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Original Article

Rotavirus epidemiology adjusted pattern in a tropical setting: mathematical correction for false positive problem relating to primary immunochromatography test surveillance

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Abstract: Background: Rotaviruses are the most common cause of acute gastroenteritis in neonates and young children worldwide. Human rotaviruses are the leading cause of acute gastroenteritis in neonates and young children worldwide. The immunochromatography test is frequently used in clinical practice to detect rotavirus infection. When the immunochromatography test is incorrectly positive, there may be a discrepancy between the two tests, the immunochromatography test and the nucleic acid test. As a result, when interpreting the findings of basic rotavirus monitoring in a system based on immunochromatography tests, we must made adjustments to address the issue of accuracy. Methods: The findings on the expected pattern of rotavirus epidemiology in a tropical setting was presented. The modified rotavirus pattern was created to address the issue of false positives. To solve the false positive issue, the modified rotavirus pattern derived from mathematical model-based correction by extracting false positivity was predicted. Results: We demonstrated an altered rotavirus epidemiology pattern in the setting studied in this study. Rotavirus has been detected in up to 19.3% of patients with rotavirus-like symptoms, with G4P [8] accounting for 6% of those infected. Conclusion: As a result, the rotavirus remains an important problem that must be addressed in the framework of this study.

Keywords: Rotavirus, diagnosis, genotype, immunochromatography

Introduction

Rotaviruses are a leading cause of acute gastroenteritis in newborns and young children all over the world [1]. Every year, million cases of gastroenteritis result from this virus infection [1]. Almost every kid is infected at least once within their first five years of life, with the peak frequency happening between the ages of six and twenty-four months [1-4]. The most common rotavirus genotype distribution has shifted over time. In such a setting, the genetic epidemiology survey on rotavirus at different periods usually provided different dominant strains. The genomic and antigenic variety of novel rotavirus genotypes and their constellations has resulted from the evolutionary dynamics of novel rotavirus genotypes and their constellations [5]. The development and implementation of an effective vaccine is one of the best solutions for reducing the disease's global burden. Species Rotaviruses are the most common cause of severe acute gastroenteritis in children under the age of five [5]. Current rotavirus vaccines were developed through serial passage of cultured cells or reassortment of human and animal rotavirus strains from wild-type rotavirus strains [5]. The reverse genetics method is a potent technique for altering viral antigenicity, growth capacity, and pathogenicity by changing rotavirus genes. Vaccines against rotavirus have been developed in recent years to help reduce the number of cases [5].

The identification of vaccine-derived viruses in stool samples following immunisation can make

diagnosing wild-type rotavirus sickness more difficult [6]. To quickly diagnose and characterise rotavirus strains in stool samples for proper patient treatment and to monitor circulating vaccine and wild-type rotavirus strains, high-throughput, sensitive, and specific approaches are required [7]. The immunochromatography test is commonly used in clinical practice to diagnose rotavirus infection. Immunochromatography tests are exceptional separation-free techniques in clinical biochemistry in which the concentration of analyte is proportional to the distance along a chromatographic strip where colour develops rather than the degree of colour development. For detection, genotyping, and full genome characterization of circulating rotavirus wild-type and vaccine strains causing medical issues, new molecular techniques are rapidly being developed that are more sensitive and specific than existing assays [7]. The accuracy of the rotavirus diagnosis, on the other hand, is critical. In any scenario, precision is critical in proving accurate information about rotavirus epidemiology. Inconsistency between the two tests, the immunochromatography test and the nucleic acid test, can occur when the immunochromatography test is falsely positive and the nucleic acid test is falsely negative [6]. Cross-reactivity with other microbes, interference, and non-specific reactivity are all possible causes of false positive antigen tests. RNA breakdown owing to lengthy storage of materials at 70°C, as well as changes in RNA viruses, are possible causes of false negative nucleic acid tests [8]. Therefore, in interpreting the results from basic surveillance of rotavirus in a setting that is based on immunochromatography testing, it is necessary to make adjustments to correct the problem of accuracy. Here, the authors report on the adjusted pattern of rotavirus epidemiology in a tropical setting. The aim is to find the expected rotavirus epidemiological pattern after adjustment for false positivity due to classical immunochromatography test surveillance.

Materials and methods

Design and setting

The setting is a tropical country in Southeast Asia with a high incidence of rotavirus disease. Regarding the immunochromatography test, the reported false positivity is up to 40% [9]. The technique used in the present report is a clinical mathematical model approach. It employs an in silico approach rather than an in vivo or in vitro approach. Therefore, it can rule out environmental confounding factors. When there is a false positive problem, the rotavirus frequency derived from the routine surveillance laboratory will be affected and inaccurate. The extraction of the false positive percentage by an arithmetic approach can solve this problem.

