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Evaluation of diagnostic tests for plague in Madagascar

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Madagascar is the country that reports the most cases of plague in the world. This is an evaluation study of plague diagnosis over a 2-year period from January 2017 to December 2018. For all suspected plague cases, peripheral RDT (performed locally), central RDT (performed in the laboratory) and PCR were performed. Parameters of the diagnostic tests used, year of study, performance and study of concordance between tests were studied. 2981 cases were collected, 21.6% were confirmed, 28.4% probable and 50% suspected. The sensitivities of peripheral RDT, central RDT and PCR were 96.55, 100 and 97.41%, respectively; the specificities were 41.43, 51.17 and 89.24%. Cohen's kappa was 0.12 between peripheral RDT and culture; 0.17 between culture and central RDT and 0.64 between CRP and culture. For pneumonic plague (PP) patient samples, sensitivities were 80.00, 66.66 and 93.33%, Cohen's kappa was 0.017 between peripheral RDT and culture; 0.013 between central RDT and culture and 0.371 between PCR and culture; sensitivities of peripheral RDT and central RDT were 75.00 and 62.50% for 2017, respectively. The null hypothesis between diagnostic tests was rejected, discordance between tests was found. Sensitivity is lowered during lung sampling and during 2017.

Key words: Plague, polymerase chain reaction, technology assessment.

INTRODUCTION

Plague is a bacterial disease caused by a small gram-negative bacillus: Yersinia pestis. It is very serious in humans as it is often fatal without prompt and appropriate treatment. Its case fatality rate is 30 to 60% for the bubonic form and almost always fatal in the pulmonary form if left untreated (OMS, 2022). Late diagnosis is one of the main causes of the spread of the disease, as it limits the effectiveness of control measures. Worldwide, between 2010 and 2015, 3248 cases of plague were recorded, 584 of which were fatal (OMS, 2022). Between 1990 and 2020, nearly 50,000 human cases of plague were reported to the World Health Organization (WHO) by 26 countries in Africa, Asia and the Americas. Plague is a disease that is still prevalent today and is one of the

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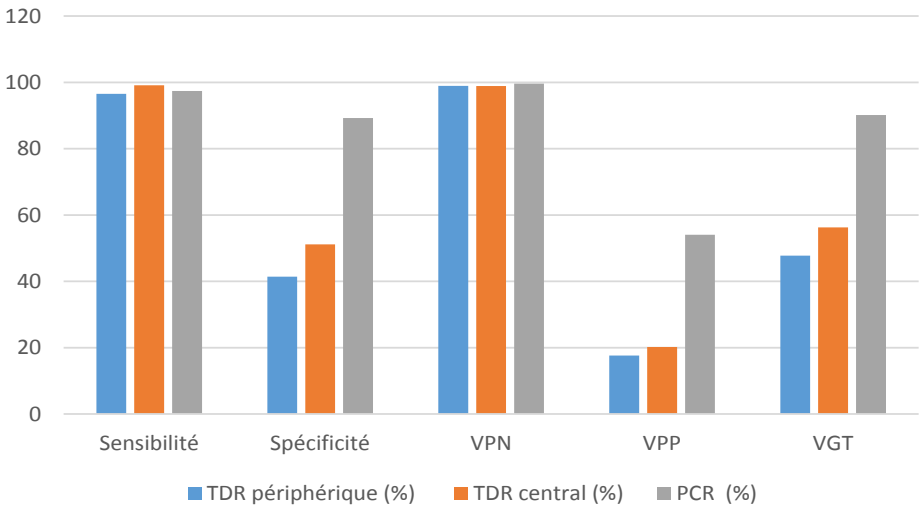

