Molecular detection of pathogens for acute gastroenteritis (AGE)

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INTRODUCTION

Acute gastroenteritis (AGE) is caused by a wide range of pathogens including bacteria.[1,2] Culture is the classical method use for diagnosis of bacterial AGE. Culture is time-consuming and labour intensive, with results available only after 3-4 days. This study aimed to compare a same-day-to-result commercial molecular method using BD MAX™ Enteric Bacterial Panel[3] for the detection of bacterial faecal pathogens against conventional culture method, and to characterise the epidemiology of bacterial AGE in Singapore.

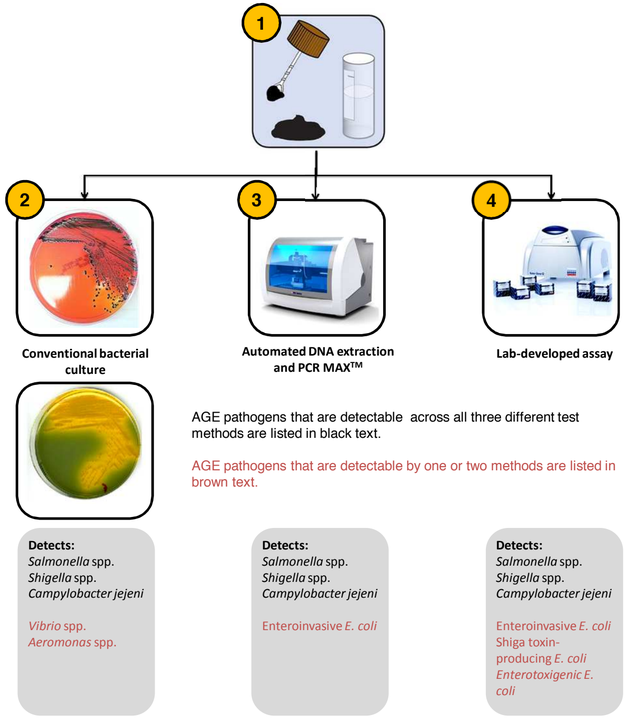
MATERIAL AND METHODS

Stool specimens received for bacterial culture between February to October 2016 were included in this study . Only specimens collected within 72 hours of patient admission were selected. Specimens were innoculated into the BD MAX™ Sample Buffer Tubes before routine bacterial culture is performed from the raw samples for *Salmonella*, *Campylobacter, Shigella*, *Vibrio* and *Aeromonas*.

DNA extraction and amplification were performed on BD MAX™ platform which detects for *Salmonella, Campylobacter*, *Shigella* spp./Enteroinvasive *Escherichia coli* (EIEC) and *Shiga* toxin-producing *E. coli* (STEC)/*Shigella dysenteriae*.

All extracted DNA obtained from the BD MAX™ extraction tubes were then separately tested using a laboratory-developed PCR assay. This laboratory-developed PCR assay also detects for enterotoxigenic *E. coli* (ETEC) which is neither available in routine culture nor on the BD MAX™ platform (Figure 1).

Figure 1: Test methodologies and bacterial AGE pathogens coverage



RESULTS

299 samples were included in the study. No pathogen was detected in 190 samples (63.5%). Of 109 pathogens detected (see Figure 2), 36 (33.0%) were missed by culture method and 15 (13.8%) were missed by BD Max™ (see Figure 3). The pathogens missed by culture include *Salmonella* (n=15), *Campylobacter* (n=11), *Shigella*/EIEC (n=4), STEC/*Shigella dysenteriae* (n=2), and ETEC (n=4). No false-positive result was found for culture method.

It was impossible for BD Max™ to detect pathogens (n=13) that were not available in this commercial panel. 2 *Salmonella* were falsely reported as negative by BD Max™. BD Max™ also reported 7 false-positive pathogens namely 1 *Salmonella,* 5 *Campylobacter* and 1 STEC/*Shigella dysenteriae*.

