Original Article

The effects of culture independent diagnostic testing on the diagnosis and reporting of enteric bacterial pathogens in Queensland, 2010 to 2014

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Abstract

Changes in diagnostic laboratory testing procedures can impact on the number of cases notified and the public health surveillance of enteric pathogens. Culture independent diagnostic testing using a multiplex polymerase chain reaction (PCR) test was introduced for the rapid detection of bacterial enteric pathogens in pathology laboratories in Queensland, Australia, from late 2013 onwards. We conducted a retrospective descriptive study using laboratory data to assess the impact of the introduction of PCR testing on four common enteric pathogens, *Salmonella*, *Campylobacter*, *Shigella* and *Yersinia*, in Queensland between 2010 and 2014.

The number of stool specimens tested and the proportion positive for each of the four pathogens increased in 2014 after the introduction of culture independent diagnostic testing. Among the specimens tested by both PCR and culture, 12% of *Salmonella* positive stools, 36% of *Campylobacter* positive stools, 74% of *Shigella* / enteroinvasive *Escherichia coli* positive stools and 65% of *Yersinia* positive stools were PCR positive only. Including those where culture was not performed, 19% of *Salmonella* positive stools, 44% of *Campylobacter* positive stools, 83% of *Shigella* positive stools and 79% of *Yersinia* positive stools had no cultured isolate available for further characterisation. The detection and tracking of foodborne and non-foodborne gastrointestinal outbreaks will become more difficult as culture independent diagnostic testing becomes more widespread. Until new techniques for characterisation of pathogens directly from clinical specimens have been developed, we recommend laboratories continue to culture specimens concurrently or reflexively with culture independent diagnostic tests.

Introduction

For bacterial enterocolitis, identification of the causative agent is important to inform clinical management and prevent ongoing transmission. Most cases only require supportive treatment.[1] For public health surveillance to examine trends in disease, detect outbreaks and monitor antimicrobial resistance, additional information about the pathogen strain or serotype is required. Traditional laboratory methods of culture followed by characterisation of isolates provide essential information for these purposes.

Culture independent diagnostic testing (CIDT) includes any laboratory test that does not require the agent to be cultured prior to identification. CIDT currently used by pathology laboratories worldwide include tests that amplify and/or detect nucleic acid (most commonly polymerase chain reaction [PCR]) and tests that detect antigens, such as enzyme immunoassays.[2-4] These tests are often fast, easy to perform, sensitive and reliable. However, they are generally unable to identify serotypes or genotypes that are used to link cases in an outbreak, or provide antimicrobial susceptibility for treatment. CIDT may also detect non-viable organisms or residual DNA and are unable to distinguish between symptomatic cases and asymptomatic carriage.[5-7] Improved diagnostic sensitivity may provide a more accurate estimate of burden of disease. In addition, the ability of multiplex PCR to detect polymicrobial infections provides new insight into diseases and pathogen interactions.[3,4] The change in methodology makes interpreting disease trends over time difficult.[3]

Clinical diagnostic pathology in Queensland (Qld), Australia, is predominantly provided by two private laboratories and one public laboratory, covering approximately 95% of the pathology market in Qld. Both private laboratories introduced a Roche PCR (https://lifescience.roche.com/en_au/brands/lightmix.html) for enteric bacterial pathogens in late 2013 and the public laboratory introduced a non-commercial in-house PCR for enteric pathogens in late 2015. These laboratories continue to culture enteric pathogens,

either concurrently with the PCR, or reflexively for PCR positive stools only. Culture alone was performed if requested.

Salmonella (non-typhoidal), *Campylobacter, Shigella* and *Yersinia* together contribute approximately 99% of the potentially foodborne bacterial infections notified to Qld Health every year.[8] We assessed the impact of changed testing procedures on these four pathogens in Qld between 2010 and 2014.

Methods

Data collection

The three participating laboratories provided data on all *Salmonella*, *Campylobacter*, *Shigella* and *Yersinia* test results for stool samples from people with Qld residential postcodes performed between 2010 and 2014, inclusive. Each laboratory provided the date the sample was received by the laboratory, the organism(s) identified by culture where applicable, and the results of PCR testing where applicable. The public laboratory, despite catering to a different population to the private laboratories, was used as a culture-only comparison group for observing the change in stool submission rates and pathogen incidence.

