GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 7, NO. 1, JANUARY 2001:13-17
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# CYTOTOXICITY OF CELL FREE FILTRATES OF CAMPYLOBACTER JEJUNI ISOLATED IN LAGOS, NIGERIA

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(Received 21 November, 1996; Revision accepted 22 June, 2000)

## ABSTRACT

Culture filtrates of *Campylobacter jejuni* strains isolated from clinical specimens in Lagos Nigeria were tested for toxic activity. Two out of five filtrates tested manifested cytopathic effect on BHK cells. The effects were mainly cytotoxic and cytotonic. Toxic activity of *C. jejuni* filtrates was much lower than toxic activity elicited by a known toxigenic *Vibrio cholerae* strain. The reduced potency of *C. jejuni* culture filtrate may thus account for lower volume of diarrhoea seen in *C.jejuni* enteritis as compared with classical cholera "rice water stool".

**Key Words:** Culture filtrate, cytopathic effect, tissue Culture, *Campylobacter jejuni.*

## INTRODUCTION

Enterotoxin production has been postulated as the mechanism of diarrhoea induction in *Campylobacter enteritis.* The enterotoxin produced is shown to be similar in many ways to cholera toxin (CT). The similarities include: induction of secretory diarrhoea by stimulating adenylate cyclase activity in the intestinal mucosa and disrupting the normal ion transport in the enterocytes (Ruiz-Palacios et al; 1983).

A cytotoxin produced by *C.jejuni* which is toxic for a number of mammalian cells has also been described. This cytotoxin was shown to be toxic to Vero, Cho and Hela cells (Guerrant et al., 1985, Johnson and Lior 1984). Johnson and Lior (1988) have also described a new heatlabile toxin which was cytolethal to Cho, Vero Hela and Hep2 cells. This toxin they referred to as Cytolethal distending toxin (CLDT) to reflect the progressive cell distention and eventual

cytotoxicity observed in all sensitive tissues cells.

The effects of *C.jejuni* extracts and culture supernatants have been described. In their report Akhtar and Huq (1989) reported that *C.jejuni* isolates from patients produced morphologic changes on HeLa, vero and Y1 adrenal cell lines.

This work was set out to examine the toxicity of culture filtrates of *C.jejuni* clinical isolates in Lagos, Nigeria on some cells lines.

### Organisms

Five strains of *Campylobacter jejuni* (1214, 1276, 2059, 1270 and 99) which had been previously isolated from clinical specimens were used. The stock cultures of the strains were originally maintained at –20°C in Ferrous bisulphite pyruvate (FBP) supplement described by George et al; (1978). The different strains were resuscicated by growing in Butzler's type medium (Coker and Dosummu-Ogunbi 1984) at 42°C microaerobically for 48 hours. The resuscicated cultures were then grown in Mueller Hinton broth (Oxoid). The broth cultures were supplemented with Ferric chloride at a concentration of 0.1mg/ml to increase toxin production as suggested by Van Heyningen and Gladstone (1953). This supplement had been found to increase toxin production in previous ileal loop assays. The broth

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cultures were again incubated at 42°C for 48 hours in candle extinction jar.

**Culture Filtrate:**

Polymyxin B which had been observed to increase toxin yield in previous experiments was added to the broth cultures at a concentration of 2mg/ml at the last 10 minutes of incubation. The broth cultures were centrifuged at 1000g for 15 minutes and the supernatants collected into sterile universal bottles. They were filtered through millipore membrane of 0.22um pore diameter (Millipore Corp. berdford Mass 01730). The sterile cell free filtrate was stored at 4°C prior to assay (Ruiz-Palacious et al 1983). Assays were however done within 48 hours of collection.

**Cells For Assays:**

Four cell lines were used for the assay, they included Vero cells; a continuous cell line established from African monkey kidney cells and Hep2 cells, a continuous cell line established from human laryngeal epidermoid carcinoma (toolan 1954). The other cells were HeLa cells which were established from primary human epitheloid cervical carcinoma cells and BHK (Baby Hamster Kidney Cells). The cells were propagated in medium 199 (GIBCO Laboratories) supplemented with 100ug/ml streptomycin sulphate and 10% tryptose phosphate broth (oxoid) with 10% fetal calf serum (GIBCO Laboratories.

