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*Full Length Research Paper*

# Plasmid profile analysis in non-O157 diarrheagenic Escherichia coli in Malaysia

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**Non-O157 VTEC is typical *Escherichia coli* (*E. coli*). A total of 50 non-O157 diarrheagenic *E. coli* isolates were examined. The organisms were isolated from GHKL, Malaysia, from patients with diarrhea. Plasmids were detected in 34 isolates with the plasmid occurrence rate (POR) of 68%. These 34 isolates appear to harbor 1 or more plasmids with the maximum of 4 plasmids. The sizes of the plasmid DNA range from the lowest 1.8 kb to the highest 96 kb. Supercoil DNA marker was used to provide reference plasmids of known molecular weight. This helped in calculating the molecular weight of the plasmids in this study.**

**Keywords:** Plasmid profile, non-O157, *E. coli.*

## INTRODUCTION

Non-O157 *E. coli* is an emerging pathogen. It is commonly present in food and food producing animals. Predictions are made of the possible increase in problems associated with these emerging pathogens (Bettelheim, 2000). In recent years, increased attention has been focused on infections caused by isolates of *E. coli* serotypes other than O157. Infections with non-O157 *E. coli* are now increasingly recognized in many countries (Schmidt *et al*., 1999). Many non-O157:H7 isolates that are associated with outbreaks do not possess either *eae* or the pO157 plasmid (Bokete *et al*., 1993) indicating that there must be additional, as yet unknown, virulence factors that distinguish pathogenic from non-pathogenic non-O157 *E. coli* strains. The virulence profiles of most non-O157 *E. coli* are unknown (Schmidt *et al.*, 1999). Therefore, easy detection, isolation, and characterization of non-O157 VTEC isolates are necessary for improving our knowledge of these organisms (Nielsen and Andersen, 2003).

Plasmid is a small, covalently closed circular, double stranded extra chromosomal DNA element. It is self-replicating independent DNA molecule. Plasmid contains the gene which is normally not essential for bacterial survival or growth. Plasmid commonly carries one or more genes which can confer advantage to the bacterial cell harboring them. Plasmid may confer toxin production, drug resistance etc. Plasmid profiling analysis sometime can be used for epidemiological analysis (Bratoeva *et al*., 1992). The size of the plasmid may vary from 1 kb to 250 kb. Different size of plasmids may be found in a single host bacterium and not all bacteria may contain plasmid. Some types of plasmid are found in large number and some are found in small number in bacterial isolates. In same bacterial cell many copies of a single plasmid can be found which can be extracted in the laboratory using different methods. Many plasmid DNA extraction methods have been established to date.

## MATERIALS AND METHODS

Fifty non-O157 *E. coli* clinical isolates were used in this study. These were obtained from General Hospital Kuala Lumpur and were kindly provided by Prof. Ansary Ahmed. A standard strain *E. coli* V517 to estimate the size of the large plasmids was kindly provided by Dr.

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Salmah Ismail. Supercoil DNA was used to provide reference plasmids of known molecular weight which was purchased from Promega, USA. Plasmid DNA extraction kit was purchased from Eppendorf, Germany. Perfectprep Plasmid Mini preparation kit from Eppendorf, Germany, was used to extract Plasmid DNA and the method was followed according to manufacturer's instructions.

**Agarose gel electrophoresis**

Plasmid DNA was resolved by electrophoresis in submerged horizontal agarose slab gel (0.7%) in Tris-Acetate buffer (TAE) (pH 8.3). The agarose (Sigma Chemical Co., USA) was dissolved by boiling in 1X Tris-Acetate (TAE) buffer (pH 8.3) followed by cooling at $50^0$C. Ethidium bromide (0.5 µg/ml) was added before casting. A 25 µl aliquot of extracted plasmid DNA was mixed with 5-10µl gel loading buffer. The DNA-dye mixture was then loaded into the well. The tank was filled with 1X Tris-Acetate (TAE) buffer (pH 8.3) to completely submerge the gel.Electrophoresis was supplied by a power pack (Model Vokam 400, Shandon Co. Ltd, England). Electrophoresis was carried out from the cathode (-) to anode (+) at a constant voltage at room temperature. The voltage used was in the range of 70 - 90. Electrophoresis was stopped when the tracking dye (loading buffer) was 5-10 mm from the anode end of the gel. The DNA-ethidium bromide complex was visualised using UV transilluminator (Model TFX, Vilber Lourmat, France). Gloves were worn at all times when handling agarose slab gels containing ethidium bromide. Photography was carried out with a Polaroid camera and Polaroid 665 black and white film. Exposure time was between 30-90 seconds. Digital camera was also used for photography and downloaded the image directly to the computer. The brightness and contrast of the image was adjusted before printing.

## RESULTS

Plasmids were detected in 34 isolates with the POR of 68%. These 34 isolates appear to harbor 1 or more plasmids with the maximum of 4 plasmids. Sixteen *E. coli* isolates (32%) did not carry any plasmids. The overall sizes of the plasmid DNA range from the lowest 1.8 kb to the highest 96 kb.

