

Journal of Microbiology and Antimicrobials

Volume 5 Number 11, November 2013

ISSN 2141-2308



*Academic
Journals*

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Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. *Antimicrob. Agents Chemother.* 51: 1281-1286.

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Journal of Microbiology and Antimicrobials

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Detection of anti-salmonella antibodies by Immunochromatographic assay at Rajshahi Medical College, Bangladesh

Bulbul Hasan, Sabera Gul Nahar, A. K. M. Shamsuzzaman, Sharmina Aftab and Abdullah Yusuf

The effect of chloroquine phosphate on the pharmacodynamic activity of ampicillin trihydrate against *Staphylococcus aureus*

Awofisayo Oladoja Abosedo and Igbeneghu Oluwatoyin Abimbola

Full Length Research Paper

Detection of anti-salmonella antibodies by Immuno-chromatographic assay at Rajshahi Medical College, Bangladesh

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Accepted 28 November, 2013

The public health burden of typhoid fever can be substantially reduced by early diagnosis and appropriate antibiotic therapy. This study was conducted in an attempt to assess the reliability of immuno-chromatographic test (ICT) for the early diagnosis of typhoid fever. Immuno-chromatographic test to detect IgM, IgG or combined IgM/IgG in serum in 1st week of fever was done for the diagnosis of typhoid fever. Blood samples were taken for culture and Widal test of 100 clinically suspected cases of typhoid fever in 1st week of illness of patients at Outpatient Department of Rajshahi Medical College Hospital (RMCH), Bangladesh. Forty (40) controls of comparable age and sex were also taken including 20 febrile (non-typhoidal) and 20 healthy persons. Blood culture positive cases (16) and Widal test positive cases (15) were considered as typhoid fever patients and the total number was 31. ICT was performed for 31 typhoid fever patients and 40 controls. In this study, out of 31 cases, ICT was found positive in 28 (90.32%) typhoid fever patient, while 03 (15.00%) of the controls also showed ICT positivity indicating its specificity of 92.50%. It is evident from this study that ICT as a reliable diagnostic tool for early diagnosis of typhoid fever was found highly sensitive, rapid and easy to perform. It can be a versatile test for the screening of clinically suspected case of typhoid fever. therefore, ICT have been found to be encouraging in this study.

Key words: Anti-salmonella antibodies, immuno-chromatographic assay, diagnostic tool, early diagnosis of typhoid fever.

INTRODUCTION

Enteric fever is still a significant public health burden in many developing countries and the incidence has been estimated at 540 cases per 100,000 of the population per year (Krishna et al., 2011). Typhoid fever is associated with significant morbidity and mortality worldwide, especially in tropical countries.

Incidence of typhoid fever has been estimated at approximately 22 million cases with at least 200,000 deaths

occurring annually (Crump et al., 2004). The disease is endemic in many developing countries particularly in the Indian subcontinent including Bangladesh (Saha et al., 1996). In Bangladesh, the overall incidence of typhoid fever is 390 cases per 100,000 populations per year (Brooks et al., 2005). Diagnosis of typhoid fever still remains a puzzle. Although culture of blood remains to be the gold standard in the diagnosis of typhoid fever,

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but facility for culture is not widely available and also time consuming. Moreover, easy and open access to antibiotics without medical prescription in community makes it difficult to isolate organisms from blood culture and alternative methods of diagnosis, like bone marrow culture may be needed, but are invasive and difficult to conduct routinely (Hayat et al., 2011). The unsatisfactory yielding rate in culture for *Salmonella* has always urged laboratory scientists to hunt for rapid and inexpensive laboratory tests for early and accurate diagnosis of patients with typhoid fever, prompting the exploration of variety of rapid antibody detection methods.

As a means of immunodiagnostic procedure, Widal test which is widely used, readily available and inexpensive has been used for many years, but with lowered specificity problems due to background levels of IgG to *Salmonella typhi* in the regions of endemicity (Kawano et al., 2007). Classically, a fourfold or greater rise demonstrated in paired sera is considered diagnostic but it is not feasible and the diagnosis become retrospective one. Thus, there is always a need for the development of a simple, rapid, reliable and sensitive diagnostic method for the early diagnosis of typhoid fever especially in the endemic areas (Hossain, 2001).

The rapid and early immunodiagnosis of typhoid fever can be done by the detection of anti-salmonella antibodies by immunochromatographic test (ICT) which does not require any specialized laboratory or highly skilled personnel and can be done in field areas also. Its usefulness has been shown to detect the anti-salmonella antibodies as early as 4 days of fever onset (Collee et al., 1996). False positivity of immunochromatographic tests in control population detecting anti-salmonella antibodies is very low (Collee et al., 1996).

Antihuman gamma globulin against IgG and IgM fixed in the strip can detect both IgM and IgG of anti-salmonella antibodies in the serum. Thus, detection of anti-salmonella antibodies (IgM and IgG) by immunochromatographic test may be an appropriate adjunct for the clinical diagnosis of typhoid fever. The purpose of the present study was to assess the reliability of immunochromatographic test (ICT) for the early diagnosis of typhoid fever.

METHODOLOGY

This prospective study was carried out from July, 2006 to June, 2007 in Microbiology Department of Rajshahi Medical College, Bangladesh. The research protocol was approved by the Institutional Review Board (IRB) of Rajshahi Medical College for issues of ethical clearance.

Study population

Patients

One hundred (100) clinically suspected cases of typhoid fever patients attending the OPD of Rajshahi Medical College Hospital

were included in this study. The patients were selected according to clinical features which include fever, chills, rigor, altered bowel habit, raised spot on the trunk, bradycardia, headache, myalgia, etc. Cases having fever with at least one of the above clinical features within 1st week of illness were considered as typhoid suspects.