**Culture Filtrate Assay:**

Cells for the assay were harvested using 0.5mg/ml trypsin and 0.5mM Versene (EDTA – Ethylene diamine tetraacetic acid). The harvested cells were then seeded unto flat bottomed 96 well tissue culture plates to form monollayers. For seeding aliquots of 0.2ml of the harvested cells were introduced unto each row (12 wells) one row being used per cell line. They were again layered with growth medium (m199 and supplements) and incubated overnight i a $CO_2$ humified chamber at 37°C. Aft 24 hours, the growth medium wa carefully pipetted out and 200 ul of tw fold diluted and undiluted samples c culture filtrate which had been brougl to 37°C carefully layered on the fres monolayers as suggested by Johnson an Lior (1986). Two control rows wer layered, one with sterile Mueller Hinto broth and the other CT from know toxigenic *V. cholerae* strain 110. Th toxigenic strain of *V. cholerae* wa isolated from a fatal case of chole involving a 38 year old lady. Th cultures were returned to the incubat for 24 hours. Cell viability wa determined by addition of trypan blue which viable cells excluded the dy The morphological changes on the cel were discerned microscopicall Cytopathic effects like rounding elongation were sought for and score positive if about 30% or more of th

Table 1 **CYTOPATHIC EFFECT OF CULTURE FILTRATE ON DIFFERENT CELL LINES**

| | Vero | Hep2 | HeLa | BHK |
|---|---|---|---|---|
| 1214 | + | + | ++ | - |
| 1276 | - | - | - | - |
| 2059 | - | - | - | - |
| 1270 | + | ++ | ++ | - |
| 99 | - | - | - | - |
| V.Cholerae | | | | |
| *Strain 110 | + | ++ | ++ | + |
| Sterile broth | - | - | - | - |

\+ Positive CPE was inferred whe 30% of cells were affected.

\- Negative CPE was inferred whe less/than 30% of cells wer affected.

Mueller Hinton broth was used.

cells were affected as suggested by Johnson and Lior (1986). The $TCD_{50}$ of the filtrates was taken as the dilution of filtrate which caused CPE on 50% of the cells.

## RESULTS

Culture filtrates of two (1214) and 1270) out of five strains assayed elicited toxicity on the cells used. Cytopathic effects (CPE) were observed on Vero, Hep2 and HeLa cells but not on BHK cells. The filtrate of known toxigenic *V.cholerae* strain 110 gave CPE on all the cells tested while the cells overlayed with only sterile broth were not affected as indicated by trypan blue exclusion. Table I shows the CPE of the filtrates on the different cell lines.

The type of effects observed were mainly cytotoxic (CTX) and cytotonic (CTN). Table II shows the distributon of these effects on the lines assayed. The highest diluton of the filtrate that caused toxicity of 50% of the cells was 1:2 for strain 1214 on vero and HeLa. Strain 1270 showed effect only in undiluted form. Table III shows the $TCD_{50}$ of C.jejuni filtrate and the known toxigenic V.cholerae filtrate.

## DISCUSSION

Elaboration of enterotoxins has been implicated as the mechanisms of diarrhoea in patients infected by C.jejuni. The C.jejuni toxin (CJT) has been shown to share functional and immunological properties with cholera toxin (CT) and heat labile Escherichia coli toxin (LT) thereby necessitating the same assays for their detection (walker et al; 1989). Tissue culture assays are thus convenient systems to test for toxin production. Chinese hamster ovary cells (CHO) were shown to be sensitive to CJT(Ruiz-Palacious et al, 1983).. In our experiments we observed that Baby hamster kidney cells (BHK) were resistant to effect of the culture filtrates. This indicates organotropism of the filtrates since CHO and BHK are both

Table II

**TYPE OF CPE CAUSED BY C.JEJUNISTRAINS ON DIFFERENT CELLS ASSAYED**

| | Vero | | Hep2 | | HeLa | |
|---|---|---|---|---|---|---|
| 1214 | $CTN^+$ | $CTX^+$ | $CTN^+$ | $CTX^+$ | $CTN^+$ | $CTX^-$ |
| 1270 | $CTX^+$ | $CTN^-$ | $CTN^+$ | $CTX^-$ | $CTX^+$ | $CTN^-$ |
| CT.* | $CTX^+$ | $CTN^-$ | $CTN^+$ | $CTN^+$ | $CTX^+$ | $CTN^+$ |

$CTN^+$ cytotonic effect (elongation of cells)

$CTX^+$ cytotoxic effect (rounding and death)

CT* cholera toxin.

Table III

$TCD_{50}$ OF FILTRATES ON DIFFERENT CELLS ASSAYED

| | Vero | Hep2 | HeLa |
|---|---|---|---|
| 1214 | 2 | Undiluted | 2 |
| 1270 | Undiluted | Undiluted | Undiluted |
| Vibrio Chol.110 | 8 | 4 | 8 |

$TCD_{50}$ was taken as reciprocal dilution of filtrate that caused 50% CPE

derived from hamsters but from different organs. The factors accounting for this form of selective toxicity may even explain the resistance of many laboratory animals to overt clinical diarrhoea in experimental infections.

