Occurrence and virulence characteristics of rotavirus and *e. coli* pathotypes in children under five years attending two sentinel sites north of Pretoria

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Background and Objectives

In low-income countries, diarrhoeal disease is the second leading cause of deaths in children under 5 years of age. Diarrhoeal diseases may be caused by different enteropathogens like viruses and bacteria including human rotavirus group A (RVA), Enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), Attaching and Effacing *E. coli* (AEEC). Current studies on the association of the afore-mentioned pathogens with diarrhoea in children under five are lacking in South Africa. We investigated the occurrence and virulence characteristics of RVA, ETEC, STEC and AEEC infections among children under five years presenting with diarrhoea at Dr George Mukhari Academic Hospital (DGMAH) and Oukasie Primary Healthcare Centre (OPHC). Age and seasonal trends of the respective enteropathogens were discussed.

Methods

This study was carried out between January 2016 and December 2017. A total of 311 diarrhoeal stool specimens were collected from the two sentinel sites (DGMAH =137 and OPHC= 174) and screened for presence of RVA, ETEC, STEC and AEEC. Rotavirus was detected using enzyme immunoassay (EIA) and positive samples were further subjected to polyacrylamide gel electrophoresis (PAGE), reverse transcription polymerase chain reaction (RT-PCR) and genotyped by PCR. ETEC, STEC and AEEC were detected by culture and multiplex PCR targeting enterotoxin encoding genes: heat labile toxin (LT), heat stable toxin (STp and STh), shiga toxins (*stx1*, *stx2*) and *eaeA* genes respectively. Data were captured and analysed using Microsoft Excel 2010 and R software version 3.3.3, 2017, Austria.

Results

Out of 311 diarrhoeal specimens, pathogens detection rate were ETEC 41.5% (129/311), RVA 11.3% (35/311), STEC 9.3% (29/311) and AEEC 5.8% (18/311). ETEC expressing STp toxin was 40% (32/80), followed by 38.7% (31/80) STh and 37.5% (30/80) LT toxin. STEC stx1 and stx2 genes were present in 83.3% (15/18) and 50% (9/18) respectively. The eighteen AEEC positives possess only eaeA gene. The RVA short electropherotypes G3P[4] was found in 28.6% (10/35) stool samples. Co-infections among the enteropathogens represented 0.6% (2/311). The majority of rotavirus, ETEC and STEC positive samples were found among age categories: $6 \le 24$ months while AEEC was common among age $6 \le 12$ months. Detection of bacterial pathogens peaked mostly during autumn and summer with ETEC accounting for 65.0% (26/40), STEC 21.4% (6/38) and AEEC 15% (6/40) while rotavirus peaked during winter months 15/27 (55.6%).

Conclusion

This study confirms that the afore-mentioned enteropathogens are associated with diarrhoea in children under five in the two sentinel sites under study. ETEC was the most predominant diarrhoeal pathogen detected followed by RVA, STEC and AEEC to a lesser extent. The diarrhoeal associated pathogens carried virulence genes that have been associated with severe diarrhoea in young children. However, differences in the age distribution and seasonal trends were observed. This study provides useful information for diarrhoeal pathogens surveillance in South Africa.