Data analysis

Samples positive for *Salmonella* Typhi or *Salmonella* Paratyphi were excluded as the public health response for these two serotypes is different from the public health response for other *Salmonella* serotypes.

The number of stool tests positive and the percent of tests positive (percent positivity) were calculated for each pathogen by month of sample receipt. The denominator data used to calculate the percent positivity was the total number of stool specimens tested for bacterial enteric pathogens at each laboratory during each month, whether they were positive or negative for any pathogen.

Percent positivity before the introduction of PCR (data from 2010 to 2012 [pre-PCR]) and after the introduction of PCR (data from 2014 [post-PCR]) were compared for all laboratories. Data from 2013 was not included in the comparison as the private laboratories introduced PCR in different months during 2013. An alternative pre-PCR period of July 2012 to June 2013 was used for *Campylobacter* to compensate for the decline in incidence in early 2012. Significance ($P \leq 0.05$) was assessed using the two sample test of proportions.

Percent positive agreement (PPA) between culture and PCR was calculated for each pathogen as a measure of agreement between these two tests. This measure is the percentage of the total number of stools where both tests were positive divided by the total number of tests positive by at least one test, where both tests were performed and is used to measure agreement when a gold standard test is unavailable for calculating sensitivity and specificity.[9] This method disregards samples that were negative by both methods in order to reduce the impact of large numbers of negative specimens. Specimens where one of the test methods was not performed were also disregarded.

All data were cleaned and analysed using StataSE 13 (Stata Corp, College Station, TX, USA) and Microsoft Excel.

Ethics approval

Human research ethics approval for the study was obtained through both the Australian National University Research Ethics Committee (reference number 2015/429) and the Royal Brisbane and Women's Hospital Human Research Ethics Committee (reference number HREC/15/QRBW/404).

Results

A total of 740,327 stools were tested for gastrointestinal pathogens at the three laboratories during the study period. The number of stool specimens submitted for testing at the three participating laboratories increased considerably following the introduction of PCR in late 2013 (Figure 1). The stool submission rate post-PCR was 3,920 per 100,000 population compared to a mean annual rate of 3,038 per 100,000 for the period pre-PCR. For the private pathology laboratories combined, the mean number of stools tested per month increased significantly by 45% ($P < 0.001$) post-PCR compared to pre-PCR. The public pathology laboratory reported a significant 9% increase ($P < 0.001$) during the same period without the introduction of PCR testing.

The number of stools that tested positive for *Salmonella* was significantly higher post-PCR when compared to the number positive during the pre-PCR period (Figure 2). Similarly, the proportion of positive stools each month increased post-PCR compared to the pre-PCR period. The proportion of stools positive for *Salmonella* at the private laboratories combined increased from 2.0% to 2.6% ($P = 0.006$) (Table 1). Public laboratory data showed a small, but non-significant increase in stools positive for *Salmonella* (2.9% to 3.5%).

Figure 1. Total number of stools tested by each laboratory, and the rate of stools tested per 100,000 population, by month and year, Qld, 2010 to 2014. The private laboratories introduced PCR in late 2013.

