Combination of biochemical fingerprinting and detection of virulence genes to trace the sources of pathogenic *Escherichia coli* in surface waters

Warish Ahmed, Jack Tucker, Ron Neller and Mohammad Katouli
Faculty of Science, Health and Education, University of the Sunshine Coast, Maroochydore DC, Qld

Summary

In all, 262 *Escherichia coli* strains (from 10 host groups) and 104 *E. coli* strains (from surface waters) were tested for the presence of 15 clinically significant virulence genes. 39 (14.9%) strains from host groups carried virulence genes and 10 (9.6%) strains from surface waters also carried same virulence genes. All clinically significant strains were typed with the biochemical fingerprinting method. Six strains from surface waters had identical biochemical phenotypes (BPTs) to strains isolated from humans, dogs, chicken and sheep. The results indicated that virulence genes detection in *E. coli* along with biochemical fingerprinting can be used to identify the sources of clinically significant *E. coli* strains in surface waters.

Aim

To use a combination of biochemical fingerprinting and the presence of virulence genes among *E. coli* strains isolated from different host groups and surface waters in order to identify the possible sources of clinically significant strains in surface waters.

Methodology

1 *Host groups and E. coli strains*

Faecal samples (n=384) from 10 host groups (including humans) were collected from Eudlo Creek catchment (southeast Qld). From each host group up to 600 isolates were characterized with the biochemical fingerprinting method (see below) and isolates were assigned to different biochemical phenotypes (BPTs).

Among the 3,728 E. coli isolates tested, 530 BPTs were found.

Only BPTs unique (n=262) to host groups were used to develop a metabolic fingerprint database.

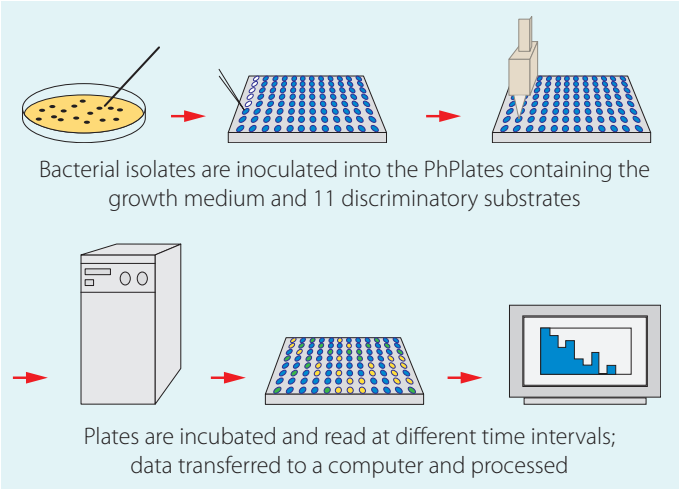
2 *Water samples*

E. coli isolates (n=550) were isolated from 5 sites from Eudlo Creek and also typed with the biochemical fingerprinting method.

Of the 282 BPTs found among water samples, 104 (36.9%) strains were randomly selected to represent all sites (i.e. up to 23 strains from each site).

3 *Source tracking*

Strains from host groups and water samples were tested for the presence of 15 virulence genes causing gastrointestinal and extra-intestinal infections. These included: attachment and effacement (*eaeA*) gene, verotoxin (VT) 1, 2 and 2e, heat-labile toxin (LT) 1, heat-stable toxins (ST) 1 and 2, enteroinvasive (Einv) gene, enteroaggregative (EAgg) gene, cytotoxic necrotizing factors (CNF) 1 and 2 gene, hemolysin A (*hlyA*) gene, (*papC*) gene, LPS O111 and O157 side chain.



Bacterial isolates are inoculated into the PhPlates containing the growth medium and 11 discriminatory substrates

Plates are incubated and read at different time intervals; data transferred to a computer and processed

Results

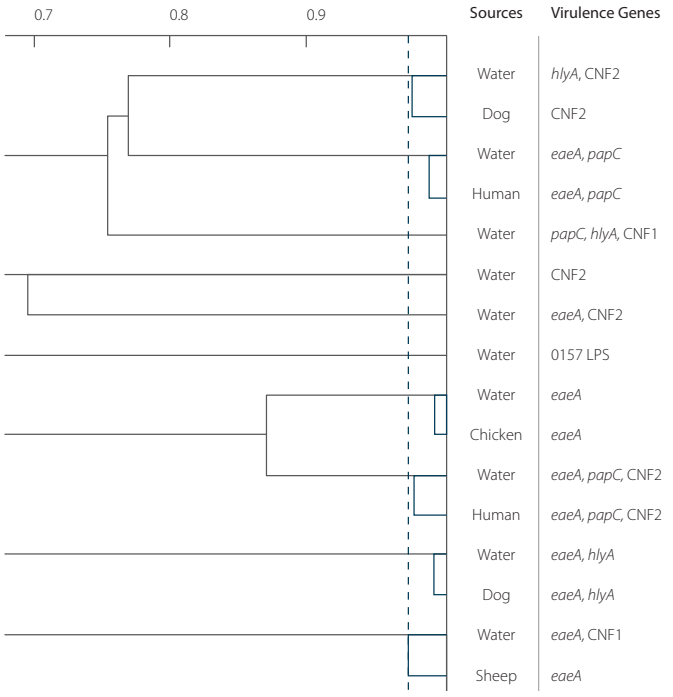
1 *Prevalence of virulence genes*

39 (14.9%) representing strains from 9 host groups and 10 (9.6%) representing strains from water samples carried virulence genes.

These included: 6 strains from human, 2 from horses, 8 from dogs, 2 from ducks, 5 from cattle, 7 from chickens, 2 from sheep and 3 from deer.

2 *Comparison of strains*

6 BPTs were shown to be identical to those of humans, dogs, chickens, and sheep with 4 BPTs also carrying same virulence genes



Conclusions

1 A combination of biochemical fingerprinting and virulence gene detection in *E. coli* can be used to identify potential pathogenic *E. coli* in surface waters.

2 It is possible to estimate the potential number of strains carrying virulence genes in a sampled water by multiplying the number of isolates/BPT.

3 This method of simultaneous identification of virulence genes along with biochemical fingerprinting can be used to assess the potential risks involved in the use of surface waters.