Controls

Twenty (20) non febrile healthy persons of comparable age and sex without having fever and past history of symptom suggestive of typhoid fever in the last six month were considered as healthy controls. Another twenty febrile controls included pulmonary tuberculosis, respiratory tract infection and urinary tract infection in which the diagnosis was confirmed earlier by relevant investigations (Hossain, 2001).

Laboratory procedure

One blood sample was taken from each suspected case at the first week of illness for culture and serological test. A second blood sample was collected from randomly selected 26 culture negative cases 7-10 days after collection of the first sample to see the rising titer. Blood sample was taken from all controls to perform ICT according to the manufacturer's instruction (IDL Biotech AB, Sweden). Blood sample was taken from all controls to perform ICT on single occasion. Blood culture was done by conventional methods using the Trypticase soya broth with sodium polyanethol sulfonate. Isolated bacteria were identified according to the recommended standard protocol. Immunochromatographic tests were done for typhoid fever patients and controls. Blood culture positive cases and cases with rising titer of widal tests were considered as typhoid fever cases. Controls include both non-typhoidal febrile controls and non febrile healthy controls.

RESULTS

A total of 100 clinically suspected cases of typhoid fever and 40 controls were studied. Among the controls, 20 were febrile controls and 20 were healthy controls. Out of 100 suspected cases of typhoid fever, blood culture positive for *S. typhi* were 16 (16%) and remaining 84 (84%) were negative (Figure 1). Widal tests were performed for all 100 typhoid suspected cases during first week of illness. Further, 26 cultures negative cases were randomly selected for paired sera in Widal test to see the rising titre for the serodiagnosis of typhoid fever. Out of 26 cases, 15 (48.38%) were found to have rising titer and considered as Widal positive patients. ICT were performed to detect IgM or IgG or both in serum in the first week of illness in the case of culture positive patients (16) and Widal positive patients (15). To evaluate the sensitivity and specificity of this device, ICT was also done in the case of healthy controls and febrile controls. ICT were found positive in 28 (90.32%) out of 31 typhoid fever patients (16 culture positive patients and 15 Widal positive patients). The tests were also positive in 3 (15%) febrile controls. None of the healthy control was positive by ICT (Table 1). Among the 16 culture positive patients, ICT were positive in 15 cases while 9 (57%) were positive

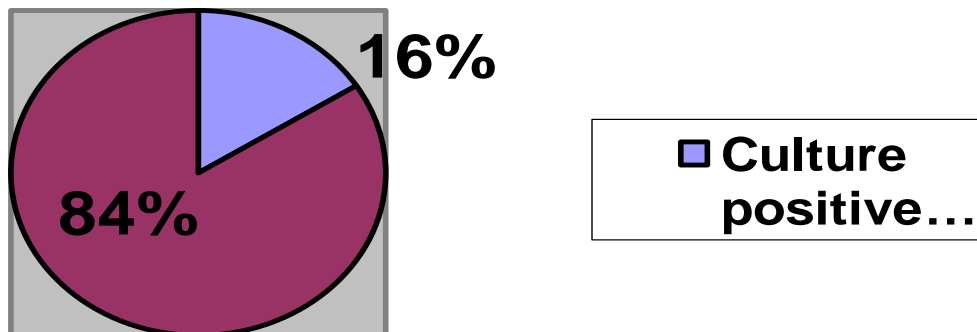


Figure 1. Rate of isolation of *S. typhi* in blood culture. Among 100 suspected cases of typhoid fever, 16 (16%) were found positive for *S. typhi* and 84 (84%) were found negative in blood culture.

Table 1. Detection of anti-salmonella antibodies by immunochromatographic assay (ICT) in typhoid patients at Rajshahi Medical College, Bangladesh.

Study group	ICT positive cases	ICT negative cases	Total cases
Typhoid patients	28 (90.3)	3 (9.7)	31 (100.0)
Febrile controls	3 (15.0)	17 (85.0)	20 (100.0)
Healthy controls	00	20 (100.0)	20 (100.0)

Figure in parenthesis indicates percentage.

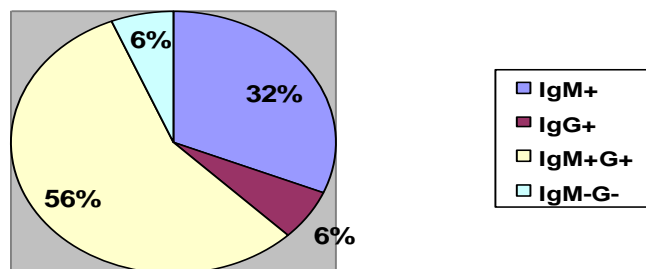


Figure 2. Results of ICT in culture positive patient. Among 16 culture positive patients, ICT were positive in 15 cases where 9 (57%) were positive for both IgM and IgG, 5 (31%) were positive for IgM, and 1 (6%) was positive for IgG only whereas 1 (6%) was negative for IgM and IgG.

for both IgM and IgG, 5 (31%) were positive for IgM and 1 (6%) was positive for IgG only, whereas 1 (6%) was negative for IgM and IgG (Figure 2). Out of 15 Widal positive patients, ICT were positive in 13 cases while 8 (54%) were positive for both IgM and IgG, 3 (20%) were positive for IgM, 2 (13%) were positive for IgG only, whereas 2 (13%) were negative for IgM and IgG (Figure 3). Among the 31 typhoid patients, 17 (54.83%) were positive for both IgM and IgG, 8 (25.80%) were positive for IgM and 3 (9.69%) were positive for IgG. Out of the 20 febrile controls, 2 were positive for both IgM and IgG and 1 showed positivity for IgG only (Table 2).

