

SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS OF CAMPYLOBACTER COLI

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Campylobacter coli were characterized using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The isolates were obtained from the faeces of diarrhoeic children with the age range of 0 to 36 months attending paediatric clinic at the Lagos University Teaching Hospital (LUTH) and Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, Nigeria. The 16 isolates studied were characterized into seven protein profiles based on their outer membrane proteins (OMPs). The flagella antigens of *C. coli* had the molecular weights of 24kDa and 84 kDa.

The SDS-PAGE proves a reliable and rapid technique for typing strains from sporadic cases.

INTRODUCTION

Compylobacter jejuni and *C. coli* are one of the major causes of diarrhoea in the human populace (1) However, the organism is poised with a variety of problems concerning speciation as a result of its expanding allelic variation. *Campylobacter jejuni* and *C. coli* are a major cause of diarrhoea (personal communication). There are a variety of typing techniques for the genus *Campylobacter*, SDS-PAGE analysis of whole cell protein is one of the several approaches taken for identification of campylobacters (2) SDS-PAGE was first introduced by Pharmacia in Australia to find out usefulness of protein banding profile in the speciation of the genus *Campylobacter*. Pharmacia examined 14 reference strains of *Campylobacter* species and 50 test strain including 30 strains of hippurate negative *Campylobacter*. The electrophoretic patterns correlated well with existing biochemical tests and with available DNA homology data. In general, each species possessed unique and reproducible protein bands that are distinct for strains of that species (3) the aim of this study is to speciate our local strains of *Campylobacter coli* using the SDS-PAGE. This technique has not been done with our Nigeria strains.

MATERIALS AND METHODS

Bacterial strains

Bacterial strains were obtained from *Campylobacter* Research Laboratory at the Lagos University Teaching Hospital (LUTH).

Preparation of sample protein

Twenty-four hour colonies were scraped into a homogeniser containing 100 μ l of sample buffer and homogenized for 5 min. The homogenate was transferred to a clean tube and 10 μ l of Tween 20 was added to solubilise the proteins. The pellet was obtained by centrifugation at 5,000 rpm for 10 min at 4 °C and the protein precipitated with cold ethanol. The pellets were then redissolved in sample reducing buffer and heated for 5 min at 100 °C. The standard protein markers were treated the same way prior to loading on the gel.

Preparation of 16% and 4% resolving and stacking gels
16% gel was prepared by mixing 13.5ml of 30% acrylamide solution, 250 μ L of 10% SDS, 6.24 ml of 3M Tris-HCl, 5.13 ml of distilled water 12.5 μ L of undiluted TEMED and 150 μ L of ammonium persulphate. 4% was prepared by mixing 1.33ml of 30% acrylamide, 100 μ L of 10% SDS, 2.5 μ L of 0.5M Tris-HCl at pH 6.8, 6ml of distilled water, 5 μ L of undiluted TEMED and 100 μ L of ammonium persulphate.

Loading of samples and electrophoresis

Samples and standards were loaded and run by the use of Laemmli's method at 50V through the stacking gel and then 100V through the resolving gel. The gel was stopped when the tracking dye was close to the end of the gel. The stacking gel was cut off while the resolving gel was stained with 0.2% of coomassie brilliant blue solution for 2h. The gel was destained overnight in 7% acetic acid in 10% methanol and the photographed.

RESULTS

The sixteen strains of *C. coli* were characterized into seven protein profiles. The first group were made up of 6 (50%) of the stains. They are IF 33, IF 79, IF 34, LA 29, LA 12 and LA 1. This group had high outer membrane protein bands (OMP) with a molecular weight of 116 kDa. The second group was made up of 2 (16%) strains having both high and low OMP with bands of 14, 18 and 116 kDa. They are LA 14 and IF 4. The third group has only one strain (IF 27). This group is made up of 4 main OMP bands of 14, 18, 24 and 116 kDa respectively. The fourth group also has only one strain (IF 32), characterized by the presence of low OMP bands of 14, and 18kDa. Group 5 has only one strain (IF 3) characterized by the presence of high and low OMP bands of 116 and 22kDa. Group 6 comprising one strain (LA 4) was characterized by the presence of 3 low and high OMP bands of 18, 84 and 116kDa. The last group, also made up of one strain (IF 28) was characterized by the presence of high and low OMP bands of 18, 23 and 116kDa (Table 1). Group 3 to 7 constituted 8.3% of the isolates.