4 ETEC were detected by laboratory-developed PCR assay.

Figure 2: Bacterial AGE pathogens detected from this study

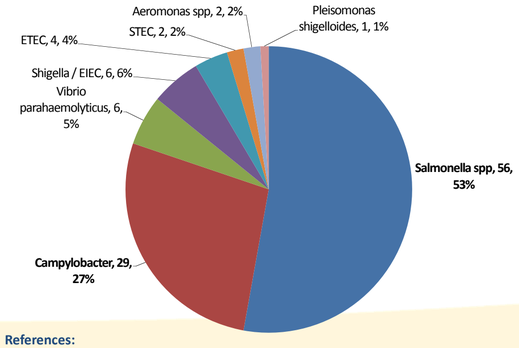
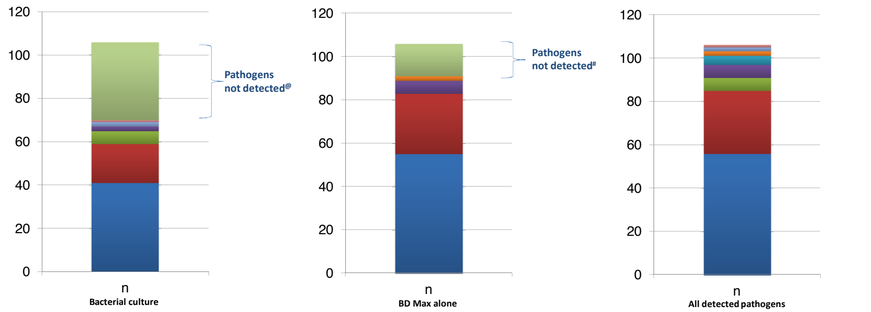


Figure 3: Comparison of culture and BD Max test results



Salmonella n=41
Campylobacter n=18
Vibrio parahaemolyticus n=6
Shigella / EIEC n=2
Aeromonas spp n= 2
Pleisomonas shigelloides n=1
@ **Pathogens not detected n=36**

Salmonella n=55
Campylobacter n=28
Shigella / EIEC n=4
STEC n=2
Pathogens not detected n=15

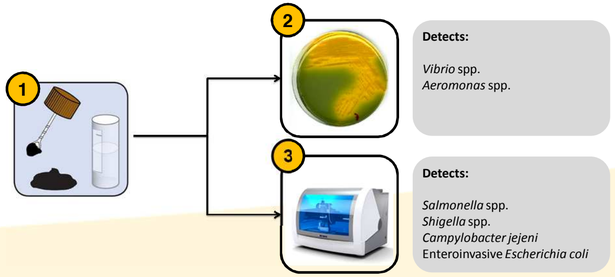
Salmonella n=56 *Campylobacter* n=29
Vibrio parahaemolyticus n=6
Shigella / EIEC n=6
ETEC n=4
STEC n=2
Aeromonas spp n= 2
Pleisomonas shigelloides n=1

CONCLUSION

BD Max™ increased the detection yield of bacterial AGE pathogens, but the absence of detection capability for *Vibrio* and *Aeromonas* affects the overall sensitivity of the system. A combination of culture for *Vibrio* and *Aeromonas* with BD Max™ usage would have provided optimum detection sensitivity. This methods combination testing (Figure 4) for bacterial AGE pathogens would have a diagnostic sensitivity of 96.2% and specificity of 96.6%.

Our study suggests that almost half of bacterial AGE were cause by *Salmonella* spp. and a quarter were cause by *Campylobacter* . Due to the low number of positive samples in this study, further work will need to be done to better understand the epidemiology of bacterial AGE in Singapore.

Figure 4: Recommended testing protocol



References:
1. Chau et al. Diarrheagenic pathogens in adults attending a hospital in Singapore. BMC Infectious Diseases (2016) 16:32
2. Eliott EJ. Acute gastroenteritis in children. BMJ. 2007;334(7583):35–40
3. BD MAX™ Enteric Bacterial Panel, Manufacturer Kit insert, May 2015

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