Modelling for prediction of the expected rotavirus epidemiology pattern

This is a mathematical model-based study for prediction. The primary epidemiological data is required for mathematical analysis. Inclusion was set for the official published data on rotavirus epidemiology pattern in the study setting, and exclusion was set for any incomplete data. The present study is a kind of descriptive statistical study using the clinical mathematical model technique. The main indicator in the present study is the frequency rate of the rotavirus strains.

In the study setting, the monitoring of the rotavirus epidemiological pattern was performed. The immunochromatography test for surveillance was generally used. The data from a previous clinical study in the setting was directly referred to and used for the present modelling study [10]. According to the reference publication [9], of 310 studied cases, 100 samples tested positive for rotavirus via an immunochromatography test. In addition, nucleic acid tests were used to examine molecular epidemiological patterns in 29 instances. The G3[P8] strain was discovered as the most prevalent (31.0 percent), followed by G1P[8], G8P[8], G9P[8], and G2P[8], which accounted for 20.8 percent, 17.2 percent, and 13.8 percent, respectively, in the nucleic acid test.

The non-adjusted pattern was initially calculated in order to find the predicted rotavirus molecular pattern. This primary data represented the pattern among the immunochromatography positive samples. Therefore, it can be converted to the frequency among overall studied samples. The final adjustment for false positivity can be done after the conversion to the frequency among overall studied samples. A correction for false positivity, 40% [9], is used to make the change. The final rotavirus epidemiology pattern was then estimated.

Table 1. Adjustment to correct for false positivity in monitoring rotavirus epidemiology pattern based on immunochromatography test surveillance

	Rotavirus pattern (% of frequency)				
	G3P[8]	G1P[8]	G9P[8]	G2P[8]	others
Background data among positive samples before adjustment*	31	20.8	17.2	13.8	17.2
Converting to represent overall studied samples	10	6.7	5.5	4.5	5.5
Adjusted data**	6	4	3.3	2.7	3.3

*Background data from the previous clinical investigation in the setting [7]. **adjusted data is based on correction for by extracting false positivity (40%).

The stepwise mathematical adjustment was performed in order to derive the final adjusted frequency value. Regarding the standard surveillance, the first step is the immunochromatography test to primarily screen for positive samples from overall specimens investigated for rotavirus as a cause of gastroenteritis. The second step is further nucleic acid testing to identify the molecular epidemiology pattern. The finalized frequency result is directly from the laboratory of sampled clinical specimens. Therefore, the adjustment has to be based on a reversing process. First, converting to represent the overall studied samples was done. Since the ratio of positive samples to overall investigated samples according to earlier mentioned data is equal to 100:310 or 1:3.1, the conversion of the frequency of each rotavirus strain to reflect the overall investigated sample in the first step is by dividing by 3.1.

The derived data from this step was then subtracted from the final adjusted frequency by the previously mentioned false positive possibility percentage (40%).

Statistical analysis

The present study used descriptive statistical analysis. The basic arithmetic calculations were performed. The path possibility distribution was used for further calculation of the frequencies in the mathematical model as earlier mentioned. Since there is no analytical statistical analysis, no statistical significance level is mentioned.

Results

Adjustment to correct for false positivity in monitoring rotavirus epidemiology pattern

The stepwise manipulation is done in order to derive the final rotavirus epidemiology pattern. The results in each step are presented in **Table 1**. The background data shows that the G3P[8] accounts for 31 percent, followed by G1P[8] (20.8 percent), G9P[8] 17.2 percent, G2P[8] 13.8 percent, and others 17.2 percent. According to the primary data, the suspicious patients with clinical symptoms to be investigated account for 19.3% of all investigated cases. After converting to represent overall studied samples, the derived pattern is G3P[8] accounts for 10 percent, followed by G1P[8] (6.7 percent), G9P[8] 5.5 percent, G2P[8] 4.5 percent, and others 5.5 percent.

Final outcomes

After adjustment to correct false positives (40%), the rotavirus epidemiology pattern, with regard to all investigated cases, is described as G3P[8] (6 percent), followed by G1P[8] (4 percent), G9P[8] 3.3 percent, G9P[8] 2.7 percent, G2P[8] percent, and others 3.3 percent.

Discussion

Rotaviruses have remained the predominant cause of acute, severe, dehydrating diarrhoea among babies and young children worldwide since their discovery in human cases about four decades ago [2]. Oral rotavirus vaccinations were prequalified by the WHO a decade ago and introduced in many countries, resulting in a significant reduction in the worldwide burden of disease, though not without hurdles in reaching global effectiveness [2]. The never-ending rotavirus diarrhoea epidemic, as well as the deaths linked to it in developing countries, has put the world on edge [2]. Vaccines against rotavirus have been developed in recent years to help reduce the morbidity associated with severe rotavirus diarrhoea [11-17]. The quality and coverage of routine rotavirus vaccinations should be assessed, and the surveillance sys-

tem needs to be reinforced to find, stop, and contain a comparable outbreak [11-17].

