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Genetic diversity, host range and molecular analysis of the virulence determinants of *Escherichia coli* O157:H7 isolated from different sources

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Two hundred and twenty samples were collected and analysed for characters of *E. coli* O157:H7. Five thousand six hundred presumptive *E. coli* O157:H7 were screened for the presence of the rfbO157 and fliCH7 gene fragments by PCR. The occurrence of the hlyA gene, the plasmid encoded eaeA gene and the shiga toxin genes (stx1 and stx2) were also determined using specific PCR analysis. ERIC, REP, ISR and BOXAIR fingerprinting analysis were performed on all positively identified *E. coli* O157:H7 isolates from the different sources. Pearson's correlation product of moment was used to determine the correlation between the *E. coli* O157:H7 isolated from the different sample sources and/or stations. The two tailed test of significance ($p<0.05$) was used. The genetic profiles generated from the ISR, REP and BOX-AIR PCR were captured using GeneTools (version 3.00.22) software (SynGene, UK). The fingerprints were compared and analysed with the TotalLab Phoretix 1D Pro software (UK). The presence, absence and intensity of band data were obtained, exported to Microsoft Excel (Microsoft Office, 2003) and used to generate a data matrix. Unweighted pair group method with arithmetic mean (UPGMA) and complete linkage algorithms were used to analyze the percentage similarity and matrix data. Relationships between the various profiles and/or lanes were expressed as dendrograms. Data from groups of related lanes were compiled and reported on cluster tables. A total 130 isolates were confirmed through PCR amplification. The prevalence of *E. coli* O157:H7 was higher in pigs and pork 88(67.7%) than in cattle and beef 36(27.7%), water 3(2.3%) and humans 1 (0.77%). Moreover, the pathogen was most frequently isolated from faecal (16.9% to 43.1%) than in meat samples (10.8% to 24.6%). A large proportion 73(56.2%) of the isolates possessed the hlyA gene while 48(36.9%) harboured the eaeA gene. Although there was no major differences in the number of isolates harbouring the stx1 and stx2 genes respectively, only a small proportion 13(10%) harboured both shiga-toxin genes. Despite this, the proportion of isolates that possessed the stx1 29(22.3%) was higher than those with the stx2 gene. None of the *E. coli* O157:H7 isolates harboured all the four shiga-toxin producing *E. coli* (STEC) virulence genes that were investigated. When comparing the proportion of isolates obtained from the different sample sources and/or stations, significant positive correlations were observed between the isolates from Mafikeng and Lichtenburg ($r=0.981$, $p<0.05$) and those from Mafikeng and Rustenburg ($r=0.991$, $p<0.05$). These results therefore indicate that the meat and faeces samples obtained from some major cities in the North-west Province were contaminated with *E. coli* O157:H7 and it is suggested that there is the need to improve the sanitary conditions in the farms, the abattoirs and the butcheries. This could reduce the transmission of *E. coli* O157:H7 to humans. Generally, all the typing methods employed were able to distinguish among isolates from particular sampling sites and/or species. However, on the basis of a comparison of the results from the four assays, ERIC PCR analysis was more discriminatory than the others for the isolates studied. ERIC fragments ranged from 1 to 15 per isolate and their sizes varied from 0.25kb to 0.771kb. A large proportion of the isolates produced ERIC banding patterns with three duplets ranging in sizes from 0.408 to 0.628kb. Eight major clusters (I-VIII) were identified. In most instances ERIC profiles were able to differentiate isolates from a particular farm; meat samples obtained from supermarkets in that particular city and/or water as they clustered together. Overall, the remarkable similarities (72% to 91%) between the ERIC profiles for the isolate from the diiferent animals species and their corresponding food products indicated that there was cross contamination. On the contrary, BOXAIR, ISR and REP PCR analysis were able to reveal close similarities in the genetic profiles of *E. coli* O157:H7 isolates obtained from the different species and/or sources. The band sizes for amplicons from the ISR PCR analysis ranged from 0.173kb to 0.878kb. However, a large proportion of the isolates had four bands ranging from 0.447kb to 0.878kb. Cluster analysis of the BOXAIR PCR profiles based on banding patterns revealed seven main groups. Clusters three (III), four (IV) and seven (VII) had large numbers of *E. coli* O157:H7 isolates in 17.9%, 16.8% and 18.9%, respectively from all the species and/or sample sources except humans. In conclusion, the results presented herein ignite the use of ERIC PCR analysis to compare the genetic profiles of *E. coli* O157:H7 from different sources in the North West province of South Africa when compared to the other typing methods.

Biography

Professor Collins Njie Ateba completed his PhD in Molecular Microbiology from the North West University in South Africa. He also received training from the Centre for Medical Genetics – Yerevan State University in Armenia, University of Tartu – Estonia and the Lethbridge Research Centre in Alberta – Canada. He has presented papers in a number of international conferences worldwide. He is currently recognized as a professional scientist by the South African Society for Scientific Natural Professions in South Africa. Prof Ateba is the head of the head of the Molecular Microbiology Laboratory at the North West University – Mafikeng Campus. He has published more than 36 papers in reputable journals and currently serving as an editorial board member of many reputable journals.

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