RAPID RISK ASSESSMENT

Risk related to the use of 'do-it-yourself' CRISPR-associated gene engineering kit contaminated with pathogenic bacteria

2 May 2017

Conclusions

On 24 March 2017, the German authorities reported the contamination of a 'do-it-yourself' bacterial gene engineering CRISPR kit produced in the US. The kit was contaminated with pathogenic bacteria (risk group 2), including some bacteria that are multidrug-resistant and ESBL-producing. The kits are still sold online and target non-professional users. The kit was declared by the producer to contain a harmless 'non-hazardous and non-pathogenic' laboratory strain of *E. coli* and to be safe for home use.

The risk of infection by the contaminating strains in the kit is low for the users of the kit, assuming that they are healthy people. The contribution of the kit to the burden of antimicrobial resistance in the EU/EEA population and environment is marginal, and the risk associated with the kits is considered very low.

At this stage, it is estimated that the distribution of such contaminated kits is very limited. The assessment of the risk associated with the DIY kits should be revised should further information indicate that the distribution of the kit extends more widely across the EU.

Option for response

As a result of this event, German authorities have prohibited the importation of the kit. National competent authorities in the EU should review the authorisation of this commercial DIY kit (CRISPR-Cas 9) product, or similar 'citizen science' biomaterials containing risk-group-1 biological agents to produce genetically modified microorganisms for educational or recreational purposes on the basis of the applicable European and national legislation. To conclude that level-1 containment is appropriate to protect human health and the environment, the assessment must show that neither the recipient microorganism, genetic vector nor DNA inserts used can endow a genetically modified microorganisms with a phenotype which would likely cause harm to humans, animals or plants.

Member States may also encourage consumers of such kits to purchase them from companies that have implemented quality control procedures and that follow proper packaging, labelling, and documentation requirements for the transport of biological materials, such as stated in the WHO 'Guidance on regulations for the transport of infectious substances' [2].

Source and date of request

Request from Europol on 11 April 2017.

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Public health issue

This rapid risk assessment aims at addressing the following risks:

- Risk of infection for users of the contaminated kit.
- Risk of releasing and spreading of antimicrobial resistant strains into the human population and the environment.
- The biosecurity risk due to dual use of these kits.

Consulted experts

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- Europol
- World Health Organization: Regional Office for Europe, Regional Office for Americas/PAHO, national IHR focal point for the United States of America.

ECDC acknowledges the valuable contributions of all experts. Although experts from the WHO reviewed the risk assessment, the views expressed in this document do not necessarily represent the views of WHO. All experts have submitted declarations of interest and a review of these declarations did not reveal any conflicts of interest.

Background information

Genetic engineering techniques are methods applied in gene editing, making directed changes to a specific gene sequence in a genome. These techniques are rapidly progressing, and some do not require specialised laboratory equipment or advanced skills to use. One of these techniques is based on CRISPR (clustered regularly interspaced short palindromic repeats).

The CRISPR genome arrays and CRISPR-associated (Cas) proteins are related to prokaryotic* adaptive immune systems [3,4]. The CRISPR are DNA segments constituted of short-base sequences that are widespread in the genomes of prokaryotes. The CRISPR-derived mechanisms protect bacteria against infection with foreign genetic material vectors (bacteriophages and plasmids) by incorporating parts of their invading DNA into the host genome [5-7]. This biotechnology, notably for the CRISPR-associated protein 9 (CRISPR–Cas9), opened a wide range of gene engineering solutions, including new potential medical applications [8]. Using the CRISPR–Cas9-derived biotechnology, highly specific DNA sequences can be edited or modulated to study genome functions with a relatively simple approach [8,9].

Recent progress in molecular biology associated with increased access to easier and cheaper technologies supported the development of a biotechnological social movement ('do-it-yourself biology', DIY) in which individuals and communities transfer biotechnology from academia or industrial laboratories to DIY biologists with little or no formal training [10]. This community of users has been significantly growing over the past decade. Various biotechnological protocols are used under DIY biology, including the use of recent CRISPR–Cas9 technology [10,11]. According to a DIY biology website, networks of DIY biology are active in several EU Members States, notably in Austria, Belgium, the Czech Republic, Denmark, France, Germany, Hungary, Ireland, Italy, the Netherlands, Slovenia, Spain, Sweden, and the United Kingdom [12]. On blogs and websites, the community shares experiences and responds to questions about their experiments and results. There is evidence that the CRISPR-associated gene engineering kit is used in the EU by non-professional microbiologists [13].

Scientific opinions on synthetic biology were recently drafted by several committees of the Directorate General for Health and Food Safety, the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), the Scientific Committee on Health and Environmental Risks (SCHER), and the Scientific Committee on Consumer Safety (SCCS) [6,7,14,15]. One of the opinions summarised the implications of expected developments in synthetic biology on human and animal health and the environment, including DIY biology (see sections on 'citizen science' and 'DNA synthesis and genome editing') [7]. The extent and probability of hazards and adverse effects of genetic engineering to human health and the environment are covered by Directives 2001/18/EC and 2009/41/EC and the guidance notes published in Commission Decision 2000/608/EC [16-18].

* A prokaryote is a unicellular organism without nucleus and other cell organelles except ribosomes.

