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HIPPURATE HYDROLYSIS AND CHRISTIE, ATKINS, MUNCH-PETERSON TESTS AS EPIDEMIOLOGICAL DIAGNOSTIC TOOLS FOR *STREPTOCOCCUS AGALACTIAE* CARRIAGE IN PREGNANCY
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ABSTRACT

Objective: **To evaluate the Christie, Atkins, Munch-Peterson (CAMP) and hippurate hydrolysis reactions as diagnostic tools for *Streptococcus agalactiae* carriage in pregnancy.**
Design: **Observational, analytical case-control study.**
***Setting*:** **Hospital-based study in a primary and a tertiary health care institution.**
Patients: **One hundred and six pregnant and 56 non-pregnant (controls) women were included in the study. The participants were of different socio-economic status. A volunteer sample was used. About 800 subjects were contacted and 162 participated in the study.**
Results: **The sensitivity of the screening test varied from 25% for the CAMP test to 77.78% for the hippurate hydrolysis reaction. The specificity was the same for both tests at (50%). A significant difference in positivity between the CAMP and hippurate hydrolysis reactions (95% confidence limit, P<0.05) was observed. The predictive values of the positive test were 66.6% (CAMP) and 87.55% (hippurate hydrolysis) while the negative test were 14.29% (CAMP) and 33.30% (hippurate hydrolysis). Pregnant women had 0.33 chances of being GBS carriers with the CAMP compared to 3.5 with the hippurate hydrolysis.**
Conclusion: **The hippurate hydrolysis test is highly recommended since the reagents are easily available and the organism was easily isolated using this method. The presence of GBS in the anorectum and endocervix is likely to induce systemic and local immunity in the female genital tract. This can contribute to the development of a mucosal vaccine for GBS diseases.**

INTRODUCTION

The association of *Streptococcus agalactiae* (taxonomically known as Group B *Streptococcus)* colonisation of the pregnant cervix and the subsequent outcome of pregnancy such as sepsis, abortion and abscesses has been reported(1,2). Group B *Streptococcus* (GBS) infections in neonates is an established fact(3,4). Few cases of GBS infections(3,5) and carriage (1,2,6) as a public health problem have been reported from Nigeria. In the developed countries, this organism is a recognised pathogen in both the neonate(1,7) and the adult(8). The organism still remains a threat to the dairy farmer in Nigeria(9). GBS infections are on the increase (8).

The laboratory methods used for the isolation of these agents include selective broth medium(10), CAMP reaction(11), hippurate hydrolysis(12), pigment production(13), agglutination test(4) and aesculin hydrolysis. A combination of any two of the above tests were found to detect 99.8% of GBS strains(14). Most laboratories in developing countries are sparsely equipped and cannot afford to run these tests on routine basis. Sodium hippurate hydrolysis has been recommended for developing countries such as those in Africa(5). Earlier studies of GBS carriage in the Jos environment in parturients(2) and nonparturients(15) have been reported. This paper evaluated the CAMP and hippurate hydrolysis tests reported in these previous studies.

MATERIALS AND METHODS

In a case-control study conducted in Jos, in 1995, a sample population of 162 was screened for GBS carriage. Cases included 106 pregnant women at different gestation ages attending the Jos University Teaching Hospital (JUTH) and the Vom Christian Hospital and 56 controls included non-pregnant women attending the Mohammed Wase Hospital (formerly Plateau State Hospital) for various health reasons. Subjects' consent was sought and gained and the purpose of the study and its advantages to them was explained. Volunteer candidates who indicated by raising their hands were included in the study. Subjects who could not understand English were communicated to through an interpreter in Hausa-the local language.

One hundred and sixty two endocervical and 162 anorectal swabs (324 swabs in all) were collected from 106

cases and 56 controls through a sterile speculum using touch light and sterile swab sticks (Antec Diagnostics, UK) with the aid of nurses and clinicians. Inoculation was carried out on 5% selective neomycin sheep red cells agar. No transport medium was used since the health institutions are not far apart. Isolates were subcultured into subsequent sheep red cells after conventional diagnosis (microscopy, colonial morphology, Gram staining and catalase test). Presumptive identification as Lancefied Group B *Streptococcus* was carried out using the CAMP(11) and hippurate hydrolysis(12) tests. The presumptive tests were carried out on cultures that exhibited the following: Gram positive cocci in chains, pairs or singles, beta-haemolysis plus those that were haemolytic but could not be eliminated by the tests mentioned above. Isolates that were both CAMP and hippurate hydrolysis negative were classified as either haemolytic or non-haemolytic streptococci.

The null hypothesis was that the validity of the CAMP and hippurate hydrolysis tests will be the same. Statistical analysis was carried out using the Chi-squared test.

RESULTS

Three hundred and twenty four swabs from the endocervix and the anorectum of 162 women, (106 (65.43%) pregnant and 56 (34.57%) non-pregnant) of different age groups and social status were screened. The mean age was 26.09 years,. A prevalence rate of 7% was observed with the distribution per health unit as shown in Table 1. A prevalence rate of 3.57% was observed in the non-parturients all from the anorectum, and 8.5% in the parturients, divided as follows: endocervix (1.89%), anorectum (6.6%) and both endocervix and anorectum (1.89%). The colonisation per site was not statistically significant (P>0.05). The CAMP test had a positivity of 30% and the hippurate test a positivity of 72.73%. Table 2 shows the GBS culture status per site (P>0.05). Two isolates from both the anorectum and endocervix were isolated from the pregnant women. Comparison of the positivity of the CAMP and hippurate hydrolysis tests of the GBS isolates is shown in Table 3 (P<0.05). The contingency table (Table 4) was used to determine the validity of the CAMP and hippurate hydrolysis tests.

