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ORIGINAL ARTICLE

Evaluation of Cortez OneStep Chlamydia Rapicard™ Insta Test for the Detection of *Chlamydia trachomatis* in Pregnant Women at Mbare Polyclinic in Harare, Zimbabwe

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ABSTRACT

Background: Cervical chlamydia infection poses high risk of pregnancy complications and neonatal infection. Reference methods for the detection of chlamydia infection are not available for routine use in developing countries. Point-of-care (POC) tests can bridge this gap. This study evaluated Cortez Onestep Chlamydia Rapicard™ insta test for the detection of *Chlamydia trachomatis* in pregnant women at Mbare Polyclinic and determined the prevalence of *C. trachomatis*.

Methods: This was a cross sectional study in 242 pregnant women aged ≥18 years attending their first ANC visit at Mbare polyclinic in Harare, Zimbabwe. Data collection form was used to obtain demographic and predisposing factors to Chlamydia infection and two endocervical swabs were collected from each patient. One specimen was examined by the POC test at the clinic and the other by SDA method in the laboratory.

Results: The sensitivity, specificity, positive and negative predictive values of the rapid kit were 71.4%, 99.6%, 90.9% and 98.3% respectively. Prevalence of *C. trachomitis* was 5.8% by SDA method.

Conclusion and Global Health Implications: The kit's sensitivity (71.4%) and specificity (99.6%) implies that the rapid test is an important test which needs further evaluations. The prevalence of *C. trichomitis* of 5.8% is comparable to studies done elsewhere in Africa.

Key words: *Chlamydia trachomatis* • Antenatal Clinic • Point of Care Tests • Rapid Test • Cortez One Step Chlamydia Test

1. Background and Objectives

Chlamydia trachomatis is an important cause of morbidity and mortality in women and neonates.[1] It is commonly associated with cervicitis, pelvic inflammatory disease (PID) and infertility. Pregnancy complications, including ectopic pregnancy, premature delivery, low birth weight, stillbirths and neonatal death have been cited in the literature.[2,3]

Other pregnancy complications include premature rapture of membranes, gestational bleeding, amnionitis, neonatal and puerperal infections.[3]

Up to 80% of women and 50% of men infected by *C. trachomatis* infections are asymptomatic, which makes clinical diagnosis of *C. trachomatis* infections difficult.[4] Women with cervical chlamydia infection at the time of delivery have 60-70% chance of transmitting the infection to the infant,[5] which may result in neonatal conjunctivitis in 35-50% of cases and neonatal pneumonia in 10-20% of cases.[6,7] Screening for chlamydia infection in pregnant women is, therefore, a high public health priority. However, due to resource limitations in developing countries, chlamydia infection in pregnant women is not routinely screened. Diagnosis is limited to syndromic approach, a diagnostic strategy whose sensitivity and specificity are very low.[8]

Available reference methods for the diagnosis of *C. trachomatis* infection including isolation cell culture, direct fluorescent antibody (DFA), enzyme immunoassay (EIA), nucleic acid probe (NAP) tests, including the APTIMA Combo 2 Assay, nucleic acid amplification test (NAAT).[7,9] However, these tests have limited utility in developing countries because of costs and long turnaround times.[10,11] For example the cost of the Becton Dickinson (BD) ProbeTec™ ET *Chlamydia trachomatis* amplified DNA assay for detecting *C. trachoatis* at the time of this study was US$10.00 per sample on a cost per test model. Therefore, rapid antigen detection tests or Point of Care (POC) tests are preferred in developing countries.

Rapid antigen tests use monoclonal antibodies in immunochromatographic strips to capture and detect chlamydial antigens from endocervical swabs. They require less expertise and give results within 30 minutes.[12] Their sensitivities, against Polymerase Chain Reaction (PCR) range between 49.7% and 95%, while their specificities between 97.9% and 100%.[13-16] Performance characteristics of different POC tests have been evaluated in various setting and these include BioStar® OIA® CHLAYMDIA test, Clearview Chlamydia, QuickVue, Chlamydia Rapid Test (CRT) and Chlamydia Dipstick Test.