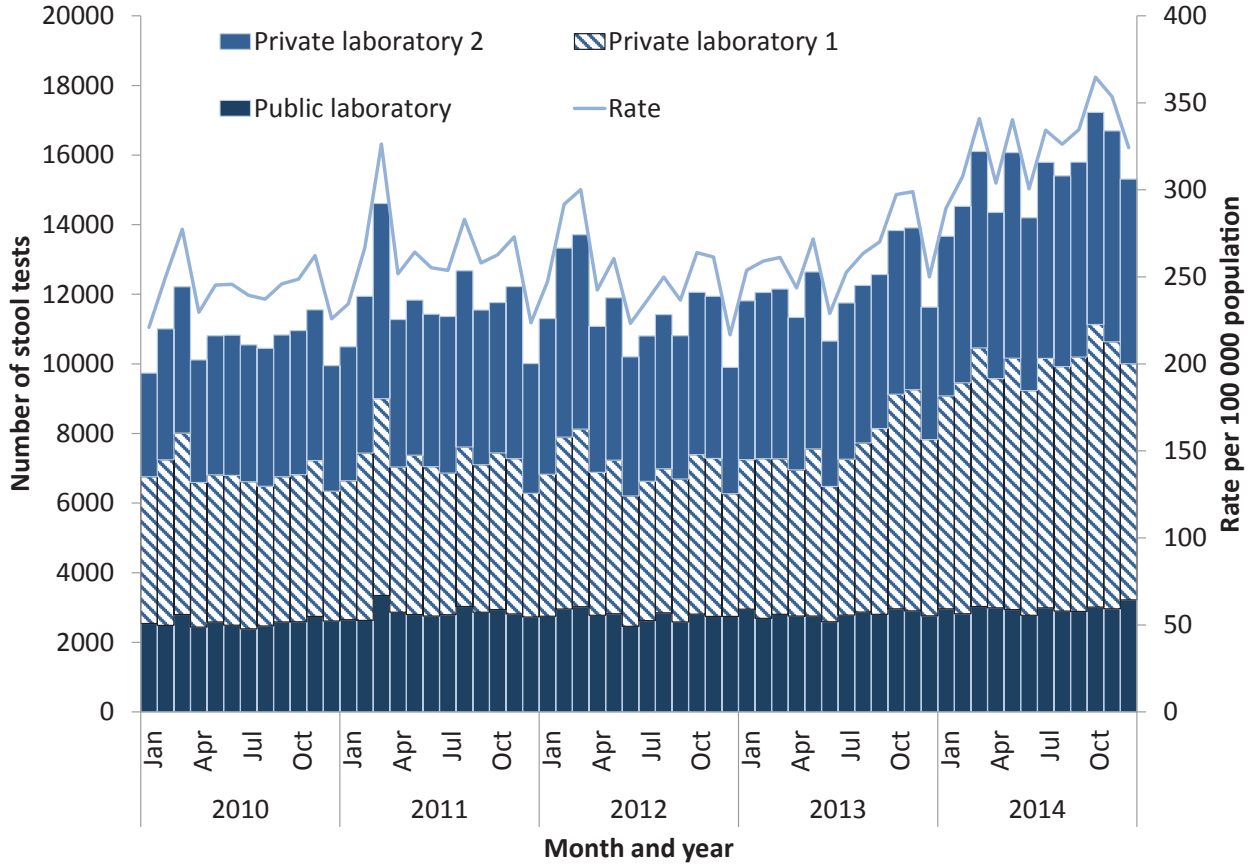


Figure 2. Number of stools positive for *Salmonella* by culture and/or PCR at the Qld private laboratories, and the proportion (%) of positive stool specimens by month and year, 2010 to 2014. The private laboratories introduced PCR in late 2013.

