Biosafety in training program on laboratory diagnosis of especially dangerous pathogens

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Abstract

Aim of study: To develop a safe training method for working with especially dangerous pathogens (RG3; KZ PGII) in KSCQZD Laboratory Central Asia training program.

Results and discussion: Trainees from Tajikistan, Kyrgyzstan and Kazakhstan participated in training on "Laboratory diagnostics of cholera," as part of a 4 month training program in laboratory diagnosis of especially dangerous pathogens. *Vibrio cholerae* is classified as belonging to the Pathogenicity Group II (equivalent to Risk Group 3).

Both lectures and hands on activities were utilized to cover the following areas of study:

•general characteristics of the bacteria

•sample collection and processing

•epidemiology of the current 7th pandemic

•current legislation of the Republic of Kazakhstan on work with especially dangerous pathogens and disinfection [1]

The laboratory portion was carried out in a dedicated bacteriological room. The avirulent strain of *V. cholerae* O1 biotype ElTor 5 was used for the laboratory training practice; it lacked the toxin but otherwise exhibited typical morphology and growth characteristics of *V. cholerae*.

Students were introduced to theoretical materials, and, afterwards were divided into smaller groups of 6, and commenced practical activities for the following 2 week period. Their laboratory diagnostic training started with characterization of known avirulent strain. After successful growth on differential media, following standard procedures, they were given an unknown sample from which they were expected to isolate and characterize the bacterium. Participating students have successfully obtained expected results for *V. cholerae* ElTor and thus gained confidence in their diagnostic skills with *V. cholerae*. None reported being sick.

Conclusion: Biological safety considerations for laboratory training program for laboratory specialists of Central Asia necessitated usage of avirulent strain of *V. cholerae* during the training process. *V. cholerae* O1 biotype ElTor 5 is a good training strain to use safely for practice activities for inexperienced students in preparation for work with especially dangerous pathogens.

Цель обучения: Разработка безопасных методов обучения при работе с особо опасными патогенами (группа риска 3; группа патогенности II в Казахстане) в подготовительной программе обучения для специалистов Центральной Азии в КНЦКЗИ.

Результаты и обсуждение: Стажеры из Таджикистана, Кыргызстана и Казахстана принимали участие в программе обучения "Лабораторная диагностика холеры", которая является частью 4-х месячной подготовительной программы по лабораторной диагностике особо опасных инфекций. *Vibrio cholerae* относится ко II-ой группе патогенности (эквивалент Риск группы 3).

Лекции и практические занятия охватывали следующие области исследования:

* общая характеристика бактерии

* забор материала и обработка

* эпидемиология современной 7-й пандемии

* текущее законодательство Республики Казахстан по работе с особо опасными патогенами и дезинфекции [1]

Лабораторная часть проводилась в специально отведенном бактериологическом зале. Для обучения по лабораторной подготовке использовался авирулентный штамм *V. cholerae* O1 biotype ElTor 5; у него отсутствовал токсин, в остальном он обладал типичной морфологией и характерным для *V. cholerae* ростом.

Студентов ознакомили с теоретическим материалом, после чего их разделили на группы по 6 человек, и далее проводили практические занятия в течении двух недель. Обучение студентов по лабораторной диагностике началось с характеристики известного авирулентного штамма.

После успешного изучения свойств культуры микроорганизма и его полной идентификации на дифференциальных средах, следуя стандартной процедуре, студентам предоставили неизвестный образец, из которого они должны были выделить и охарактеризовать возбудителя. Участвующие студенты успешно получили ожидаемый для *V. cholerae* ElTor результат и таким образом приобрели уверенность в своих диагностических навыках по работе с *V. cholerae*. Никто из студентов не заболел.

Выводы: Соображения биобезопасности для программы по лабораторной подготовке для лабораторного персонала Центральной Азии требовали использования авирулентного штама *V. cholerae* в процессе обучения. *V. cholerae* O1 biotype ElTor 5 является подходящим штамом для безопасного использования во время практических занятий при подготовке неопытных студентов к работе с особо опасными патогенами.

Introduction

Cholera is an acute diarrhea caused by ingestion of food or water contaminated with the bacterium *Vibrio cholerae*. Every year, there is an estimated 3–5 million cholera cases and 100,000–120,000 deaths from cholera. Cholera is thought to have its ancestral home in the Ganges Delta of the Indian subcontinent. In the nineteenth century, pandemic waves of cholera spread to many parts of the world. In 1961, a massive epidemic began in Southeast Asia; this is now recognized as the beginning of the seventh cholera pandemic. This pandemic was caused by the El Tor biotype of toxigenic *V. cholerae* O1. It spread rapidly through South Asia, the Middle East, and southeastern Europe, reaching Africa by 1970. In January 1991, epidemic cholera appeared in South America in several coastal cities of Peru and spread rapidly to adjoining countries. Isolates of *Vibrio cholerae* serogroup O1 are classified into two biotypes, El Tor and classical, on the basis of several phenotypic characteristics. Currently, the El Tor biotype is responsible for virtually all of the cholera cases throughout the world, and classical isolates are not encountered outside of Bangladesh. In addition *V. cholerae* O1 is classified into 3 serotypes, Inaba, Ogawa and Hikojima, based on agglutination in antiserum. Although *V. cholerae* will grow on a variety of commonly used agar media, isolation from fecal specimens is more easily accomplished with specialized media. Alkaline peptone water (APW) is commonly used as an enrichment broth [2] .