However this study evaluated Cortez Onestep Chlamyidia Rapicard™ insta test for the detection of *Chlamydia trachomatis* in pregnant women in Harare, Zimbabwe and determined the prevalence of *C. trachomatis* in the pregnant women from the test conducted. This POC test is manufactured by Cortez Diagnostics, Inc. and the literature on the test was obtained on the company website (www.rapidtest.com/chlamydia) and on the kit package insert. To the best of our knowledge, we believe this test has not yet been evaluated elsewhere except by the manufacturer which makes us first to evaluate this test kit other than the manufacturer.

2. METHODS

2.1. Study setting

Mbare is a densely populated suburb in Harare, Zimbabwe with an estimated population of 300,000 people. Mbare Polyclinic, is a primary health care facility in Mbare. This study was conducted at Mbare Polyclinic's antenatal clinic (ANC). The clinic has three days set aside for women attending their first ANC visits during the week one day for repeat visits.

2.2. Study design

This was a cross sectional experimental study in pregnant women attending first ANC visit at Mbare Polyclinic.

2.3. Study population and sample size

The target population were pregnant women 18 years or older attending ANC visits between January and April 2012. Eligible women should not have received erythromycin, azithromycin, or amoxicillin within the previous one month. A total of 242 consenting met the selection criteria and were included in the study.

2.4. Chlamydia trichomitis detection using Cortez OneStep Chlamydia *RapiCard™ insta* test

This is a rapid immunoassay for direct qualitative detection of *Chlamydia trachomatis* antigen from endocervical or endourethral swab specimens. The assay is based on chemical extraction of a

carbohydrate antigen from *C. trachomatis* followed by qualitative detection of *C. trachomatis* utilization using migratory colour immuno-assay technology. The test takes about 20 minutes.

Extraction buffer A was mixed by swirling and six drops of the buffer were added to each of the labelled test tubes. Patient swab specimens were placed in the test tubes and swirled briefly to mix the sample with the buffer. The swabs were incubated for 5 minutes at 25°C. Thereafter, 6 drops of Extraction Buffer B were added to each of the test tubes. The swabs were twirled vigorously for 10 seconds and removed from the test tubes. The swabs were discarded after all the liquid was removed from the swabs by pressing the swabs against the test tube walls. The test tubes were recapped and further mixed by gently swirling the tubes. Seven drops of the swab extract were dispensed into the sample wells of the test device. The results were read after 15 minutes and recorded on the patients' laboratory request forms.

A positive result was interpreted as two rose pink bands appearing after 15 minutes, one in the control zone and another in the test zone indicating the presence of *C. trachomatis* antigen in the sample. One rose pink band in the control zone indicated a negative result. No pink band in the control zone with or without a pink band in the test zone was interpreted as an invalid result.

2.5. Chlamydia trichomitis detection using BD ProbeTec™ ET Chlamydia trachomatis amplified DNA assay

The BD ProbeTec™ ET *Chlamydia trachomatis* amplified DNA assay is based on the simultaneous amplification and detection of target Deoxyrobonucleic Acid (DNA) using amplification primers and a fluorescent labeled detector probe. The BD ProbeTec™ ET *Chlamydia trachomatis* (CT) Amplified DNA Assay, when tested with the BD ProbeTec ET System, uses Strand Displacement Amplification (SDA) technology for the direct, qualitative detection of *Chlamydia trachomatis* DNA in endocervical swabs. The detection procedure was performed according to the manufacturer's instructions. Briefly, each swab was inserted into a tube with 1 ml of the diluents and mixed by swirling to extract the endocervical secretion from the swab. The contents were vortexed for 5 seconds to mix the fluid and the sample. After vortexing the tubes were placed on the lysing rack, locked into position lysed at 114°C for 30 minutes. After lysing, the tubes were left 25°C for 15 minutes to cool.