Figure 1. Global evaluation of the performance of diagnostic tests for plague in Madagascar (n = 1009).
Source: Authors

re-emerging diseases (Institut Pasteur, 2021). It remains a public health problem in many countries. In the DRC, between 2020 and 2021, 578 cases and 44 deaths related to plague were reported in the entire province of Ituri (Nations Unies, 2021). In Madagascar, cases of plague are reported every year with a seasonal upsurge between August and April. Pneumonic plague is one of the most severe forms of plague and in this country, a total of 30 cases of pneumonic plague were reported on 03 September 2021, of which 12 were confirmed and 7 were deaths (CFR=23%) (Morvan, 2021). To confirm its diagnosis, the main diagnostic methods are: Microscopy of a smear after Gram or Giemsa or Wayson staining, the rapid diagnostic test (RDT) by detection of the F1 antigen specific to *Y. pestis*, the ELISA test by detection of the anti-F1 antibody, culture and detection by molecular technique or polymerase chain reaction (PCR) (Aubry and Gaüzère,2021). Diagnostic tests must be evaluated against the gold standard for the isolation of the bacteria by bacterial culture. For years, microscopy or RDT has been evaluated to confirm the diagnosis of the plague. This study is the first to evaluate the performance of both RDT and PCR. The objective of this study is to define the null hypothesis of RDT and PCR versus bacterial culture for the diagnosis of plague in Madagascar.

METHODS

This is a retrospective analytical plague diagnostic evaluation study spanning a 2 year period from January 2017 to December 2018. The study was conducted within the Directorate of Epidemiological Health Surveillance and Response (DVSSER) by exploiting the plague database containing epidemiological and biological data. The diagnostic tests used for plague confirmation were peripheral RDT (performed locally), central RDT (performed in the laboratory), PCR and bacterial culture. The latter 3 were performed in the central plague laboratory hosted by the Pasteur Institute of Madagascar (IPM). The study included suspected plague cases for which all tests were performed and excluded cases with incomplete data. This was an exhaustive study and the parameters studied were the different diagnostic tests used (peripheral RDT, central RDT, PCR, Culture), the clinical forms (PP, BP), the evaluation of the performance of the diagnostic tests and the study of the concordance between the tests. Peripheral RDTs are RDTs that are performed at the bed of the patient, while central RDTs are RDTs performed in the laboratory. Data were processed and analyzed on Epi info 2012 version 3.5.4 software with a significance level below 0.05.

RESULTS

During the two years of the study, 2981 cases of plague were notified, 644 cases were confirmed (21.6%), 845 cases were classified as probable (28.4%) and 1492 cases were classified as suspect (50%). Of the 2981 cases, 2915 cases received peripheral RDT, 2556 cases received central RDT, 2826 PCR cases, and 1751 bacterial culture cases. One thousand nine (1009) cases had complete results on peripheral RDT (635 positive cases: 62.93%), central RDT (547 positive cases: 54.21%), PCR (209 positive cases: 20.71%) and bacterial culture (116 positive cases: 11.5%). The peripheral RDT, the central RDT and the PCR were evaluated relative to the bacterial culture which is the gold standard for the laboratory diagnosis of plague (Figure 1).

The study of the concordance of the plague diagnostic tests with the gold standard, the bacterial culture was carried out. Between bacterial culture and peripheral TDR, Cohen's kappa = 0.12, between bacterial culture and central TDR, Cohen's kappa = 0.17, between

Table 1. Study of the concordance between diagnostic tests for plague with the gold standard.

	Peripheral RDT			Central RDT			PCR		
	Bacterial culture			Bacterial culture			Bacterial culture		
	Positive (n)	Negative (n)	Total (n)	Positive (n)	Negative (n)	Total (n)	Positive (n)	Negative (n)	Total (n)
Positive	112	523	635	111	436	547	113	96	209
Negative	4	370	374	5	457	462	3	797	800
Total	116	893	1009	116	893	1009	116	893	1009
Cohen's Kappa		0.12			0.17			0.17	

Source: Authors

Table 2. Performance (%) of plague diagnostic tests according to the type of sample.

	Peripheral RDT		Central RDT		PCR	
	Bubo	Sputum	Bubo	Sputum	Bubo	Sputum
Sensitivity	99.00	80	100	66.66	98.01	93.33
Specificity	40.38	41.86	59.23	47.86	79.23	93.36
Predicted positive value (VPP)	39.21	3.15	48.79	2.94	64.7	25.22
Predicted negative value (VPN)	99.05	98.88	100	98.37	99.03	99.83

Source: Authors

Table 3. Study of the concordance of diagnostic tests for plague according to PP patient samples.