In the Republic of Kazakhstan, *Vibrio cholerae* is classified as belonging to IInd Pathogenicity Group, which is equivalent to the Risk Group 3 in the United States. Only laboratory personnel who completed specialized training are qualified to work with *Vibrio cholerae* or other especially dangerous pathogens (EDP) in Kazakhstan and in some of the neighboring countries. A national training center for laboratory diagnosis of EDPs, M. Aikimbayev's Kazakh Science Centre for Quarantine and Zoonotic Diseases (KSCQZD) has established a four-month EDP training program. The program provides knowledge, technical and biosafety skills needed for EDP diagnosis.

Trainees from Tajikistan, Kyrgyzstan, Kazakhstan participated in training on "Laboratory diagnostics of cholera," as part of a 4 month training program in laboratory diagnosis of especially dangerous pathogens.

Vibrio cholera has a virulence, high variability features helping to survive in the environment under certain circumstances. The training curriculum includes [2]:

*microbiology of *Vibrio cholerae* (including genetic features, variability, virulence);

*laboratory diagnostics (sampling; isolation of culture; identification, detection of virulence and toxigenicity);

*epidemiological features of 7th pandemic;

* legislation of the Republic of Kazakhstan in the area of especially dangerous pathogens and disinfection(1)

* practical training.

Biosafety training is a critical component of the program. Identifying workplace biorisks, conducting risk assessment, using Personal Protective Equipment (PPE) and good laboratory practices are emphasized during the didactic training. Laboratory diagnostic skills and biosafety skills are demonstrated during the practical exercises. Trainees have the opportunities to exercise their biosafety skills while conducting hands on diagnostic procedures(3).

Methods and materials

The training program was developed based on knowledge, and skills required for conducting EDP diagnostic procedures. Microbiological laboratory techniques, culture media selection, aseptic technique, microorganism identification, and good laboratory practices were the main objectives of the program. Other objectives included the utilization of critical and analytical thinking skills in the application of principles and regulations in order to ensure quality assurance, accuracy, and validity of laboratory tests. In addition, the following biorisks related to EDP diagnostic procedures and risk mitigation skills were identified and covered by the program.

- Inhalation risks (i.e. aerosol production) when using loops, streaking agar plates, pipetting, making smears, opening cultures, taking blood/serum samples, centrifuging, etc.
- Ingestion risks when handling specimens, smears and cultures
- Risks of percutaneous exposures when using syringes and needles
- Handling of blood and other potentially hazardous pathological materials
- Decontamination and disposal of infectious materials

An avirulent strain of *V. cholerae* O1 biotype El Tor 5 was selected for the laboratory training practice. Lacking cholera toxin, the organism exhibits typical morphology and growth characteristics of *V. cholerae*.

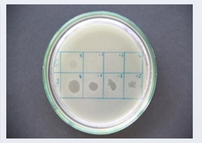
The strain # 5, *V. cholerae* serogroup *O1*, isolated from the environment was taken for the research. The strain had the morphological, biochemical, and serological features which were typical for *V. cholerae* serogroup *O1*. These characteristics are motility, colony morphology, biochemical activity on the differential diagnostic media; positive agglutination reaction with diagnostic cholera sera of "O" and Inaba, and absence of agglutination with cholera sera of Ogawa, RO and O139; sensitivity to phage of El Tor, and resistance to the classic phage; growth on medium with polymyxin, positive reaction of Voges-Proskauer, positive agglutination with guinea pig erythrocytes and positive hemolytic activity in Graig reaction (4).

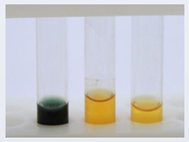
Results and discussion

The *V. cholerae* O1 biotype El Tor 5 were cultured and characterized by various standard diagnostic procedures including culture, serology, and Polymerase Chain Reaction (PCR). The consistent morphology, growth characteristics, and standard diagnostic test results generated by the trainees suggested *V. cholerae* O1 biotype El Tor # 5 was an ideal strain to use for *V. cholerae* laboratory diagnosis training and for general EDP diagnostic training and biosafety practical training. Some examples of diagnostic test results generated by the trainees are illustrated above to the right:

Diagnostic reagents used for laboratory exercises may include: Cholera anti-serum "O", "Ogawa", "Inaba", cholera phages C (classical) and ElTor, nutrient media (Hiss medium, Hottinger agar, 1% peptone water), and erythrocytes for diagnosis provided by KSCQZD.

Trainees conducted practical exercises. All potential infectious materials were handled under BSL-2 conditions. Their diagnostic and biosafety skills were evaluated.





Colony morphology (smooth, transparent, with even edges)

Test with a classic and ElTor phages (positive with ElTor)

Determination of Heiberg group (sucrose + + mannose, arabinose -)

Although *V. cholerae* O1 biotype El Tor 5 was considered an avirulent strain and was isolated from environment, potential health risks from exposure to the organisms were evaluated and considered.
In order to minimize the potential risks, biosafety precautions were taken and BSL-2 practices were followed.
All trainees, who were diagnostic specialists from Tajikistan, Kyrgyzstan, and Kazakhstan had successfully completed their EDP diagnostic specialization courses at KSCQZD. They have demonstrated their knowledge, technical and biosafety skills needed for safe EDP diagnosis. A series of practical exercises were safely conducted. In the near future, a new BSL-3 laboratory training facility funded by the Defense Threat Reduction Agency (DTRA) will be completed at KSCQZD. Future trainees will have the opportunity to learn and exercise their biosafety skills at this new training facility.

Conclusions

Biosafety training is an important component of our four-month EDP training program. Laboratory diagnostic skill training and biosafety practical training can be successfully conducted simultaneously.

V. cholerae O1 biotype ElTor 5 is an ideal strain to be used for *V. cholerae* laboratory diagnostic training. It also can be used for general EDP diagnostic training and biosafety practical training.

References

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