After cooling, 150 µl of lysed sample was transferred to the priming microwells using a multi-channel micropipette. The priming plate was covered with a priming cover and incubated at 25°C for 20 minutes. After 20 minutes of incubation, the priming microwells with the cover removed and empty amplification microwells plates were placed in priming/warming heater (set at 72.5°C and 54°C) respectively. The plates were incubated for 10 minutes. At the end of the 10 minutes, 100 µl was transferred to the corresponding amplification microwells. Immediately after the transfer, the amplification microwells were sealed with an amplification sealer and placed into instrument. The instrument was immediately activated for automated amplification and detection of target DNA for 60 minutes. Interpretation of the test results was done automatically by the instrument according to method other than acceleration (MOTA) scores.

2.6. Treatment

Those who tested positive with either of the two tests were put on a 7-day treatment course of erythromycin, 500mg four times daily, by ANC midwives.

2.7. Statistical data analysis

Descriptive summary statistics of the data were obtained using Stata version 12 statistical software. Univariate logistic regression analysis ($p<0.05$ at 95% confidence interval (C.I) was performed to identify factors associated with *C. trachomatis* infection and multivariate analysis ($p<0.05$ at 95% C.I) was performed to adjust for confounding risk factors associated with *C. trachomatis* infection. The performance characteristics, including sensitivity, specificity, and positive and negative predictive values were calculated by standard methods.

2.8. Ethical considerations

Ethical approval was obtained from the Joint Research Ethics Committee (JREC) and the Harare City Health Department. Participants were given informed consent forms written in the language they preferred between English and vernacular (Shona). Signed informed consent forms and the results were kept in custody by the researcher. anonymous identity numbers were used on the samples to conceal the participants' identities. Participants were also informed that their participation was voluntary and that they could decline to answer certain questions, including that they could withdraw their participation at any time without affecting the care they receive at the clinic.

3. Results

3.1. Characteristics of the study participants

Two hundred and forty two pregnant women with a median gestation age of 30 (IQR: 26-34) weeks were enrolled to participate in the study. They had a median age of 25 (IQR: 21-31) years. Two hundred and thirty seven (98%) of the women were married. Thirty (12%) of these women had previously been treated for STI.

3.2. Prevalence of C. trachomatis in the study participants

Using the SDA reference method, 14/242 (5.8%) of the women had positive results for *C. trachomatis,* while 11/242 (4.6%) of the women tested positive for *C. trachomatis* using the rapid test method. However, the results of the two tests were not statistically different (p=0.683).

Notably, the positivity rate in 18-25 age group was 6.6% using the rapid test and 7.4% using the SDA reference method. In addition, the 26-30 age had a positivity rate of 3.8% using the rapid method and 5.8% using the SDA reference method. Those aged above 30 years had a positivity rate of 1.5% using the rapid test and 2.9% using the reference method. All women who tested positive for *C. trachomatis* with both tests were married.

Women in the first trimester had a similar positivity rate of 11.1% using the reference method and the rapid method. However, women in the second trimester had positivity rates of 9.5% and 7.9% using the reference and the rapid method, respectively. Women in the third trimester had the least positivity rates of 4.1% and 2.4% using the reference and the rapid method, respectively. However, the positivity rates were not statistically different between the semesters (p=0.229).

Table 1 presents results of logistic regression analysis of factors associated with *C. trachomatis* infection in the pregnant women. When adjusted for other factors, the 18-25 age group was 2.7 times more likely to be positive for chlamydial infection when compared to the ages above 30. However, age was not a statistically significant predictor of chlamydial infection (p> 0.05). The 26-30 age group had a lower risk of infection (OR=2.1) compared to the ages above 30. When adjusted for other factors, being in the first trimester was 2.8 times more likely to be infected with *C. trachomatis* CI (OR: 0.3 – 26.4) when compared to being in the third trimester.