DISCUSSION

In the present study, out of the 100 clinically diagnosed typhoid fever, 16 were blood culture positive for *S. typhi*. Similar findings were also reported from another study in Bangladesh where the isolation rate is 14% (Begum et al., 2009). This lower values in positivity of blood culture could be due to the rampant use of antibiotics by private practitioners. This value is too low to satisfy the criterion of a diagnostic test, irrespective of the reasons for its low yield (Beig et al., 2010). In this study, ICT was done on 31 typhoid fever patients (16 blood culture positive patients and 15 Widal positive patients) and was found positive in 28 patients in the first week of illness. A positive IgM or IgG either alone or in combination, was regarded as a positive ICT in this study. Detected IgM antibody is suggestive of recent infection and IgG indicates a current or previous infection (Parry et al., 2011). The reason for considering IgG as positive for ICT is that when typhoid infection occurs the immune response mechanism produces both IgM and IgG as well. That is why positivity of IgG is considered as positive ICT. Fifteen (93.75%) out of 16 culture positive patients were found positive for ICT in our study. The only patient who tested negative in ICT in this group was a 3 year old child diagnosed in the 2nd day of fever which also had insignificant titer (TO/TH=20/20) in Widal test. The negative ICT in this case may be due to the inadequate antibodies

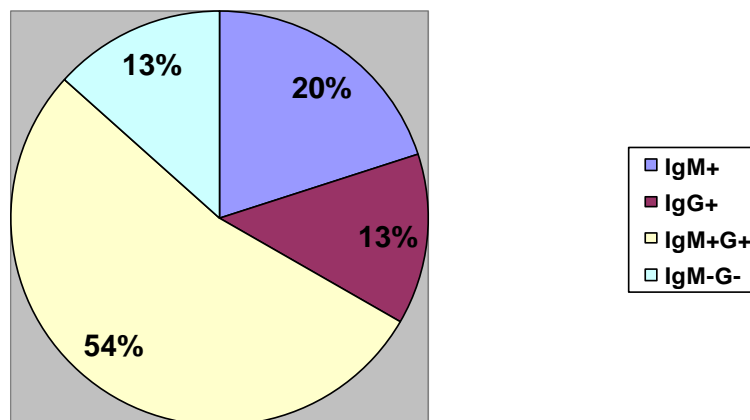


Figure 3. Results of ICT in Widal positive patient. Figure shows that out of 15 Widal positive patients, ICT were positive in 13 cases whereas 8 (54%) were positive for both IgM and IgG, 3 (20%) were positive for IgM, 2 (13%) were positive for IgG only whereas 2 (13%) was negative for IgM and IgG.

Table 2. Positivity of IgM, IgG or combined IgM/IgG in ICT among typhoid patients and controls.

Study group	ICT			Total ICT positive
	M+	G+	M+G+	
Typhoid patients	8	3	17	28 (90.3%)
Febrile controls	0	1	2	3 (15.0%)
Healthy controls	0	0	0	0

Table 3. Validity of ICT for detection of typhoid fever.

Test validity	Result (%)
Sensitivity	90.3
Specificity	92.5
Positive predictive value	90.3
Negative predictive value	92.5
Accuracy	91.5

production to be detectable by ICT (Table 3).

On the other hand, 13 (88.88%) out of 15 Widal positive patients were found positive for ICT. Only 2 Widal positive patients were found negative for ICT. The negative ICT in these cases were perhaps due to the antibodies which did not yet reach detectable levels in the first week of fever. Among the 20 non-typhoidal febrile patients, ICT was found positive for 3 (15%) cases.

In this study, the sensitivity, specificity, positive and negative predictive value of the test was found as 90.32, 92.50, 90.32 and 92.50%, respectively. The results are remarkably consistent with the findings of another investigator in Bangladesh who noted sensitivity, specificity, positive and negative predictive value as 91.42, 90.00, 88.88 and 92.30%, respectively (Begum et al., 2009).

It is generally stated that, conventional blood culture is

time consuming procedure with a very low yielding rate though it is considered still as the gold standard for the detection of typhoid fever. During blood culture it is important to consider adequacy of blood and it should be at least 1:5 dilution with the tryptic soy broth with blood. It is not a routine practice in common laboratory while Widal titer in a single blood sample is only presumptive. Whereas almost certain diagnosis of typhoid fever can be done by doing ICT in a single blood sample in the first week of illness. Thereby, ICT is a more sensitive and specific test which is easy to perform and more reliable as compared to the Widal test and is it useful in early therapy and similar report was also mentioned in another study (Khoharo, 2011).

Although, bacterial isolation remains to be conclusive for the diagnosis of typhoid fever but failure to perform culture does not rule out the existence of infection. Laboratory diagnosis of typhoid fever always put a dilemma since conventional blood culture is a cumbersome, time consuming procedure with unsatisfactory yielding rate. Though Widal test is performed widely it has some limitation due to poor standardization and difficulty in interpretation on a single sample (Kawano et al., 2007). Considering the practical situation of laboratory diagnosis, detection of anti-Salmonella antibodies by ICT has been found to be quite reliable, easy to perform and may be a good adjunct to clinical suspect in early days of

fever.

Conclusion

The study concludes that ICT is a reliable diagnostic method for early and rapid detection of typhoid fever and was found to be highly sensitive, rapid and easy to perform. It can be a versatile test for the screening of clinically suspected case of typhoid fever. So, ICT have been found to be encouraging in this study.