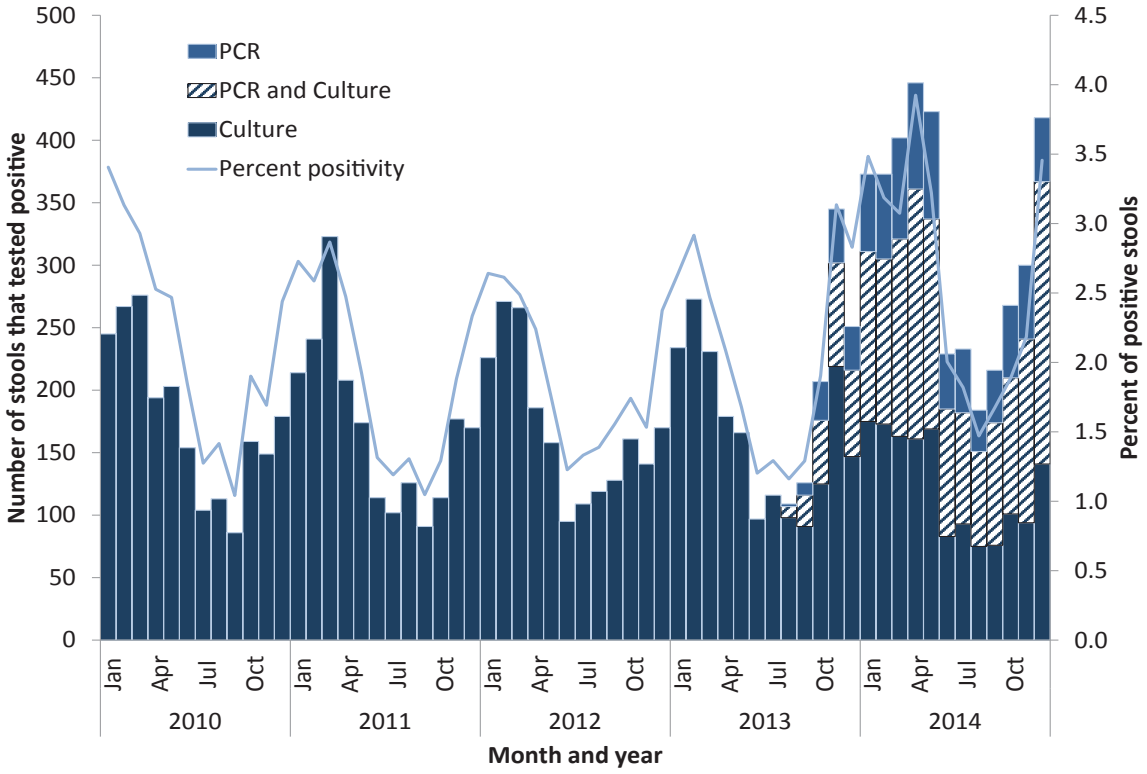
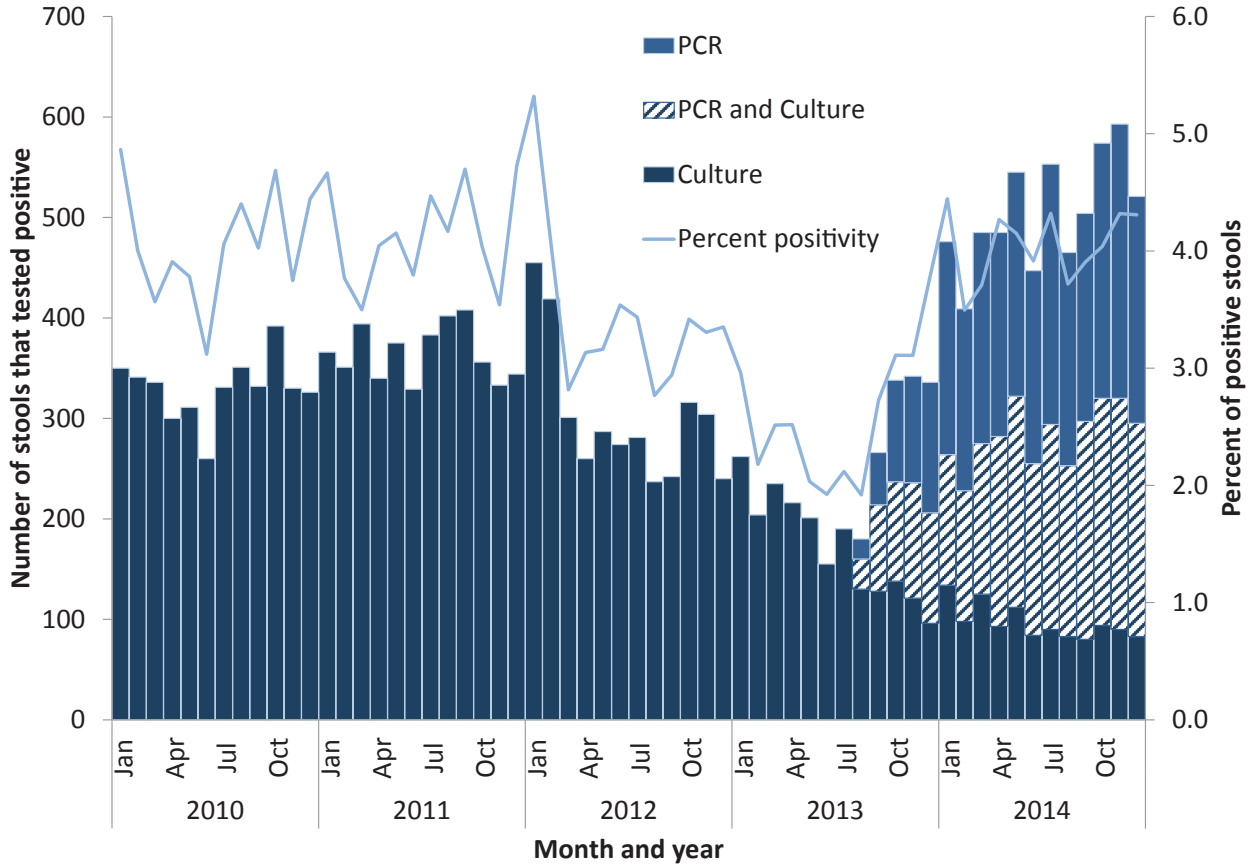