3.3. Diagnostic capability of Cortez OneStep Chlamydia Rapi CardInsta test

Table 2 presents the performance characteristics of the Cortez OneStep Chlamydia RapiCard Insta test method against the SDA test method. Out of the

Table 1: Factors associated with *C. trachomatis* infection in pregnant women attending ANC at Mbare Polyclinic

Variable included	Univariate analysis		Multivariate analysis	
	OR	95% CI	AOR	95% CI
Risk factor				
Age (years)				
18-25	2.6	0.55 – 12.53	2.7	0.56 – 13.32
26-30	2.0	0.33 – 12.56	2.1	0.33 – 13.40
>30	1	-	-	-
Gestational age (weeks)				
13	2.9	0.30 – 26.59	2.8	0.30 – 26.41
14-27	2.5	0.79 – 7.60	2.5	0.78 – 7.76
>28	1			
Previously treated for STI				
Yes	1.19	0.25 – 5.60	1.3	0.25 – 6.29
No	1			

14 true positives, the Cortez OneStep Chlamydia RapiCard™ Insta test was able to detect 10 true positives (71.43%). The rapid test was able to detect 227 of 228 (99.56%) of the true negatives. Therefore, Cortez OneStep Chlamydia RapiCard™ Insta test had a sensitivity ratio of 71.4% and a specificity ratio of 99.6%. The negative and positive predictive values were 98.3% and 90.9% respectively.

4. Discussion

4.1. Prevalence of C. trachomatis

The results of this study by the rapid (4.6%) and the reference method (5.8%) were not statistically different. This implies that the Cortez OneStep Chlamydia RapiCard™ Insta test is a valuable test for the detection of *C. trachomatis* in this population. These findings corroborate those of other studies reviewed by Wilson et al[17] and other studies in Africa.[1,18-20] These studies reported prevalence of *C. trachomatis* infection ranging from 3 to 31% in sub-Saharan Africa. Munjoma et al reported a seroprevalence of *C. trachomatis* of 4.1% in HSV-2 infected pregnant adolescent women.[21]

Women in the 18-25 age group had higher prevalence (7.4%) compared to the 26-30 age group which had a prevalence rate of 5.8%. According to Mayaud et al, younger age is an important factor associated with *C. trachomatis* infection.[22] This implies that age needs to be considered in screening programmes for *C. trachomatis*. De Muylder et al reported that the presence of chlamydial antibodies were higher in older ages.[23] In relation to marital status in this group, only married women tested positive for chlamydia. The same was for a study done in Nigeria, where married women had higher prevalence (38.4%) compared to the unmarried women.[24] Women in the first trimester tested positive (11.1%).This emphasizes the need to screen for *C. trachomatis* during the first ANC visit, and repeat the test only in the third trimester as recommended by the CDC.[25] There is need to encourage pregnant women to register early for ANC. The low prevalence in the third trimester may be due to use of antimicrobial agents such as amoxycillin to treat other infections. Only two of the women who tested positive for *C. trachomatis* had a history STIs. As also shown in a study by Javato-Laxer and colleagues, previous STI did not appear to increase the risk for *C. trachomatis* infection.[5]

4.2. Performance of Cortez OneStep Chlamydia RapiCard™ insta test

The Cortez OneStep Chlamydia RapiCardInsta Test correctly detected 71.4% of pregnant women with *C. trachomatis* infection and 99.6% of those without the infection. This implies that it is an important test for the detection of genital chlamydia infection.

The high NPV of 98.3% means that by using the rapid test in diagnosis of *C. trachomatis* in pregnant

Table 2: Performance of Cortez OneStep Chlamydia RapiCard™ insta test

Samples Tested (n)	POC Results	SDA Results		Sensitivity	Specificity	PPV	NPV
		+	-				
242	+	10	1	10/14=71.4%	227/228=99.6%	10/11=90.9%	227/231=98.3%
	-	4	227				

PPV=Positive Predictive Value, NPV=Negative Predictive Value

	Gold standard		Total
	S+	S-	
Clinical Test			
T+	a=True Positive	b=False Positive	a+b
T-	c=FalseNegative	d=True Negative	c+d
Total	a+c	b+d	

Sensitivity=a/a+c, Specificity=d/b+d, Positive Predictive Value (PPV) = a/a+b, Negative Predictive Value (NPV) = d/c+d

women, the majority of those with no *C. trachomatis* infection will be correctly identified. However the lower PPV of 90.9% means a relatively lower probability of detecting those pregnant women with the infection than when SDA is used.