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Full Length Research Paper

The effect of chloroquine phosphate on the pharmacodynamic activity of ampicillin trihydrate against *Staphylococcus aureus*

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Accepted 6 December, 2013

The effect of chloroquine phosphate on the pharmacodynamic activity of ampicillin trihydrate against *Staphylococcus aureus* NCTC 6571 was investigated using physicochemical and microbiological assay techniques. The physicochemical method was thin layer chromatography and ultraviolet spectrophotometry in a condition simulating normal body temperature and pH. The microbiological assay compared the zones of inhibition of *S. aureus* NCTC 6571 by ampicillin trihydrate alone and in combination with chloroquine phosphate using the agar diffusion method. The kill kinetics was determined to give a dynamic assessment of the bactericidal activity of ampicillin trihydrate alone and in combination with chloroquine phosphate at various concentrations. The results showed that the zone of inhibition of *S. aureus* NCTC 6571 by ampicillin trihydrate in the presence of chloroquine was smaller than that produced by ampicillin alone. The kill kinetics revealed a significant increase in the percentage survivor of the organisms in the presence of chloroquine than with ampicillin alone. The results of the study suggest that there is no chemical interaction between the two drugs *in vitro* however, an antagonistic pharmacodynamic interaction exists between ampicillin trihydrate and chloroquine phosphate *in vitro* on *S. aureus* NCTC 6571.

Key words: Ampicillin trihydrate, chloroquine phosphate, drug-drug interaction, antagonism, antimalarial, antibiotic.

INTRODUCTION

Malaria amongst other protozoa infections is a major parasitic disease in the tropical and subtropical regions of the world (Bremar, 2001). It constitutes a major health hazard causing a high mortality and morbidity among children and pregnant women in the region (Snow et al., 2005). Likewise, bacterial infectious disease especially diarrhoea and respiratory tract infections are major contributors to the mortality and morbidity associated with infections caused by bacteria in the region (Mathers et al., 2009; Black et al., 2010; WHO, 2010). An important

symptom of these infections is fever and in some cases they are found co-occurring together (Akpede and Sykes, 1993; Berkley et al., 1999; Ayoola et al., 2005; Okunlola et al., 2012). This probable co-occurrence and presentation of fever as symptom in both cases makes physicians to treat malaria and bacterial infections in patients presenting with fever with or without diarrhoea or respiratory tract infections. A common feature therefore in the prescription from most physicians' desk is anti-malarials with antimicrobials for the treatment of per-

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ceived bacterial infections in individuals with symptoms of malaria pending the results of the culture of any probable offending bacteria (Akoria and Isah, 2005).

Drug-drug interaction studies on antimicrobials and antimalarials have shown the possibility of reduced or enhanced activity of one of the drugs in the presence of the other (Crowle and May, 1990; Kazzim et al., 2006). These interactions could be pharmacokinetic, physico-chemical or pharmacodynamic. Pharmacokinetic interactions are caused by the effects of one drug on the absorption, distribution, metabolism, or elimination of the other drug (Rowland and Tozer, 1995; Kashuba and Bertino, 2005). Physico-chemical interactions could be due to chemical interactions between the drugs such as the physical adsorption of one drug by the other, while pharmacodynamic interactions could be due to antagonism or synergism between co-administered drugs (Kazzim et al., 2006). *In vivo* studies have shown a reduction in the bioavailability of ampicillin co-administered with chloroquine (Ali, 1958). This *in vitro* study was carried out to determine if any chemical or pharmacodynamic interaction exists between ampicillin and chloroquine when administered together.

MATERIALS AND METHODS

Chemicals

Chloroquine phosphate powder, a white, odourless, hygroscopic crystalline powder was obtained from Bond Chemical Industry, Aawe, Oyo State, Nigeria while Ampicillin trihydrate powder, a white, odourless, crystalline powder was purchased from Sigma Aldrich. Reagents used included hydrochloric acid, Silica gel GF₂₅₄, Acetic acid, acetone, Iodine vapour, ammonium chloride, ethyl acetate, Phosphate Buffered Saline all of which were of analytical grade.

Qualitative assay of the pure powders

The content of drug sample in each of the powders was determined according to official methods (BP 2013, U.S.P 2003).

Drug-drug interaction studies

Physicochemical method

Ultraviolet spectrophotometry: A solution containing 0.05%w/v ampicillin trihydrate powder in 0.1 M hydrochloric acid (solution I) and another containing 0.00625%w/v chloroquine phosphate powder in 0.1 M hydrochloric acid (solution II) were prepared. A third solution containing equimolar mixture of solutions I and II was prepared and the ultraviolet spectrum each at 200-400 nm was determined using an ultraviolet spectrophotometer.

Thin layer chromatography: A chromatographic system consisting of Silica gel GF₂₅₄ (pre coated plates, 0.25mm) as stationary phase and acetic acid: acetone (95:5v/v) as mobile phase employing ultraviolet lamp (254 nm) and iodine vapour for detection was used.

A solution containing 0.0323 g chloroquine phosphate powder

dissolved in 5 ml of acetone : 0.1 M ammonium chloride : ethyl acetate, 3:1:1 was prepared (solution A). The ampicillin sample was a solution of 0.0229 g ampicillin trihydrate powder in 5 ml of acetone : 0.1 M ammonium chloride : ethyl acetate, 3:1:1 (solution B). A third sample was a mixture of chloroquine phosphate and ampicillin trihydrate in 5 ml of acetone: 0.1 M ammonium chloride : ethyl acetate, 3:1:1 (solution C). Each solution was filtered and labelled appropriately.

Each of the samples were incorporated into tubes containing 5 ml of freshly prepared phosphate buffered saline (pH 7.4) and other tubes containing 5 ml of 0.1 M hydrochloric acid placed in a thermostated water bath at 37°C. 1 ml samples were then withdrawn from each of the reaction mixtures at 0, 15, 30, 45 and 60 min and analysed using the TLC system.