The strains of *C.jejuni* used were from clinical specimens diarrhoeagenic stools. Two out of five (40%) of the culture filtrates produced positive CPE. In another study Mathan et al; (1984) found that the proportion of enterotoxigeni-

strains among patients was 35% while Leunk et al (1988) working with *C.pylori* observed 55% toxicity. The proportion ot toxigenic strains detected by routine assays thus appears to depend on different factors. Loss of toxicity during laboratory passage, growth medium, sensitivity of assay and potency of the toxins in the assayed filtrate may limit the detection of toxigenic strains.

he watery diarrhoea observed in enteritis associated with *C.jejuni* does not cause as much dehydration as seen in V.cholera and enterotoxigenic *E. Coli*. This indicates minimal electrolyte imbalance (Karmali and Fl;eming 1979). This is further buttressed by the 1-1.3 fold increase in Cyclic adenosine monophosphate (cyclic AMP) caused by *C.jejuni* enterotoxin as compatred with 10-15 fold increase caused by V.cholera (Johnson and Lior 1984). In determing $TCD_{50}$ for our filtrates we observed that V.cholerae filtrate was more than four times as potent as our C.jejuni filtrate. Thus reconfirming that the CJT though similar to·CT is of lower potency. The role of the toxins produced by C.jejuni may be more of tissue damage. However, the manifestation of a particular form of symptom would still depend on the host immune status and virulence of the strain involved.

## ACKNOLWEDGEMENTS

We are very grateful to Dr C.P. Iiobi of Dept. of Medical Microbiology Medical School. University of Birmingham U.K. for kindly providing the cell lines used. We are also grateful to Professor S.J. Utsalo, Department of Medical Microbiology, College of Medical Sciences, University of Calabar for kindly providing the toxigenic *Vibrio cholerae* strain.

## REFERENCES

Akhtar, S.Q. and Hug F., 1989. Effects of Campylobacter jejuni extracts and culture supernatants on cell culture. J.Trop. Med. Hyg 92:80-85

Coker, A.O. and Dosunmu-Ogunbi, O., 1984 Isolation of Campylobacte jejuni in Lagos nigeria a "Nev bacterial agent of diarrhoea E afr. Med. J. 61:52-55.

George, H.A., Hoffman, PS. Smibert, R.M. and Trieg, N.R. 1978 Improved media for growth an aerotolerance of Campylobacte fetus J.Clin Microbiol 8:36-41.

Guerfant, R.L. Pennie, R.A.Bbarret, L.J. and O'Brien, A., 1985. Studie of cytotoxin from campylobacte jejuni in Pearson, A. D., Skirrow M.B., Lior, H. and Rowe, R (eds) Camplylobacter III Publi Health Laboratory Service London P. 150.

Johnson, W.M. and Lior, H. 1989. Toxin produced b Campylobacter jejuni an Campylobacter coli Lance 1:229-230.

Johnson, W.M. and Lior, H. 1986. Cytotoxic and cytotonic factor produced by Campylobacte jejuni, Camppylobacter Coli an Campylobacter laridis J. Cli Microbiol. 24:275-281.

Johnson, W.M. and Lior, H., 1988. A new heat labile cytoletha distending toxin Campylobacte spp. Microbial. Pathogenicity 4:115-126.

Karmali, M.A. and Flemming, P.C., 1979; Campylobacter enteritis i children J. Paed. 94:527-533.

Leunk, R.D., Johnsoon, P.T. David, B.L., Kraft, W. G. and Morga D.R. 1988. Cytotoxic activity i broth-culture filtrates c Campylobacter Pylori. J. Mec Microb. 26:93-99.

Low, D.C., Lawande, R.V. and Wall C.,

1981 Campylobacter entritis in Northern Nigeria (letter) Trans. Roy. Soc, Trop. Med. Hyg. 75:754 – 758.

Mathan,V.I., Rajan, D.P., Klipstein, P.A. and Engert, R.E., 1984. Enterotoxigenic Campylobacter jejuni among children in South India. Lancet II 981.

McCardell, B.A., Madden, J. M. and Lee E. C., 1984. Production of cholera like toxin by Campylobacter jejuni/coli Lancet I 448 – 449.

Ruiz- Palacious, G.M., Torres, J., Escamilla, N.I. Ruiz-Palacies, b. and Tomayo, J., 1983. Cholera like enterotoxin produced by Campylobacter jejuni. Characterization and Clinical significance Lancet II 250-251.

Toolan H.W., 1954. Transportable human neoplasm maintained in cortisone-treated laboratory animals: NSI; Hep2; Hep3 and HembRhl · Cancer Research. 14:660 – 666.

Van heyningen W.E. and Gladstone .P., 1953. The neurotoxin of Shigella Shigae III. The effect of iron on production of the toxin Brit. J. Exp. Pathol. 34:221 – 229.

Walker, R.L., caldwell, M.B., Lee, E.c. Guerry, P., trust, T.J. and Ruiz-Palacios G.M., 1986. Pathophysiology of Campylobacter enteritis. Microbilogical Reviews 50:81-94.