## DISCUSSION

Perfect prep Plasmid Mini preparation kit from Eppendorf, Germany, was used to extract Plasmid DNA and the method was followed according to manufacturer's instructions. The exact procedure was followed and no further modification was done. This isolation technique gave plasmid DNA bands in patterns, and this technique was found to be stable, i.e., reproducible recovery of all plasmid DNA was observed from all the *E. coli* isolates. Therefore, this kit was used for plasmid profiling in this study. Firstly, the plasmids of all the 50 *E. coli* isolates were extracted. The experiment was repeated few times for the confirmation of the findings. After purification the plasmids were analyzed to estimate the approximate size by using the graphical method of Aiij and Borst (1972).

The largest plasmid observed was 96 kb (60 Md) plasmid. This plasmid was carried by 5 non-O157 *E. coli* isolates. These *E. coli* isolates that harbored 96 kb plasmid did not show similar plasmid profiles. Wan Himratul (1999) also showed a similar finding where 2% of the different non-O157 *E. coli* isolates tested were found to carry this type of plasmid. This 96 kb size plasmid is commonly found in O157:H7 strains and it contains genes encoding a hemolysin (enterohemolysin). This plasmid is also reported to be widely distributed among non-O157 VT- producing *E. coli*. Even in Germany, approximately 90% of the VT-positive isolates were found to harbor this type of plasmid (Nataro and Kaper, 1998). One gene on a 96 kb plasmid was reported to encode a type VI pilus and a positive transcriptional activator of multiple chromosomal and plasmid virulence genes (Kaper *et al*., 1997). In addition it was also reported that a gene on this 96 kb plasmid encode for the fimbriae which is responsible for the adherence to the Henle intestinal cells in tissue culture (Karch *et al.,* 1987). Further studies especially in fimbriae and adhesin are needed to find out the role of this 96 kb plasmid isolated from the non-O157 *E. coli* isolates in this study.

The plasmid profile analysis revealed the presence of a 7.2-kb plasmid DNA harbored by 8 non-O157 *E. coli isolates*. This result is in agreement with Wan Himratul (1999) findings where different strains were used. This 7.2-kb plasmid from non-O157 was previously shown to be associated in VT1 production. DNA hybridization studies showed that, this 7.2 kb plasmid to be hybridized against VT1 probe (Wan Himratul, 1999).

Four of the *E. coli isolates* harbored a 6.6 kb plasmid. This size plasmid was also reported by Wan Himratul, (1999) and a plasmid of similar size was also reported by Scaletsky *et al*., (1995). Scaletsky *et al.,* (1995) reported the 6.6 kb plasmid to be very significant in its pathogenesis. Bacteria containing this plasmid synthesize a protein of 32 kDa (pl 4.93) which seemed to be required for cell invasion. They isolated this 6.6 kb plasmid from an O111:H- EPEC that is capable of conferring to an avirulent, non-adherent *E. coli* k12 strain (DK1) the capacity to invade epithelial cells. Similar studies are required to observe the pathogenesis of the 6.6 kb plasmid harbored by the *E. coli* isolates in this study.

The most common plasmid found was a 4.6 kb plasmid, which was harbored by 12 *E. coli* isolates. Wan Himratul, (1999) also showed a similar finding in Malaysia when

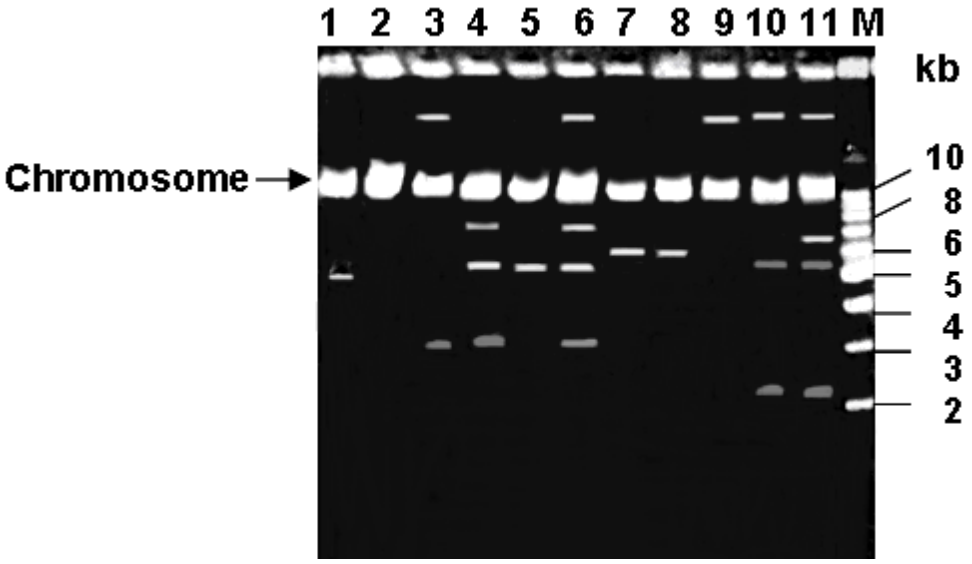


**Figure 1.** Agarose (0.7%) gel electrophoresis of plasmid DNA extracted from non-O157 *E. coli* isolates and supercoil (M) as standard marker. This is a representative picture of all the organisms with plasmids.