Group No.	No of Isolates	Sizes (kDa)
1.	6	116
2.	2	14,18,116
3.	1	14,18,116
4.	1	14,18
5.	1	22,116
6.	1	18,84,116
7.	1	18,23,116

Table 1: Table showing the groupings of 13 *C. coli* isolates according to SDS-PAGE profiles.

Diagram: Picture

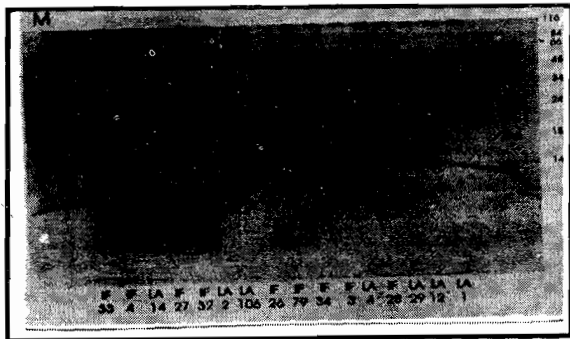


Fig. 1 Coomassie blue-stained SDS-PAGE of OMPs from 16 *Campylobacter coli* isolates. The numbers at the bottom identify the isolates. Lane M: molecular weight marker for the proteins in Kda

DISCUSSION

The ability of polyacrylamide gel electrophoresis of whole cell proteins to identify *Campylobacter* spp has been established in several studies (4). Analysis of outer membrane proteins (OMP's) was performed by SDS-PAGE from 16 isolates of *Campylobacter coli*. *Campylobacter coli* were clearly differentiated into seven subgroups. The results of this work differ from that of Derclaye et al. (5), where twenty – two isolates were only grouped into two. In this study, the common bands seen were 116kDa, 18kDa and 14kDa respectively.

In a report by Derclaye et al. (5), the commonest bands were 37, 55kDa for *C. jejuni* reference stains, while 25 and 84 kDa were present for *C. coli* reference strain. In our study, only one strain had 84kDa while three strains had an OMP of 22, 23 and 24kDa. In another report by Penner et al. (6), approximately 60-62 kDa of protein bands were visualized in *C. jejuni* and *C. coli*. They explained that the protein bands visualized were flagella antigens by the use of acid-glycine extract in detecting serum antibodies that are common antigens associated with flagellin. Logan and

Trust (7) reported that glycine extraction fraction contained flagellin antigen of approximately M_r 31 and 62kDa, while saline extraction was approximately M_r 22, 27 and 45 kDa. All these studies from previous workers showed a different molecular weight from our result possibly as a result of the different technique used (ethanolic and heat stressed) and also environmental variation amongst strains. The use of a probe to check for cross reaction within various isolates is suggested to see which of them share common antigenic determinants. The profile generated from SDS-PAGE is relatively simple and materials and equipments required are generally less costly than those needed for other genomic techniques. In addition the profiles are stable and reproducible, methodological differences between laboratories have little effect on identification.

SDS-PAGE is a valuable tool for the rapid identification of *Campylobacter* species in Nigeria, however, excellent results will be obtained when combined with serotyping as a confirmatory procedure, furthermore, in the developing countries where there are not much funds to carry out meaningful research it proves a reliable means for identifying *Campylobacter* species.

REFERENCES

- Blaser MJ. *Campylobacter* and related species. In Mandell, Douglas and Banett's Principles and Practice of Infectious Diseases. (eds Mandell, GL; Bennett JE and Dolin RP) pp 1995; 1948-1956.
- Patton CM, Wachsmuth IK. Typing schemes: Are current methods useful? In: Nachamkin I, Blaser MJ and Tompkins LS eds. *Campylobacter jejuni*, current status and future trends. ASM Press 1992; 110-128.
- Ruiz-Palacios GM, Calva E, Ruiz-Pelacios BR. Proceeding of the Vth International Workshop on *Campylobacter* infection. National Institute of Nutrition, Mexico, 1989.
- On SLW. Identification methods for campylobacters, helicobacters and related organisms. In: Newell DG, Ketley J. and Feltham RA eds. New York, Plenum Press 1996: 221-226.
- Derclaye I, Delor I, van Bouchaute M, Moreau P, Wauters G and Cornelis GR. Identification of *Campylobacter jejuni* and *C. coli* by gel electrophoresis of the outer membrane proteins. J. Clin Microbiol 1989; 1072-1076.
- Penner JL, Kujanczyk LA, Mills SD. Identification of antigen common to different species of the genus *Campylobacter*. J. Clin Microbiol. 1988; 26, 1411-1413.
- Logan SM and Trust TJ. Molecular identification of surface protein antigens of *Campylobacter jejuni*. Infect. Immun. 1983; 42, 675 –682.