Table 1. Proportion of stools positive and monthly mean for each pathogen before and after introduction of PCR, by whether the laboratory had introduced PCR.

Pathogen	Type of laboratory	Pre-PCR		Post-PCR		
		%	Monthly mean	%	Monthly mean	P value†
Salmonella	Private (PCR)	2.0	173	2.6	322	0.006
	Public (no PCR)	2.9	78	3.5	103	0.22
Campylobacter	Private (PCR)	2.8	241	4.0	505	<0.001
	Public (no PCR)	3.2	89	3.1	91	0.72
Shigella	Private (PCR)	0.03	3	0.32	40	<0.001
	Public (no PCR)	0.14	4	0.16	5	0.97
Yersinia	Private (PCR)	0.06	5	0.39	49	<0.001
	Public (no PCR)	0.07	2	0.12	4	0.63

† Two sample test of proportions

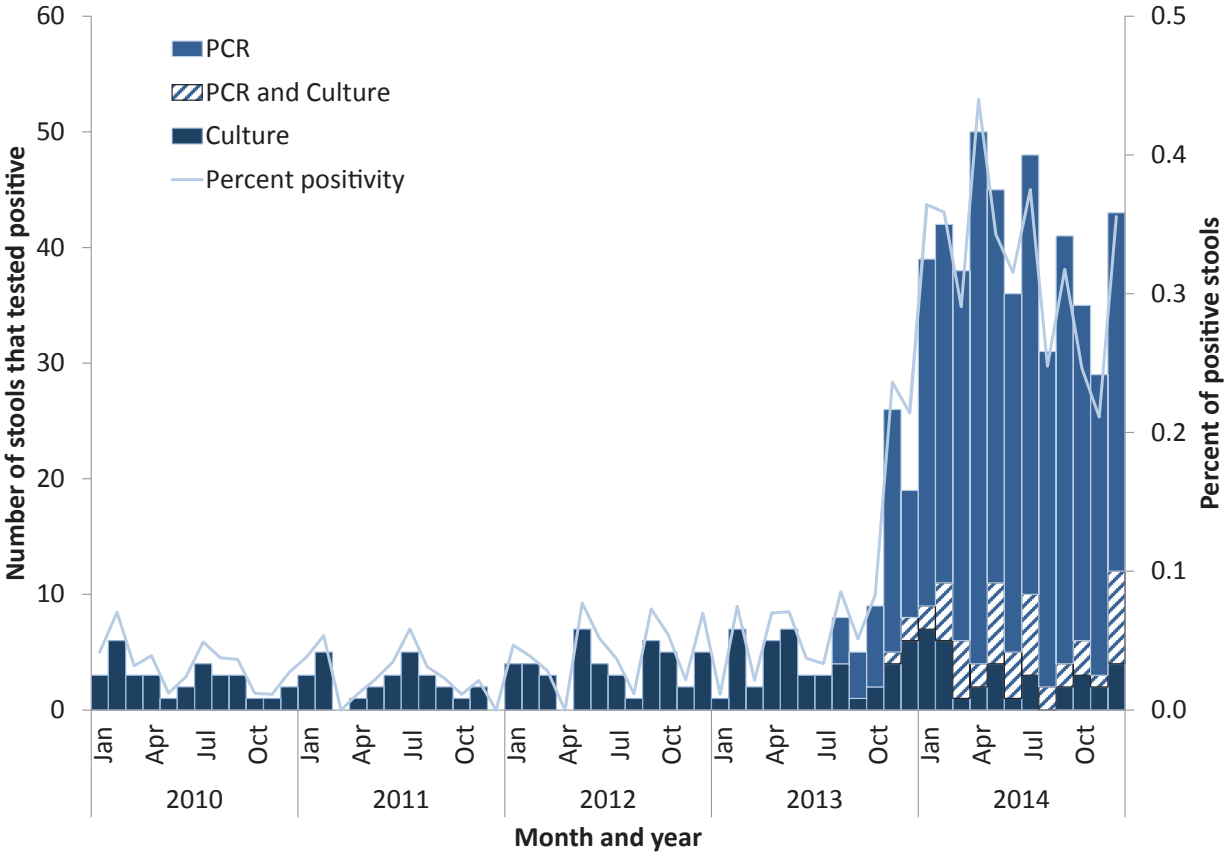
Figure 3. Number of stools positive for *Campylobacter* by culture and/or PCR at the Qld private laboratories, and the proportion (%) of positive stool specimens, by month and year, 2010 to 2014. The private laboratories introduced PCR in late 2013.