Event background information

On 24 March 2017, the Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL, Bavarian Health and Food Safety Authority) issued a press release warning about the risk of exposure to several pathogenic bacteria associated with the use of a specific DIY bacterial gene engineering CRISPR kit manufactured by a US company [19]. According to the manufacturer, the kit allows users to perform a molecular biology experiment to make a

> 'genome mutation (K43T) to the *rps*L gene, changing the 43rd amino acid, a lysine (K) to a threonine (T), thereby allowing the bacteria to survive on a kanamycin and streptomycin culture media which would normally prevent its growth' [20].

The kit is based on CRISPR–Cas9 system. To perform the experiment, the kit should contain the Cas enzyme (i.e. endonuclease protein), exogenous genetic material (i.e. RNA and DNA sequences), and a recipient bacterium able to acquire the exogenous genetic material [20]. The recipient bacterium included in the kit is described as a harmless laboratory strain of *Escherichia coli (E. coli)* HME63. However, analysis performed by LGL on two kits ordered from the US in November 2016 and in March 2017 revealed the presence of the facultative pathogenic bacteria of risk group 2.

According to the Genetic Engineering Act in Germany, DIY genetic engineering kits should only be used in genetic engineering installations [21]. Moreover, the risk-group-2 bacteria that were detected in two kits are pathogens according to the German Infection Protection Act. Their import, export, storage or handling require permission by the competent authority [22]. Consequently, LGL recommends not opening the kit, not to perform the experiments, and to contact the public health authority [19,23,24]. After detecting risk-group-2 bacteria in the kits, the LGL stated that the risk of infection cannot be excluded during the manipulation of these kits, even if the spread of an infection in the general population remains unlikely [19].

ECDC threat assessment for the EU

Risk of infection for users of the contaminated kit

The isolates of *Klebsiella pneumoniae, Enterobacter* spp. and *Enterococcus faecalis* that have been detected as contaminants in the kits are bacterial species that are part of the normal human gastrointestinal as well as environmental microbiome. They are also facultative pathogens with the ability to cause severe human infections in certain conditions, especially in healthcare settings or when natural host defences are impaired. They belong to biological risk group 2, a group which requires handling in containment facilities with appropriate safety measures (e.g. worker protection) (Directive 2000/54/EC) [25].

In the ECDC point prevalence survey of healthcare-associated infections 2011–2012, *Enterococcus* spp., *K. pneumoniae* and *Enterobacter* spp. were the third, fifth and eighth most frequently encountered microorganisms associated with healthcare-associated infections in European acute care hospitals [26]. The most frequently associated infections were surgical site and urinary tract infections for *Enterococcus* spp., respiratory tract and urinary tract infections for *K. pneumoniae*, and surgical site and lower respiratory tract infections for *Enterobacter* spp. [26]. In addition, some of the *K. pneumoniae* and *Enterobacter* spp. isolates detected in the kits were multidrug-resistant and ESBL producing bacteria that lead to resistance to penicillins and cephalosporins. The detection of imipenem-resistant *K. pneumoniae* likely implies the carriage of carbapenemase genes encoding resistance to the last-line agents that are used in hospitals for treatment of severe infections.

The risk of infection for users of the kits who are unaware of the contamination with pathogenic agents is low because the manipulation of the kit does not involve percutaneous injury-prone manipulations. However, infection resulting from the contamination of broken skin or mucous membranes may occur, even though the kit recommends and provides disposable gloves. Furthermore, the kit includes lyophilised materials that need to be reconstituted, which may lead to contamination of the mucosae of the eyes, mouth and nose. Finally, the risk of infection may be increased for immunocompromised or immunosuppressed persons.

Risk of releasing and spreading antimicrobial-resistant strains into the human population and the environment

The ESBL and carbapenemase resistance genes carried by the pathogenic bacteria present in the contaminated kits are usually located on plasmids which can be transmitted easily between bacteria of the same species and to other bacteria of the same family of *Enterobacteriaceae* that inhabit the human gastrointestinal tract. Bacteria with resistance can persist for several months in the intestinal tract of asymptomatic carriers. If a carrier develops severe illness and requires antimicrobial treatment, there is a potential risk that the antibiotic-resistant bacteria proliferate and subsequently cause multidrug-resistant infection [27].

The population prevalence of intestinal carriage of ESBL-producing bacteria is already high in EU/EEA countries [28]. Carriage may occur following exposure via household contacts, food, environment, international

travel, and transmission in healthcare settings. Therefore, the contribution of the contaminated kits to the overall risk of acquisition of ESBL-producing bacterial pathogens at population level is likely to be negligible. Because the prevalence of carriage of carbapenemase resistance genes among the general population is low in many European countries, the contribution of the contaminated kit to the general burden of carbapenem-resistant *K. pneumoniae* is proportionally higher than for ESBL, but remains very low [29,30]. Carbapenem-resistant *K. pneumoniae* strains have caused multiple outbreaks after introduction into EU/EEA hospitals [31-35].

Multidrug-resistant bacteria acquired from the kits by human carriers could be introduced in hospital settings, as they could be introduced by any carrier who acquired these bacteria from other sources via travel, household or healthcare contacts. Adherence to standard infection control measures and enhanced control measures for multidrug-resistant bacteria can limit the spread of multidrug-resistant bacteria within hospitals.

Finally, used material from the contaminated kit should be disposed appropriately to avoid the release of multidrug-resistant bacteria into the environment. However, the contribution of the kits to the quantity of multidrug-resistant bacteria in the environment remains negligible.

In conclusion, the potential contribution of the contaminated kit to the increasing burden of antimicrobial resistance in the EU/EEA is marginal, and the associated public health risk is considered very low.

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