Diversity of *Salmonella*, *Shigella* and *Escherichia coli* strains in response to a multiplex real-time PCR kit Amplidiag® Bacterial GE

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When suspecting bacterial gastroenteritis, culture is the standard procedure for diagnostics. In recent years, molecular methods for gastrointestinal pathogen have quickly emerged. Amplidiag® Bacterial GE is a multiplex real-time PCR kit designed to detect the most common bacteria causing gastroenteritis. The PCR assay panel includes eight pathogen targets: four pathovars of *Escherichia coli* EHEC (*stx1*, *stx2*), EPEC (*eae*) , ETEC (*est*, *elt*) and EAEC (*aggR*), *Salmonella* spp. (*invA*), *Shigella*/EIEC (*ipaH*, *invE*), *Yersinia enterolitica/pseudotuberculosis/pestis* (*rumB*, *virF*) and *Campylobacter jejuni/coli* (*rim*M, *gyrB*).

MATERIAL - METHODS

	6 *S. sonnei*	23 *S. flexneri*	20 *S. boydii*	11 *S. dysenteriae*
biotypes	a, e, f, g	1, 2a, 2b, 3, 4, 5, 6	1 à 20	SD1 à SD10
ipaH	+			
Shiga-toxine variants Intimine eae	-	3 strains stx1a	1 strain eae	2 strains stx1a

	81 *S. enterica*	4 *S. salamae*	3 *S. arizonae*	4 *S. diarizonae*	5 *S. houtenae*	1 *S. indica*	2 *S. bongori*
Sérotypes	40	4	3	4	5	1	2
MLST	62 populations						
invA	+						

	101 STEC (Shiga-toxine stx1 et stx2)		2 EPEC (intimine eae)	9 EAEC (enteroagggative aggR)	8 ETEC (entérotoxine st et lt)		29 *E. coli*
Serotypes	O157:H7, O26:H11, O80:H2...		O103	O15, O86, O60…	O6, O8…		O25, O9..
MLST	60 MLST populations						
Shiga-toxine variants	+	stx1a, stx1c, stx1d	-	-	-		-
		stx2 : a à f					
Eae variants	eae β, eae γ, eae ξ…. (60 strains)		+	-	-		-
enteroaggrative	2 souches aggR		-	+	-		-
Enterotoxines	2 strains stb et 2 sta1 1 strain stab + sta1		-	-	+	ltcA	-
						sta1/stb	

307 well characterized and sequenced strains were included in this study : 149 *E. coli* containing different genes content, 60 *Shigella* (different species, serotypes and biotypes), 90 *Salmonella* (different serotypes and species) and 9 other bacteria causing diarrhea. Amplidiag® Bacterial GE PCR was performed according to the manufacturer's instructions with CFX96 (Bio-Rad®). Whole-genome sequencing (WGS) was carried out on all the strains by Illumina platform. The sensitivity and specificity of Amplidiag® Bacterial GE were determined by comparing the results obtained by WGS of all the strains tested.

AIMS

The aim of the study was to validate the specificity of this multiplex PCR on all the variants of genes targeted on a representative panel of strains of *Escherichia coli*, *Shigella* and *Salmonella*. The study was conducted in the French National Reference Center, Institut Pasteur, Paris.

RESULTS

Sample : ADN from bacterial culture
Cut-offs modification : Cq < 20 = positive results

%	ipaH	stx1	*stx2*	Eae	aggR	st/lt[2]	invA
Sensibility	98	100	100	100	100	69	100
Specificity	100	100	100	99,6	100	100	100
Predictive Positive Value	100	100	100	98,5	100	100	100
Predictive Negative Value	99,6	100	100	100	100	98,6	100

Presomptive enteropathogenic bacteria targeted by Mobidiag	What mobidiag target gene detection do you expect?
Salmonella spp	*invA* +
Yersinia spp	*Target Yer* +
Campylobacter spp	*Target Camp* +
Enteropathogenic *E. coli* : EPEC	*eae* + AND *stx1* - AND *stx2* -
Enterotoxigenic *E. coli* : ETEC	*st_lt* + AND *stx1* - AND *stx2* -
Enteroaggregative *E. coli* : EAEC	*aggR* + AND *stx1* - AND *stx2* -
Enteroinvasive *E. coli* : EIEC/*Shigella* spp	*ipaH* + AND *stx1* - AND *stx2* -
Shigella stx1+	*stx1* + AND *ipaH* +
Shiga-toxin E. coli : STEC *or* *Enterohaemorragic E. coli : EHEC*	*Stx1* + OR/AND *stx2* + OR/AND *eae* + OR/AND *st_lt* + OR/AND *aggR* + AND ipaH -

The use of Amplidiag® Bacterial GE PCR directly on strains needs to be adapted. Our results showed that for the eight targets the specificity reached 100% after a cut-off modification of positive signal (Cq<25). With this condition, we confirmed a perfect discrimination between *Shigella* and *E. coli* populations, the detection of all *Salmonella* strains, the distinction of stx1 and/or stx2 producing *E. coli* and all the targets has been revealed whatever the variants. An algorithm is also proposed to help microbiologist in their interpretation, in particular to limit the over-interpretation of co-infections due to the presence of multiple gene targets.

DISCUSSION – CONCLUSION

This study demonstrates that Amplidiag® Bacterial GE kit is a valuable tool in the detection of all diverse populations of *E. coli*, *Shigella* and *Salmonella* causing diarreal infections. This work help the microbiological interpretation of multiple positive analytes in a single specimen and highlight the necessity of monitor the potential for agent evolution. A culture-based approach is still necessary for specific pathogens to ideally complete virulence potential, culture base susceptibility tests for the need of surveillance and outbreak detection.

References

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