The number of stools positive for *Campylobacter* was higher post-PCR when compared to the monthly number positive pre-PCR (Figure 3) and the proportion of stools that tested positive increased significantly from 2.8% to 4.0% (P <0.001) when comparing 2012/13 pre-PCR period with the post-PCR period (Table 1). The public laboratory showed a non-significant decrease in the proportion of *Campylobacter* positive stools per month when the 2012/13 pre-PCR period was compared with post-PCR (Table 1).

The proportion of stools positive for *Shigella* at the private laboratories combined increased significantly from 0.03% pre-PCR to 0.32% post-PCR (P <0.001) (Figure 4, Table 1). The public laboratory showed only a slight increase in the proportion of stools positive per month post-PCR compared with pre-PCR (Table 1). The increase was not significant.

Figure 4. Number of stools positive for *Shigella* by culture and/or PCR (*Shigella*/EIEC) at the Qld private laboratories, and the proportion (%) of positive stool specimens, by month and year, 2010 to 2014. The private laboratories introduced PCR in late 2013.



The proportion of stools positive for *Yersinia* at the private laboratories combined increased from 0.06% pre-PCR to 0.39% post-PCR (P <0.001) (Figure 5, Table 1). The public laboratory showed no significant difference in the proportion of stools positive per month post-PCR compared with pre-PCR (Table 1).

Table 2 shows the number and proportion of positive test results that were concordant (both tests gave the same result) or discordant (one positive result and one negative result) for PCR and culture at the private laboratories combined in 2014. Of those stools that tested positive for *Salmonella,* 17% had discordant results. More so, 36% of stools positive for *Campylobacter*, 74% of stools positive for *Shigella* and 65% of stools positive for *Yersinia* had discordant results.

The PPA for samples positive by culture and PCR (at the private laboratories combined) in 2014 was 71% (1,641/2,316) for *Salmonella*, 51% for *Campylobacter* (2239/4427) and 12% (48/400) for *Shigella*. PPA was not calculated for *Yersinia* as culture for this pathogen was not routinely performed prior to the introduction of PCR.

Discussion

Introduction of PCR diagnostic testing of stools by the two Qld private pathology laboratories significantly impacted public health surveillance of *Salmonella, Campylobacter, Shigella* and *Yersinia* by markedly increasing the number and proportion of stools testing positive for these four pathogens. The changing baseline will have a major impact on interpretation of surveillance data and the reduction in the number of isolates available for characterisation will reduce the ability to detect clusters of related cases.

Much of the increase in the proportion of stools testing positive for *Salmonella* in 2014 can be attributed to the introduction of PCR and increase in testing in the private laboratories. At least 12% of *Salmonella* positive stools identified at the private laboratories in 2014 would not have been identified if PCR had not been in use (PCR positive, culture negative). Further characterisation of *Salmonella* strains by typing was unable to be performed for almost one fifth of stools as they were PCR positive only (culture negative or no culture performed) for *Salmonella* in 2014. Although maintaining concurrent or reflexive culture for PCR positive stools will not

Figure 5. Number of stools positive for *Yersinia* by culture and/or PCR at the Qld private laboratories, by month and year and the proportion (%) of positive stool specimens, 2010 to 2014. The private laboratories introduced PCR in late 2013.