Agar diffusion assay

The test plates were prepared by inoculating 0.2 ml of an overnight culture of *S. aureus* NCTC 6571 into 20 ml of cooled molten Mueller Hinton Agar (Oxoid), the plates were allowed to set and harden. Holes 7 mm in diameter equidistant to one another were then bored into the agar using sterilized cork borer. Samples of ampicillin at concentrations of 1 - 6 µg/ml were introduced into the wells of the agar plates. Similarly, the organism was tested against mixtures of ampicillin and chloroquine containing 1- 6 µg/ml of ampicillin and 0.08 µg/ml of chloroquine. The drugs were allowed to diffuse for 1 h before incubation right side up at 37°C for 24 h. The zones of inhibition produced after incubation was measured in mm. The inhibition produced by the pure ampicillin alone was compared with that of the mixture of ampicillin and chloroquine. The minimum inhibitory concentration (MIC) of the pure ampicillin was graphically determined and compared with that of the mixture of ampicillin and chloroquine.

Kill kinetics

The reaction medium was prepared by transferring 1 ml of an inoculum of 10⁸ organisms/ml of *S. aureus* NCTC 6571 into flasks containing different concentrations of ampicillin and chloroquine to obtain a final concentration of 10⁷ cells per ml in the reaction media. The drugs were tested at concentrations of 5 µg/ml for ampicillin and 0.01 - 0.3 µg/ml for chloroquine. A reaction medium containing each of the two drugs alone and another containing none of the drugs were included in the experiments as controls.

The reaction flasks were incubated at 37°C. At 0 and 6 h, 1 ml samples were withdrawn from the reaction media, introduced into a neutralising mixture containing Penase (Difco) and viable counts were determined using the pour plate technique. The plates were incubated at 37°C for 24 - 48 h and number of colony forming units was counted.

Statistical analysis

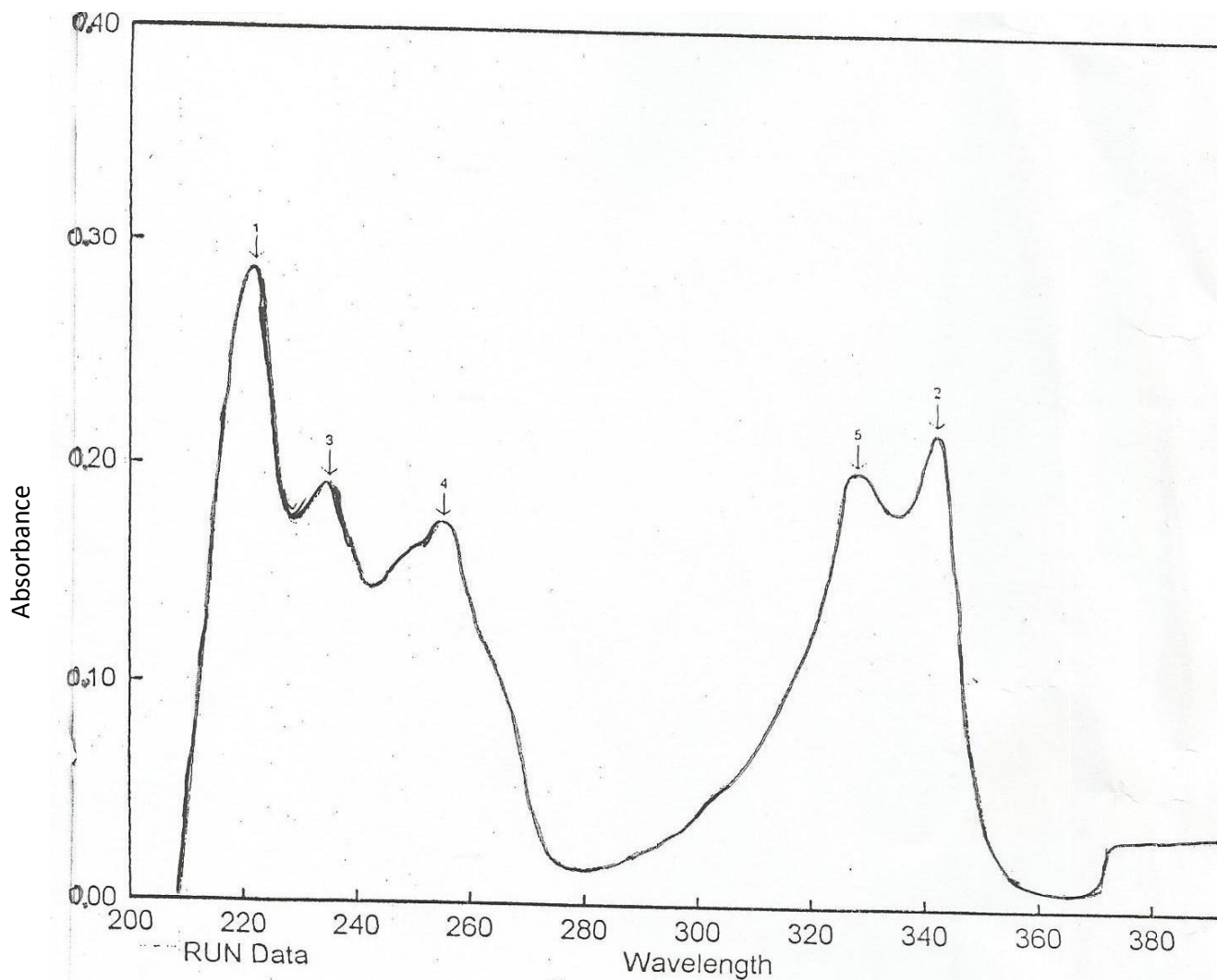
Data obtained from the study was analysed statistically using paired t-test at 95% confidence interval. A p value ≤ 0.05 was considered significant.

RESULTS

The result of the thin layer chromatography of each of the drug samples is presented in Table 1 while the ultraviolet spectra are presented in Figures 1 and 2, respectively.

