was done in the capital of the Democratic Republic of the Congo, Kinshasa.

In-silico analysis and antibody binding assays revealed nucleotide variations that could affect the epitope binding site of a key component of the experimental therapeutic monoclonal antibody cocktail ZMapp. This further highlights the dangers of using monospecific monoclonal antibody therapeutics to treat viral infections. A successful phase 3 trial of the recombinant viral-based vaccine VSV-ZEBOV[2] was done during the 2013–16 west African EBOV outbreak. The authors' in silico analysis of the glycoprotein sequence of the "Tumba" strain suggested only a minor potential effect on the immune profile of the target antigen, which is based on the 1995 Kikwit outbreak strain of EBOV. The authors also evaluated the effects of genome sequence variations on the performance of several EBOV molecular diagnostics, concluding that they would not have a negative effect on their performance.

Although the retrospective sequencing of the diagnostic samples started only 3 weeks before the outbreak was declared over, Mbala-Kingebeni and colleagues propose a protocol for the rapid assessment of pathogen sequence on the efficacy of current medical countermeasures, which could have an effect on future incidents.

The 2013–16 west African outbreak was associated with more than 28 000 cases of EVD, which resulted in more than 11 000 fatalities.[3] Permission from local government to ship samples to Europe and North America enabled early mass sequence analysis by the European mobile laboratory (EMLab)[4] and others,[5] which revealed important factors about the timing of the initial spillover event, mutation rate, suitability of available medical countermeasures, and the transmission dynamics during the first year of the outbreak. A subsequent review of approximately 2000 sequences from the outbreak provided greater insight into contributions of geographical, political, economic, and social factors on transmission throughout the region.[6]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