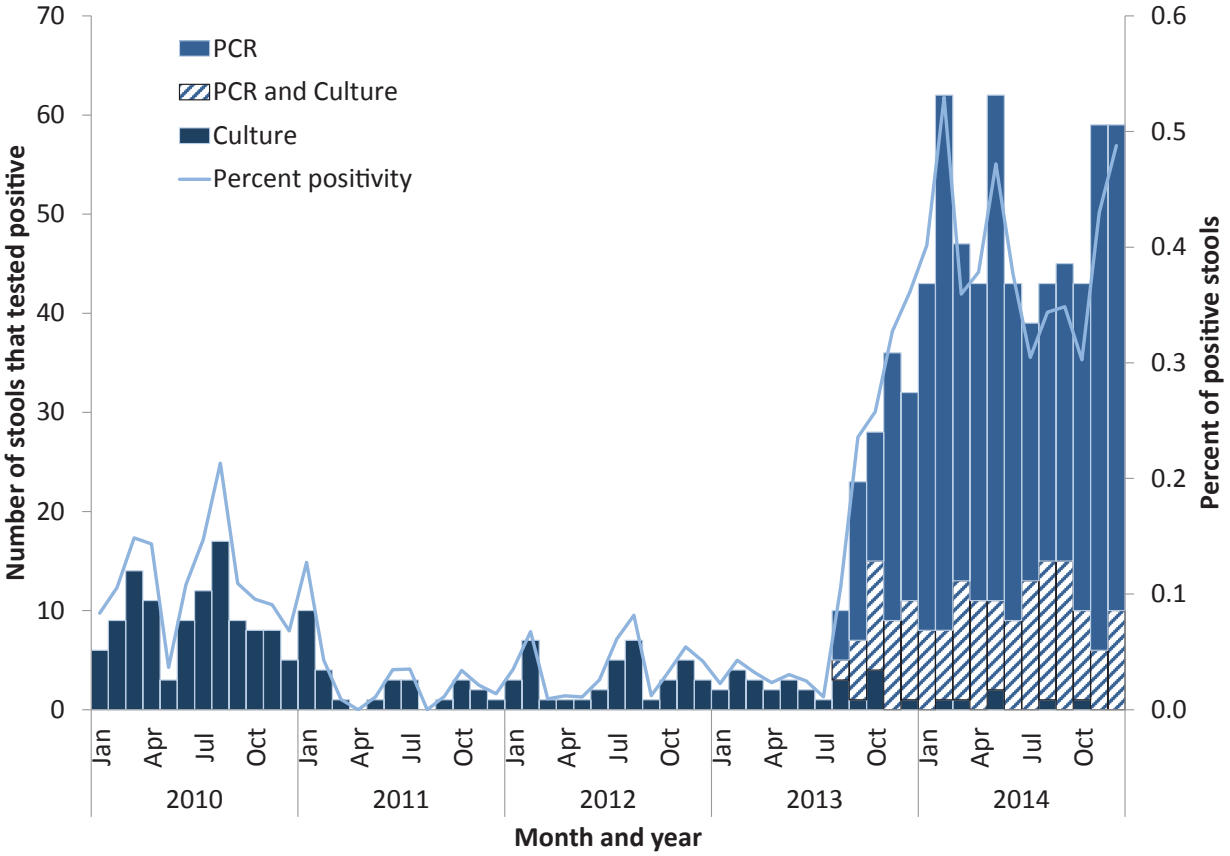


Table 2. Number and proportion of positive stools by test type (private laboratories only), 2014. Shading shows discordant results.

PCR	Culture	*Salmonella*		*Campylobacter*		*Shigella*		*Yersinia*	
		n	%	n	%	n	%	n	%
+	+	1,641	42	2,239	37	48	10	123	21
+	-	482	12	2,176	36	352	74	379	65
-	+	193	5	12	0	0	0	1	0
+	ND*	238	6	476	8	42	9	80	14
ND*	+	1,311	34	1,154	19	35	7	5	1
Total		3,865	100	6,057	100	477	100	588	100

* ND – not done

always result in an isolate,[3] it remains of public health importance to attempt to acquire an isolate for as many cases of salmonellosis as possible.