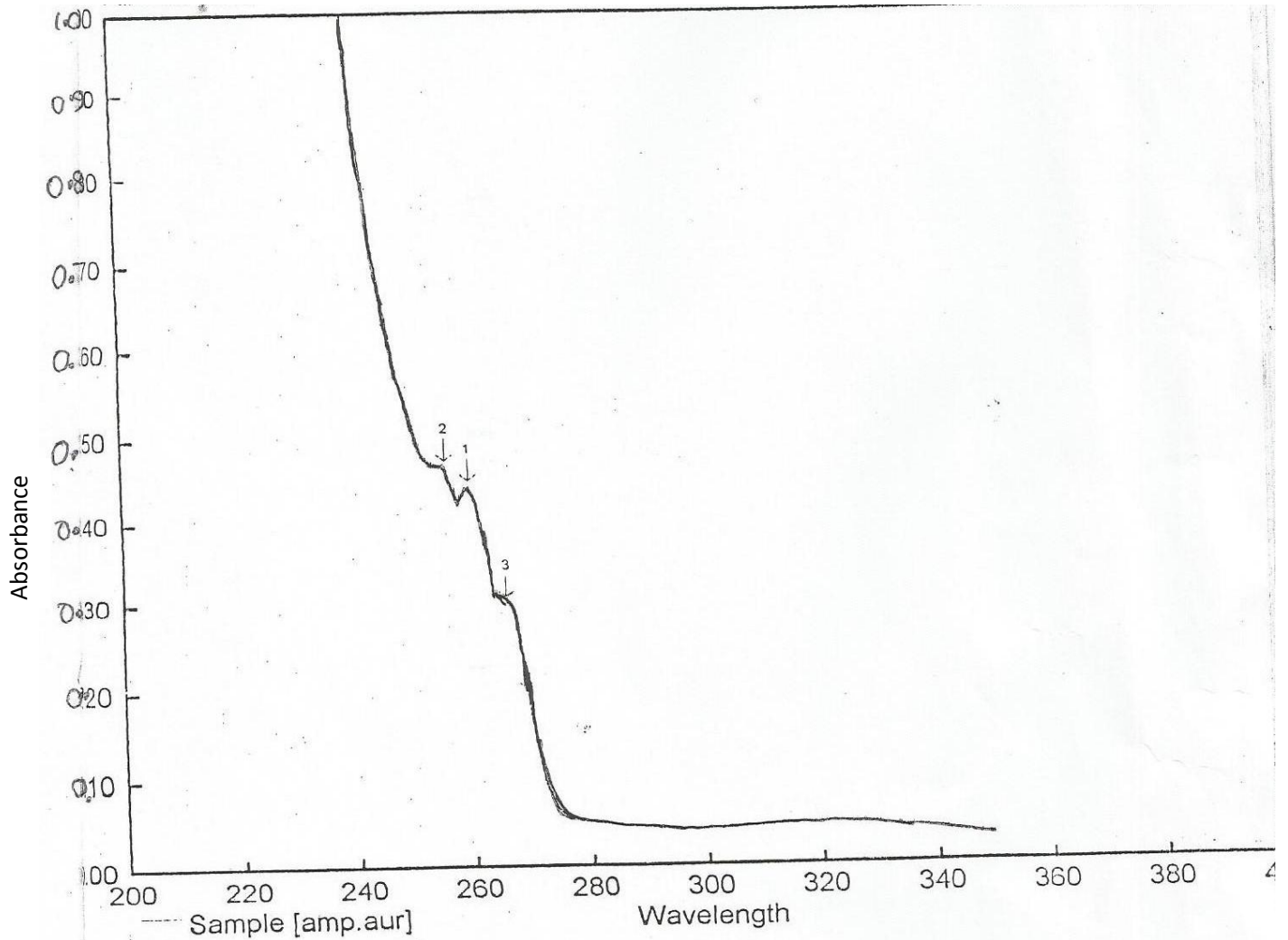
Table 1. Thin layer chromatography of the pure ampicillin trihydrate and chloroquine phosphate samples.

Drug sample	Solvent front (cm)	Distance moved (cm)	R _f
Ampicillin	9.60	1.44	0.15
Chloroquine phosphate	9.60	4.32	0.45



METHOD		PEAK TABLE	
Start : 200	Graph High: .4	Wavelength	Abs
Stop : 400	Graph Low: 0	1	222
Interval : 1	Peak Pick Mode: Manual	2	342
Mode: Absorbance	Bandwidth: 2 nm	3	235
Sample ID: Chloroquine		4	255
		5	328
		6	

Figure 1. The ultraviolet spectrum of pure chloroquine phosphate in 0.1 M hydrochloric acid.



METHOD		PEAK TABLE	
Start : 190	Graph High: 1	Wavelength	Abs
Stop : 400	Graph Low: 0	1	260
Interval : 2	Peak Pick Mode: Manual	2	256
Mode: Absorbance	Bandwidth: 2 nm	3	266
		4	

Figure 2. The ultraviolet spectrum of pure ampicillin trihydrate in 0.1 M hydrochloric acid.

The content of chloroquine phosphate in the powder, obtained from the assay procedure was 101.52% w/w while the content of ampicillin trihydrate in the powder, obtained from the assay procedure was 99.70% w/w.

The ultraviolet spectrum obtained for the solution mixture of chloroquine phosphate and ampicillin trihydrate (solution III) is presented in Figure 3 which was

comparable with those of the individual drugs (Figures 1 and 2), for chloroquine phosphate and ampicillin trihydrate, respectively. The TLC result of the drugs in phosphate buffered saline and in 0.1 M hydrochloric acid is presented in Table 2.