Before a rotavirus vaccine programme can be implemented, data on the present burden of rotavirus disease, as well as the distribution and frequency of circulating rotavirus strains in different parts of the country, is required [5]. After the advent of rotavirus vaccinations, diversity in group A rotavirus strains has been a growing concern around the world [5].

Despite vaccination, clinical problems due to rotavirus infection is still observed. There is still a requirement to have a surveillance system to monitor the rotavirus epidemiology pattern, which is useful for planning for disease control. Basically, high-throughput, sensitive, and specific techniques are required to swiftly diagnose and characterize rotavirus strains in stool samples for proper patient treatment, as well as to monitor circulating vaccine and wild-type rotavirus strains [7]. In clinical practice, the immunochromatography test is routinely used to identify rotavirus infection. Immunochromatography tests are unique separation-free procedures in clinical biochemistry, with analyte concentration proportional to the distance along a chromatographic strip where colour develops rather than the degree of colour development. New molecular approaches that are more sensitive and specific than existing assays are being rapidly developed for detection, genotyping, and whole genome characterization of circulating rotavirus wild-type and vaccine strains, causing medical concerns [7]. Rotavirus diagnosis accuracy, on the other hand, is crucial. Precision is crucial in providing precise information on rotavirus epidemiology in any scenario. When the immunochromatography test is incorrectly positive, there may be inconsistency between the two tests, the immunochromatography test and the nucleic acid test.

As a result, when interpreting the findings of basic rotavirus monitoring in a system based on immunochromatography tests, adjustments must be made to address the issue of accuracy. To solve the false positive issue, the modified rotavirus pattern is used. The present study is based on the basic concept that any laboratory investigation can generate a false positive possibility. The problem in laboratory investigation may be corrected by good quality control, but there is still the possibility of a false positive result. In the current report, the authors used a mathematical technique for adjustment of the rotavirus frequencies derived from molecular epidemiology surveillance. The authors present their findings on the expected pattern of rotavirus epidemiology in a tropical setting.

This study's findings indicate that the rotavirus epidemiology pattern differs between before and after adjustment. Prior to adjustment, background data from positive samples typically reveals high frequencies of various rotavirus strains. However, the reported data typically only includes the rate of the chosen studied positive samples, so it cannot represent the rate of all positive samples as a whole. As a result, the result must be modified to reflect the rate across all samples. At this stage, the authors can demonstrate that the rotavirus epidemiology is changing at a decreasing rate. In addition, the false positive still needs to be adjusted for. The final rate will be determined following the corrections step and can accurately reflect the epidemiology pattern among the overall positive samples. The current study can demonstrate that, in actuality, a stepwise adjustment can lead to a rate that is significantly lower. It can show that up to 19.3% of patients presenting symptoms suspected of rotavirus infection have carried rotavirus and the most common carried strain is G4P[8], which accounts for 6% of the total. Therefore, the rotavirus is still an important problem to be managed in the study setting.

Based on the authors' own views, the exact genetic epidemiology of rotavirus is very important for local public health policy planning. It is necessary to have the data corrected for possible false positives. The current study is a good example. To understand the broader influence of the rotavirus vaccines on locally circulating strains, it is crucial to comprehend the strain diversity before and after vaccine introduction [18]. As suggested by Gibory et al. [19], genotyping is required to identify rotavirus strains, and it is vital to prevent reporting of false-positive cases of active rotavirus infection in newly vaccinated individuals [20]. The detection of changes in genotypic patterns and the provision of diagnostic laboratories with quality assurance through the reporting of instances of wildtype, vaccine-like, or false positive rotavi-

rus results are both dependent on ongoing rotavirus monitoring [20].

The shortcomings of this study are due to the nature of the retrospective study. The mathematical model analysis makes use of primary data for predictive purposes. The actual genetic epidemiology might change, and it requires a continuous follow-up study to monitor the situation. Future similar research should be performed to maintain the most updated data on the genetic epidemiology of rotavirus. Since rotavirus infection is still an important public health problem worldwide, molecular epidemiology surveillance is still an important and necessary clinical investigation. Based on the authors' expectation, the problem of laboratory accuracy will remain an important issue to be managed and the adjustment for correctness should be continuous in clinical laboratory.

Conclusion

The findings on rotavirus epidemiology in a tropical setting is shown. The modified rotavirus pattern is utilised to address the false positive issue. The authors may exhibit the altered rotavirus epidemiological pattern in the analyzed scenario in this research. It has been shown that up to 19.3 percent of patients with symptoms suggestive of rotavirus infection have rotavirus, with G4P[8] accounting for 6 percent of those infected. As a result, the rotavirus remains a significant issue that must be addressed in the research context. Finally, this report can give a clue for further implementation of the mathematical model technique to adjust the derived laboratory in other laboratories that might have the problem of false positive results in surveillance of rotavirus molecular epidemiology.

Disclosure of conflict of interest

None.

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