	Peripheral RDT			Central RDT			PCR		
	Bacterial culture			Bacterial culture			Bacterial culture		
	P	N	T	P	N	T	P	N	T
P	12	368	380	10	330	340	14	42	56
N	3	265	268	5	303	308	1	591	592
T	15	633	648	15	633	648	15	633	648
Cohen's kappa		0.017			0.013			0.371	

P= positive, N= negative, T= total.
Source: Authors

bacterial culture and PCR, Cohen's kappa = 0.64 (Table 1).

The sensitivities of the peripheral RDT, of the central RDT, of the PCR according to the types of samples have been shown in Table 2.

Regarding the concordance of diagnostic tests for plague, for lung samples, Cohen's kappa = 0.017 for bacterial culture and peripheral RDT, 0.013 for bacterial culture and central RDT and 0.371 for bacterial culture and PCR (Table 3). For the bubonic samples, we found respectively a Cohen's kappa 0.448; 0.448 and 0.667 (Table 3).

Concerning the concordance of the diagnostic tests for plague, for the BP patient samples, we found respectively a Cohen's kappa 0.448; 0.448 and 0.667 (Table 4).

DISCUSSION

Madagascar is one of the 46 member states of the African region of the World Health Organization (WHO) which monitors the disease through integrated disease surveillance and response. This system aims to monitor diseases with potential epidemics including 28 diseases and plague is one of these diseases. Globally, 10 of the 33 countries with a plague outbreak have reported human cases in the past 5 years. The regions that have reported these cases are limited to sub-Saharan Africa, Asia, and North and South America. Madagascar remains, with the Democratic Republic of Congo (DRC), the country which reports the most cases of plague in the world. In 2008, 2683 cases were notified and 5 countries

Table 4. Study of the concordance of diagnostic tests for plague according to BP patient samples.

	Peripheral RDT			Central RDT			PCR		
	Bacterial culture			Bacterial culture			Bacterial culture		
	P	N	T	P	N	T	P	N	T
P	100	155	255	101	106	207	99	54	153
N	1	105	106	0	154	154	2	206	208
T	101	260	361	101	260	361	101	260	361
Cohen's kappa		0.448			0.448			0.667	

P= positive, N= negative, T= total.
Source: Authors

(Madagascar, Uganda, Peru, DRC and United Republic of Tanzania) alone reported 98% of global cases. Ten years later, the total number of cases in the world is 10 times less and they are reported in only 5 countries (Bertherat, 2019).