The result of the zones of inhibition (mm) produced by the drugs against *S. aureus* NCTC 6571 is presented in

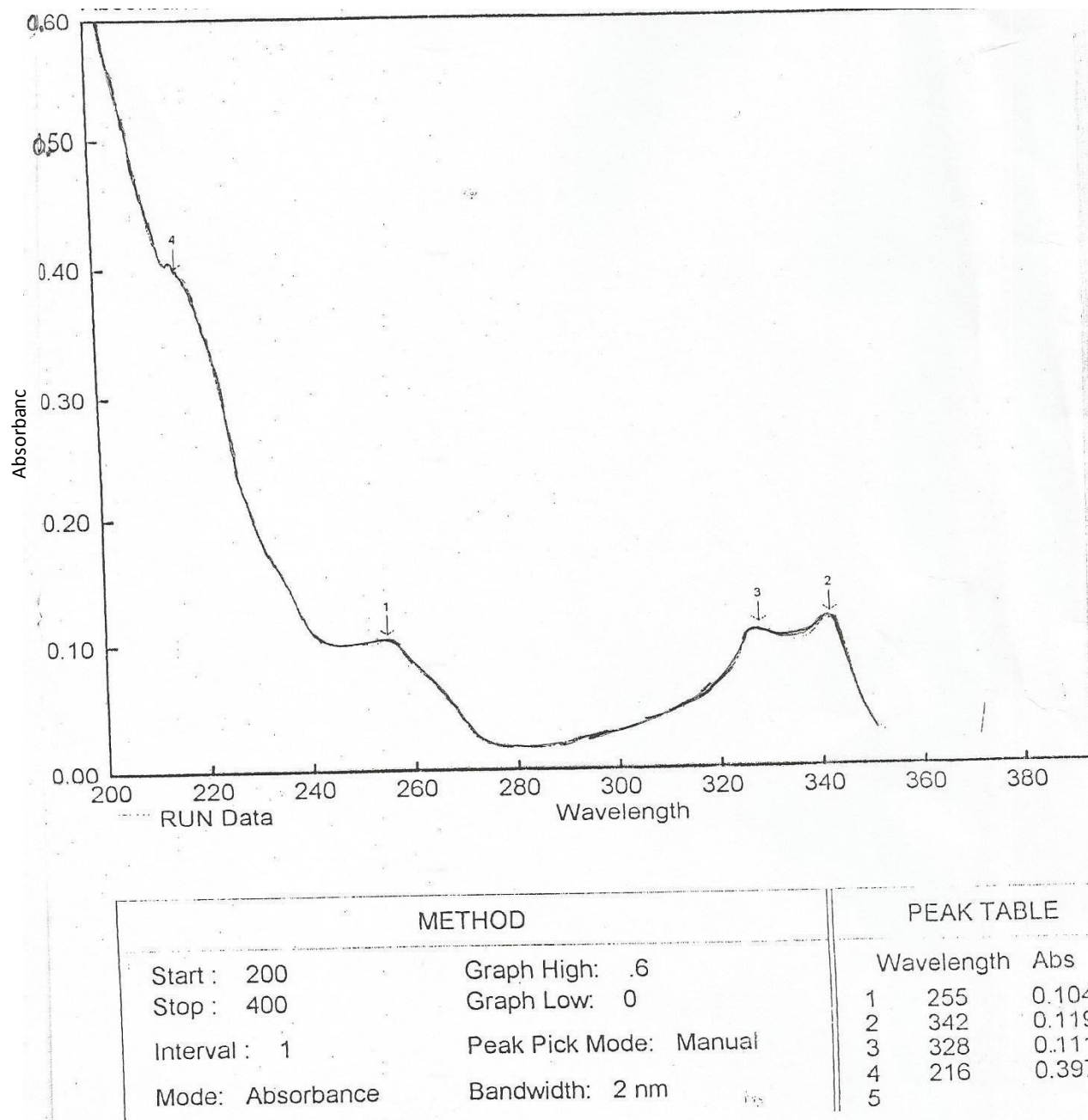


Figure 3. The ultraviolet spectrum of equimolar mixture of chloroquine phosphate and ampicillin trihydrate solution in 0.1 M hydrochloric acid.

Table 3. Table 4 shows the result of viable bacterial count after a contact time of 6 h in ampicillin alone and in a mixture of ampicillin and chloroquine.

DISCUSSION

The results of the thin - layer chromatography of the two drug samples, ampicillin trihydrate and chloroquine phosphate as well as the UV spectra were similar to that

of the official specifications showing that the tested drug samples were in a high state of purity. It also verified the absence of degradation products in the powder samples (BP 2013). The use of thin - layer chromatography (TLC) was to detect and monitor possible *in vitro* chemical interaction between chloroquine phosphate and ampicillin trihydrate, which may ultimately affect the activity of ampicillin trihydrate *in vitro*. This is based on possible structural interaction between chloroquine phosphate and ampicillin trihydrate. The two drugs were however

Table 2. Thin layer chromatographic determination of ampicillin/ampicillin-chloroquine mixture in phosphate buffered saline (PBS) (pH 7.4) and 0.1 M hydrochloric acids (pH 2.0).

Drug	Time (min)/ distance moved (cm)									
	PBS (pH 7.4)					0.1 M hydrochloric acids (pH 2.0)				
	0	15	30	45	60	0	15	30	45	60
Amp trihydrate	0.67	0.67	0.67	0.58	-	0.62	0.62	0.62	0.62	0.62
Chloroquine phosphate	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Amp trihydrate +	0.67	0.67	0.67	0.58	-	0.62	0.62	0.62	0.62	0.62
Chloroquine phosphate	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07

Table 3. Zones of inhibition produced by test drugs against *S. aureus* NCTC 6571.