The sensitivity of the Cortez OneStep Chlamydia RapiCardInsta test in this study was less (71.4%) than that the rate reported in the manufacturer's package insert of 97.2%. The difference in the sensitivity reported in this study may be due to the comparison of the Cortez OneStep Chlamydia RapiCardInsta Test with SDA. The manufacturers used the latex OneStep immunoassay as the gold standard. The manufacturer's sensitivity claims was higher than the values reported by other studies (3.3-18.8%).[26]

The various rapid tests that have been evaluated for use in the diagnosis of *C. trachomatis* showed different sensitivities in detecting *C. trachomatis*. The sensitivity of 71.4% for Cortez OneStep Chlamydia test in this study was similar to the sensitivity of 73.8% demonstrated by the BioStar® OIA® CHLAYMDIA test.[27]

Studies of other rapid tests in developing countries reported lower sensitivities, ranging from 49.7 to 53.5%.[16] QuickVue had a sensitivity of 92% when its performance was compared to NAAT.[14] In another study the sensitivity of QuickVue Chlamydia was 27% which is lower than the sensitivity reported in this study.[28]

4.3. Limitations

We used a gold standard that is different from the one used by the manufacturer. This test was only evaluated for endocervical swab specimens from pregnant women. The sensitivity and specificity of the test was not evaluated on urethral swabs or vaginal swabs. This evaluation was not conducted using pure isolates of *C. trachomatis*.

5. Conclusions and Global Health Implications

The results of this study shows that the Cortez OneStep Chlamydia test is an important test which needs further evaluation before it can be used for routine screening of *C. trachomatis* infection. The prevalence of *C. trachomatis* in pregnant women in this population was 5.8% by SDA assay. Given the prevalence rate found in this study, there is need for our health policy makers to consider including screening for *C. trachomatis* in pregnant women attending ANC in Zimbabwe. With studies showing an increase in the prevalence of *C. trachomatis* in pregnant women, ranging from 2 to 35%,[20] and POC tests showing as high as 13.3% in Nigeria,[20] the introduction of POC tests for screening of *C. trachomatis* will improve the detection and timely treatment of the infection in asymptomatic pregnant women in resource limited countries like in sub-Saharan Africa.

Compliance with Ethical Standards

Conflict of Interest: *The authors declare that there is no conflict of interests regarding the publication of this paper.* ***Ethics Approval:*** *This study was approved by a recognized Institutional Review Board.* ***Acknowledgments:*** *For granting us the opportunity to carry out this study at Mbare polyclinic, we wish to extend our sincere gratitude to the Harare City Health Department. This project was made possible by the staff at the polyclinic which spared their precious busy schedule to help in data and specimen collection and for this I thank them so dearly. We would also like to thank the UZ-UCSF Laboratory Management for allowing us to use their laboratory and the equipments for the BD ProbeTec™ ET Chlamydia trachomatis Amplified DNA Assay.* ***Funding:*** *No funding or assistance was received from the manufacturer. This study was funded by authors of this paper. All the reagents and consumables used in this study were the sole responsibility of the authors. There was no financial support from the manufacturer or any institution.*

Key Messages

- The prevalence of *C. trachomatis* found in this study (5.8%) was high.
- It is essential to screen for *C. trachomatis* at antenatal clinics in Zimbabwe.
- There is need to evaluate more point of care tests for affordability of screening of *C. trachomatis* in resource limited settings to find a more sensitive and specific test.

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