During this study, 2981 cases of human plague were notified, 21.60% were confirmed (bacterial culture positive or RDT positive with PCR positive), 28.34% were classified as probable (RDT positive or PCR positive) and 50.05% were classified as suspect (clinical picture suggestive of plague with a favorable epidemiological link). This prevalence is similar to the study carried out previously in Madagascar from 1998 to 2016 including 13234 cases reported to the National Plague Program (PNLP) (Andrianaivoarimanana et al., 2019) and to that of Uganda from 2008 to 2016 with 255 cases of human plague reported. For this country, the diagnostic methods for plague were serology and bacterial culture (Forrester et al., 2017). In Madagascar, the plague is an endemic disease, with a seasonal upsurge between August and April (Rajerison et al., 2020). When comparing the results of plague diagnostic tests with the gold standard, the positivity rate is not similar; it is 62.93% for peripheral RDT, 54.21% for central RDT, 20.71% for PCR against 11.50% for the gold standard which is bacterial culture. We can therefore say that there are many false positives, especially for RDT. Similarly, the percentage of true negatives is underestimated. Regarding the overall performance of plague diagnostic tests in Madagascar, sensitivities were excellent at 96.55, 100 and 97.41%. On the other hand, specificities were low for RDT, 41.43% for peripheral RDT, 51.17% for central RDT and 89.24% (high) for PCR. Their PPV was low and their NPV was high. These sensitivities of RDTs are similar to those of previous study (Chanteau et al., 2003; Andrianaivoarimanana et al., 2019). On the other hand, the specificity is different from that observed in previous studies which found a specificity of RDTs at 100% (Chanteau et al., 2003). Concerning PCR, the sensitivity is similar to that found by some authors in the literature with sensitivity of between 80 and 95% (Matero et al., 2009; Loiez et al., 2003). The sensitivity can be altered (35 to 50%) for samples collected and transported under non-optimal conditions (Rahalison et al., 2000). This result is different from that observed by some authors (Matero et al., 2009; Loiez et al., 2003) who also found a PCR specificity of 100%. In this study, the pla gene was also used with other genes, including the caf1 genes for real-time PCR and the caf1 and inv genes for conventional PCR. PCR performance depends on the amplification target used and the quality of the sample, and can be further improved by changing the amplification targets. In general, high sensitivity of diagnostic tests was related to limited specificity, these results are similar to previous studies (Lutkenhoner and Basel, 2013). An ideal diagnostic test has high sensitivity coupled with high specificity. However, it is extremely difficult to meet these criteria. It is often necessary to find a reasonable balance between sensitivity and specificity. If highly pathogenic bacteria such as *Yersinia pestis* are detected in the field, sensitivity appears to be a priority, with positive samples usually being confirmed by more sophisticated laboratory methods. When rapid test kits are applied, the likelihood of false negative results should be taken into account. The low sensitivity of the tests used in the field makes it necessary to retry even negative samples in the laboratory with all the negative financial consequences. At the level of health systems, when consulting a patient with suspected plague, it is preferable that a test is available on site with a rapid result such as the RDT and that this RDT has a high sensitivity allowing immediate treatment of the patient after. This RDT will be confirmed by a more specific test such as PCR and this will gradually eliminate cases of false positive RDTs. For the diagnostic tests of the plague in Madagascar, the sensitivities of the RDTs were excellent; on the other hand their specificities were low at 41.43% for the RDT carried out at the level of health systems and at 51.17% for the RDT carried out at the level of health systems of the central plague laboratory. This means that there are many healthy people who have received treatment for plague without being sick, unnecessary treatment. On an individual level, these people will be frustrated, stressed and may experience

side effects from prescribed medications. Collectively, the effect can be positive in sensitizing the community on brush clearing, pest control, and deforestation and bushfire avoidance. The total overall value for RDTs was low, 47.77% for peripheral RDT and 56.29% for central RDT; on the other hand, it is 90.18% for PCR. Therefore, it will be necessary to search the market for RDTs which will have the best performance, especially in terms of specificity.

A gold standard or reference test is the best diagnostic test already available for the pathology of interest and which can be used in a "reasonable" situation, that is to say applicable in patients in the clinic or in research (Versi, 1992). Here, the bacterial culture is the gold standard recommended by the WHO; it allows the isolation of *Yersinia pestis*. Agreement between judgments (diagnostic tests) is defined as the conformity of two or more pieces of information that relate to the same subject. The rate of agreement is therefore estimated by the Kappa coefficient proposed by Cohen (1960). It is an index that varies between 0 and 1, which reflects a level of agreement or concordance as much as its value is close to 1. In this study, between peripheral RDT and bacterial culture, Cohen's kappa was 0.12; between central RDT and bacterial culture it was 0.17 and between PCR and bacterial culture it was 0.64. It can be said that there is a poor degree of agreement between peripheral RDT and bacterial culture and also between central RDT and bacterial culture; in contrast, there is a good degree of agreement between PCR and bacterial culture. These RDTs are therefore not recommended as diagnostic tests for plague because of this discrepancy. It is best to search the market for RDTs that will match the best bacterial culture. It is best to search the TDR market that will have the best matches with the bacterial culture.