Table 1

GBS isolated in the different hospitals

Hospital	No. of Patients(%)	No. of isolates (%)	P-values
JUTH(Pregnant woman)	74 (45.7)	6 (3.7)	P>0.05*
Vom Christian Hospital (Pregnant woman)	32 (19.7)	3 (1.85)	P>0.05**
Mohammed Wase Hospital (non-pregnant women)	56 (34.6)	2 (1.23)	
Total	162 (100)	11 (7)	

*P-Value when pregnant women and isolates in the two hospitals were compared

**: P -value when the cases and controls were compared in terms of the number of patients and the isolates.

Table 2

GBS culture status according to site

Site	Pregnant		Non-pregnant	
	No. tested	GBS positive No. (%)	No. tested	GBS positive No. (%)
Endocervix	106	2 (1.89)	56	0 (0.0)
Anorectum	106	7 (6.6)	56	2 (3.57)
Total	212	9 (8.5)	112	2 (3.57)

Table 3

Comparison of CAMP and hippurate hydrolysis tests of GBS isolates

Test	No. tested	No. positive (%)	No. negative (%)	P-value
CAMP	10	3 (30)	7 (70)	$p < 0.05$
Hippurate Hydrolysis	11	8 (72.73)	3 (27.27)	

Table 4

Contingency table of the CAMP and hippurate hydrolysis tests

		Exposure		
	Test	Pregnant	No Pregnant	Total
CAMP test	Positive	2	1	3
	Negative	6	1	7
	Total	8	2	10
Hippurate Hydrolysis	Positive	7	1	8
	Negative	2	1	3
	Total	9	2	11

A sensitivity of 25% and 77.7% was observed for the CAMP and hippurate hydrolysis tests respectively. The specificity of the tests was the same (50%). The predictive values of the positive test were 66.6% (CAMP) and 87.5% (hippurate hydrolysis) and for the negative tests 14.29% (CAMP) and 33.3% (hippurate hydrolysis). Pregnant women were 0.33 more likely to be *Streptococcus agalactiae* carriers than non-pregnant ones when the CAMP test was used compared with the hippurate hydrolysis test. Pregnant women were 3.5 more likely to be carriers of GBS with the hippurate hydrolysis than the CAMP test.

DISCUSSION

The importance of early detection of asymptomatic people in the community through screening in order to reduce morbidity and mortality from communicable diseases is a vital part of public health. The use of the CAMP and hippurate hydrolysis tests for the identification of *Streptococcus agalactiae* has been investigated recently in this environment(2,15). The positivity of the CAMP test was very low as well as the specificity of the two tests. A combination of the two methods was found to detect 99.8% of GBS strains(14). Darling(16) stated that all GBS strains are

CAMP positive, a 95% positivity was reported for the hippurate hydrolysis test(12,17). The low CAMP result (30%) could be due to lack of a known beta-lysin producing *Staphylococcus aureus.* The *Staphylococcus aureus* species used in this study were locally obtained in the laboratory and it is likely that some were not beta-lysin producing organisms. Many technical reasons including the method of collection and treatment of the specimens, method of identification and perhaps environmental factors could be responsible for the difference in the results compared to the work of other authors.

Lowering the criterion to define exposure, that is, pregnancy would have increased the sensitivity because the number of false negatives would be less. This could not be done because there is only one criterion for pregnancy, and no-pregnancy. Conversely, raising the criterion to define caseness will increase the specificity of the screening test by decreasing false positives. The low sensitivity of the CAMP test implies there were a lot of false negatives. This low sensitivity means failing to detect some true carriers. The specificity of the two tests (50%) was low which could have led to the mislabelling of some patients as likely or unlikely carriers.

The CAMP test was a paralleled test (an individual was considered a probable carrier when the test was positive). Due to preservation problems only ten isolates were tested for the CAMP test compared to eleven for the hippurate hydrolysis test. The hippurate hydrolysis reaction acted as a serial test because all positive results on the CAMP test were re-evaluated on it. The "yield" of these tests (66% for CAMP and 87.5% for hippurate hydrolysis) indicate the likelihood that an individual with a positive test was a carrier. Pregnant women were of the high risk group (odd ratio =3.5) using the hippurate hydrolysis reaction. The ecological conditions of the pregnant cervix and the acidic milieu created by the lactobacilli and a glycogen-rich mucosa seem conducive to the growth of GBS(7).

These screening methods were however not very acceptable to the target population and the health care providers because of the complexity and duration in collecting endocervical and anorectal swabs. The CAMP test seems more expensive in terms of resources (neomycin sheep red cells, known beta-lysin producing *Staphylococcus aureus* and time). The hippurate hydrolysis reaction can be read in less than three hours and its reagents are easily available. Together with its high sensitivity, positivity and positive predictive value, hippurate hydrolysis test thus holds promise as a screening test for future epidemiological studies.

In conclusion, because of the poorly equipped bacteriology laboratories in Africa, sodium hippurate hydrolysis method is highly recommended. *Streptococcus agalactiae* was easily recognised by sodium hippurate hydrolysis and its reagents are easily available.

The colonisation of GBS in the anorectum and the endocervix and its possible induction of systemic and local immunity in the female genital tract(18) might have implications for the development of a mucosal vaccine for GBS diseases.

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