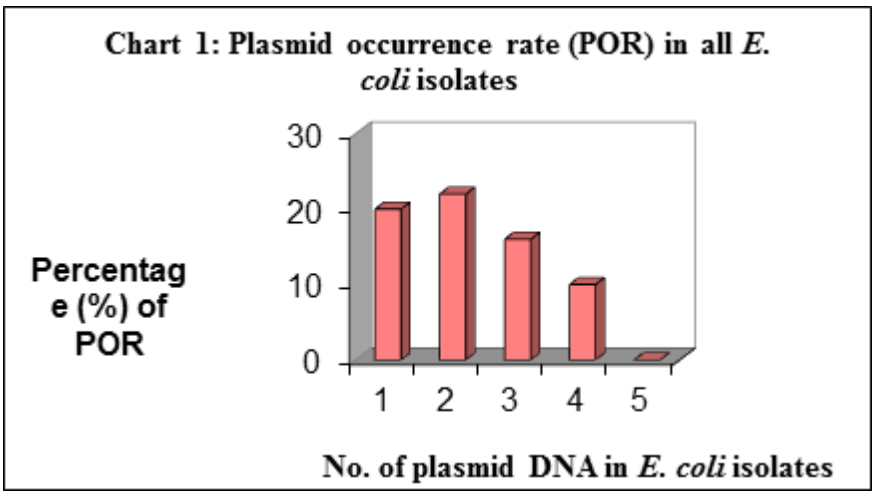


**Chart 1.** Plasmid occurrence rate (POR) in all *E. coli* isolates

tested different isolates, where 16 diarrheagenic *E. coli* were found to harbor a 4.6-kb plasmid. Another common plasmid found was 5 kb plasmid. The significance of these plasmids is yet to be known. Gyles *et al.* (1974) found plasmids coding for heat stable enterotoxins was in the range of 3.2-12 kb. It suggests that any of this plasmid might be involved in producing heat stable enterotoxin but requires further study to confirm its association with the toxin production.

Seven*E. coli* isolates harbored two plasmids of 3.4 kb and 1.8 kb. One possible explanation for this 3.4 kb plasmid, is that may be a dimer of 1.8 kb plasmid, in which there is only one plasmid DNA that is present in two possible forms namely super helical of covalently closed circular (CCC) and relaxed form or open circular (OC). Two *E. coli* isolates were found to harbor only 3.4 kb plasmid without harboring 1.8. Wan Himratul (1999) also showed that these two plasmid 3.4 kb and 1.8 kb were harbored by two different *E. coli* isolates. These findings indicate that these two 3.4 kb and 1.8 kb plasmids may be of different origin and unrelated, not a dimer. But the significance of these two plasmids is yet to be reported.

Four *E. coli* isolates were observed to have identical plasmid profiles carrying four different size plasmids of 5.8, 4.6, 3.4 and 1.8 kb. Another 4 *E. coli* isolates also

showed to harbor identical plasmid profiles carrying 3 different size plasmids of 7.2, 6.6 and 5 kb. Many other isolates also showed identical plasmid profile among them. Identical plasmid profiles have been reported when the isolates are in same serogroups (Fernandes *et al.,* 1992). Fernandes *et al.,* (1992) reported the usefulness of plasmid profile analysis to differentiate strains of *E. coli*. They showed no common plasmid profiles among strains of distinct serotypes. However, they grouped most of the strains within a few major profiles. They also reported that plasmid profile analysis is very useful to differentiate strains within specific *E. coli* serotypes. All the *E. coli* isolates in this study need to be further serotyped to provide a finer plasmid profiling analysis as to differentiate the strains within the specific serotype. This will also lead to a better understanding of the plasmid profiles among the respective serotypes. There are several common small plasmids of similar sizes among many of the *E. coli* isolates, but the significance of these plasmids is yet to be determined.

No plasmid bands were detected in 16 (32%) of the *E. coli isolates*. This finding also agrees with the findings of Wan Himratul (1999) where 22% different strains of non-O157 *E*. *coli* isolates were shown to be plasmidless. Plasmid occurrence rate is normally significant for the epidemiological studies where large number of isolates is tested. Plasmids are known to be associated with different virulence property of the bacterial isolates. In this study POR is significant for the basis of further studies and each plasmid needs to be characterized to detect their role in pathogenesis (if any).

## CONCLUSION

POR in this study was found to be 68%. POR is usually more significant for epidemiological studies where a large number of isolates are tested. Large number of isolates is needed to be tested to use this POR as an epidemiological tool for non-O157 *E. coli* isolates.

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