The sensitivities of the diagnostic tests were low for peripheral RDT and central RDT in PP patient samples at 80.00 and 66.66%, respectively. The sensitivities were high during the PP patient samples, 99.00 and 100% and during the PCR regardless of the type of sample, 93.33% during the pulmonary samples and 98.01% during the BP. Low sensitivity to PP could be related to the very short reaction and relative insufficient affinity and avidity of the antibodies used. An additional reason could be the low volume of the lung sample. During PB and PP patient samples, the specificities of the peripheral RDT for these two types of sampling were 40.38 and 41.86%, respectively; for the central RDT, they were 59.23 and 47.86% and 79.23 and 93.36% for the PCR. The specificity of RDTs regardless of the type of sample is limited; it was higher for BP than for PP. This study is similar to that observed previously between 2002 and 2007 which found a specificity of 60.5% against 44.7% (Andrianaivoarimanana et al., 2019). For PCR, the specificity is higher than that of RDTs although it is not excellent, unlike RDT; it is higher in PP than in BP.

During PP patient samples, the sensitivities and specificities of RDTs were low; therefore, true positive cases were diagnosed with false negative. This is serious because these are cases of PP with a high risk of lethality in the absence of prompt treatment within 48 to 72 h. For those around the undiagnosed, untreated patient, no preventive measures have been taken and since the transmission of PP is aerial, it will be transmitted to the family, to those around them and with the risk of an epidemic PP difficult to manage after. Since the sensitivity of RDTs in PP patient samples is limited, their specificity is also limited. The use of these RDTs when PP is suspected requires a question; it will be necessary to search the market for RDTs that will have the best sensitivities and specificity. Otherwise, for the suspicion of this clinical form of plague, PCR should be used first if it has better sensitivity and specificity. In this study, the latter were respectively 92.30 and 93.36%. An improvement in the molecular technique to increase their performance could be done and in this case PCR would be used on the first line in a case of PP. According to the literature, the performance of PCR depends on the amplification target used; perhaps changing the targets could help improve their performance (Matero et al., 2009). During this study, for PP patient samples, the degrees of agreement between the diagnostic tests and the gold standard were very poor with a Cohen's kappa of 0.017 and 0.0013 for the RDTs and the bacterial culture and of 0.371 for PCR and bacterial culture. The RDTs used are therefore not recommended in the first line in cases of suspected PP. Other suppliers of RDTs with a good match with the bacterial culture for PP patient samples should be sought in the market. It is also important to improve the quality of the sample as this contributes to the improvement of the tests. Training of health workers on the quality of the sample is essential. They need to know the ideal type of sample, how to properly sample, and how to store and ship samples. Health workers also need to be trained in the use and interpretation of RDT. On the other hand, during the PB patient samples, the concordances between the diagnostic tests and the gold standard were acceptable. The degrees of agreement between RDTs and bacterial culture were moderate with a Cohen’s kappa similar to 0.448; agreement was good between PCR and bacterial culture with a Cohen’s kappa of 0.667. It is therefore always recommended to use RDT if BP is suspected.

This study remains limited due to the retrospective nature of the study; therefore not all suspected cases of plague during this study period received 2 RDTs, PCR and bacterial culture at the same time. The evaluation of the performance of each of these tests was therefore not likely to be exhaustive on all suspected cases of plague. Some cases have been tested with RDTs and bacterial culture, some do not have bacterial culture results and some do not have PCR results.

Conclusion

Plague is a disease that still exists in Madagascar and in rare countries in the world. Test performance was performed on 1009 cases that had complete results and were assessed against bacterial culture. A poor degree of agreement was found between the RDT and the gold standard. On the other hand, there is a good degree of agreement between the PCR and the gold standard. In PP patient samples, this agreement was very poor to poor for diagnostic tests for plague. The sensitivity of RDT was low for PP patient samples. The null hypothesis was rejected, a discrepancy in diagnostic tests was observed, it will be necessary to search the market for RDT that will have the best sensitivities and specificity, especially in the event of suspected PP. Training of health workers in plague endemic areas on the quality of samples, performing RDT and their interpretations is necessary. The performance of PCR can be further improved by changing the amplification targets.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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