Concentration (ug/ml)	Diameter of zone of inhibition (mm)					
	Ampicillin alone			Ampicillin with 0.08 ug/ml chloroquine		
	1	2	Mean	1	2	Mean
6.0	35	35	35	31	30	30.5
5.0	34	33	33.5	27	28	27.5
4.0	34	32	33.0	26	26	26.0
3.0	30	31	30.5	24	23	23.5
2.0	28	28	28.0	21	22	21.5
1.0	20	19	19.5	14	14	14.0
PBS (pH 7.4)	0	0	0	0	0	0
Chloroquine 0.08	0	0	0	0	0	0

Table 4. Viable count and percentage survivor of *S. aureus* NCTC 6571 in the presence of ampicillin and chloroquine phosphate after a contact time of 6 h.

Concentration ug/ml		Viable count		Survival (%)
Ampicillin	Chloroquine phosphate	T = 0 min	T = 6 h	T = 6 h
0	0	3.3×10^7	3.8×10^7	115.2
0	0.3	3.3×10^7	3.0×10^7	93.8
5	0	3.3×10^7	9.0×10^6	27.3
5	0.02	3.3×10^7	1.9×10^7	57.6
5	0.1	3.3×10^7	1.8×10^7	54.5
5	0.2	3.3×10^7	1.7×10^7	51.5
5	0.3	3.3×10^7	1.9×10^7	57.6

separately resolved throughout the sampling period of 0 - 30 min implying that the two drugs did not interact chemically in phosphate buffer saline at pH 7.4 and 37°C. Furthermore, the gradual decrease in the R_f value of ampicillin trihydrate by the 45th min of sampling could possibly be due to the degradation of the drug. This is confirmed by the 60th min of sampling in which there was a final disappearance of the spot suggesting the degradation of the ampicillin trihydrate at 37°C in phosphate buffer saline at pH 7.4. Chloroquine phosphate was however stable throughout the sampling period of 60 min and is likely stabilized by the presence of buffer at 37°C being a phosphate salt itself. On the other hand, in 0.1 M HCl (pH 2.0) at 37°C, the R_f of

ampicillin decreased which could be attributed to the protonation of the amine side chain conferring greater stability on the drug molecule in the acidic environment. The fact that both were again resolved separately on the TLC plate suggests the absence of chemical interaction between ampicillin trihydrate and chloroquine phosphate in acidic media. This is within the detectable limit of the thin layer chromatographic technique.

The agar diffusion method revealed that the chloroquine phosphate affected the antibacterial activity of ampicillin trihydrate by a reduction in the zone of inhibition obtained, when compared with pure ampicillin alone. The observations made with the phosphate buffered saline solution of the drugs at 37°C, which simu-

lates *in vivo* body temperature and plasma pH of 7.4 showed that chloroquine is likely to cause a reduction in the antibacterial activity of ampicillin *in vivo*. Moreover, the analysis of the kill kinetics results showed that ampicillin produced a significant reduction in the percentage survivor of the test organism. The presence of chloroquine however significantly reduced the bactericidal effect of ampicillin though increase in the concentration of chloroquine phosphate in the presence of ampicillin trihydrate did not significantly increase the degree of antagonism of ampicillin trihydrate activity against *S. aureus* NCTC 6571. Chloroquine has similarly been reported to antagonise the activity of ciprofloxacin against a strain of *Pseudomonas aeruginosa* in a study by Adegbolagun et al. (2008).

The modification in the *in vitro* antimicrobial activity of ampicillin trihydrate caused by the presence of chloroquine phosphate is an indication of a pharmacodynamic interaction between the two drugs. This pharmacodynamic interaction is between unrelated classes of compounds since ampicillin trihydrate is an antimicrobial agent and chloroquine phosphate is an antimalarial agent. Since chloroquine phosphate inhibited the activity of ampicillin trihydrate, it can be said to have an antagonistic pharmacodynamic interaction with ampicillin trihydrate.

The reduction of ampicillin bioavailability on co-administration with chloroquine *in vivo* was attributed to slower gastric emptying and inhibition of gut motility produced by chloroquine (Ali, 1985). However, *in vitro* pharmacodynamic reduction in the activity of ampicillin trihydrate against *S. aureus* NCTC 6571 cannot be attributed to these factors. A different mechanism entirely is therefore responsible for this *in vitro* antagonistic action of chloroquine phosphate against ampicillin trihydrate. A possibility is a competitive inhibition of the transport mechanism responsible for the uptake of ampicillin into the bacterial cell wall where it elicits its action. There is however the need for further work to investigate the mechanism of pharmacodynamic interaction between the two drugs.

The result of this study therefore suggests that there is no form of physicochemical interaction between ampicillin trihydrate and chloroquine phosphate *in vitro* in acidic medium of 0.1 M HCL (pH 2.0) or in phosphate buffer saline pH 7.4 both at 37°C but chloroquine phosphate antagonizes the pharmacodynamic activity of ampicillin trihydrate against *S. aureus* NCTC 6571 *in vitro*.

ACKNOWLEDGEMENT

The authors wish to acknowledge Bond Chemical Industry, Aawe, Oyo State, Nigeria for the gift of chloroquine phosphate.

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UPCOMING CONFERENCES

ICMVM 2013: International Conference on Molecular Virology and Microbiology, Bangkok, Thailand, 24 Dec 2013

ICMVM 2013 : International Conference on Molecular Virology and Microbiology



ICBHES 2014: International Conference on Biological, Health and Environmental Sciences, 20 Jan 2014

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ICBHES 2014: International Conference on Biological, Health and Environmental Sciences, 20 Jan 2014

International Conference on Food, Biological and Medical Sciences (FBMS-2014), Bangkok, Thailand, 28 Jan 2014

February 2014

Australian Society for Antimicrobials 15th Annual Scientific Meeting, Melbourne, Australia, 20 Feb 2014

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