The sharp decline in the number of *Campylobacter* positive stool specimens during 2012 and 2013 may reflect a real drop in the incidence of campylobacteriosis following commencement of the national Primary Production and Processing Standard for Poultry in 2012 after a two year implementation period.[10] The decrease was observed in notification data in other jurisdictions.[11] There was a significant increase in the proportion of positive stools in 2014 at the private laboratories that was not evident at the public laboratory. This suggests any increase in the total number of stools positive for *Campylobacter* in 2014 was primarily a result of the introduction of PCR at the private laboratories. The PPA for *Campylobacter* was 51%; almost all of the positive stools with discordant results had a negative culture. *Campylobacter* is difficult to culture as it requires specific environmental conditions (microaerophilic) and fresh stool samples in order to recover viable bacteria for culture,[12] and culture based methods are less sensitive for detecting *Campylobacter* than PCR.[13,14]

Current PCR methods for identifying *Shigella* are unable to distinguish between *Shigella* species and enteroinvasive *Escherichia coli* (EIEC) as both are closely genetically related.[15,16] However, EIEC typically causes milder symptoms than *Shigella*[17] and is not notifiable in Australia. As 74% of stools positive for *Shigella* by PCR did not yield a culture, the large increase in the number of stools positive for *Shigella* may in part be due to cases of EIEC being detected by the *Shigella* PCR. However, since *Shigella* can be difficult to culture,[18] the possibility that a proportion of the PCR positive samples were true cases of *Shigella* cannot be discounted. The case definition for *Shigella* in Qld requires isolation of the pathogen, so only those samples with an associated isolate are notified to the Qld Notifiable Conditions System and the National Notifiable Diseases Surveillance System.[19] Thus, in 2014, over 80% of stools positive for *Shigella* by PCR were not notified or were rejected from the notification system. Nevertheless, although these possibly false positive samples were rejected when an isolate was unable to be obtained, they had already resulted in an increase in workload at both primary and reference laboratories where isolate characterisation is performed.

Not all pathology laboratories routinely use culture methods to select for *Yersinia* unless specifically requested by the clinician. However, as *Yersinia enterocolitica* is included in the multiplex PCR test, it was detected even when it was not specifically requested. This may explain some of the increase in the number of tests positive for *Yersinia* after introduction of PCR, and is a reflection of the true incidence of *Yersinia* in Qld. However, 65% of all stools positive for *Yersinia* were positive by PCR and negative by culture, suggesting a much higher sensitivity of PCR than culture for *Yersinia*.

An additional explanation for the increase in stools with a positive culture in Qld during the study period may be associated with the nature of reflexive testing with laboratory technicians searching more intensely for pathogens when they are aware of PCR positive samples.[20]

The proportion of tests positive by PCR only in Qld is higher than that seen in a United States (US) study, where 5% of *Salmonella* infections, 24% of *Campylobacter* infections, 14% of *Shigella* infections and 9% of *Yersinia* infections were positive by CIDT methods only (including PCR and other methods) in 2015.[4] However, the US study only included tests done in a subset of the population, and adoption of PCR (compared with other CIDT) has been slow in some areas.[4] Indeed, the proportion of salmonellosis cases in the US diagnosed by CIDT alone increased substantially in 2015 compared with 2012-2013.[2]

All three laboratories in the current study reported an increase in the total number of stool specimens tested for enteric pathogens, although the percent increase at the public laboratory, where PCR wasn't introduced, was considerably lower than that reported by the private laboratories. It is likely that the increase is predominantly due to the availability of the more rapid PCR test leading to more requests for stool testing by general practitioners.

A limitation of this study is that only one year of data was available after the introduction of PCR at the private laboratories. Comparison of multiple years of data would ensure that the increased incidence of the pathogens in 2014 was not an anomaly. Publicly available notification data suggests that notifications of these four pathogens in Qld has stabilised at a higher rate than the pre-PCR period (data available from http://www9.health.gov.au/cda/source/cda-index.cfm). In addition, only Qld data was analysed, and only for four enteric pathogens. This study should be extended in the future to other jurisdictions across Australia, and for other pathogens. However, the results of this study can be used by laboratories and public health units to inform the way forward for diagnosis, referral and subtyping of enteric pathogens.

We have shown that the introduction of PCR has led to an increase in the detection of these four pathogens in stools, leading to an increase in incidence. Furthermore, PCR introduction has been associated with an increase in the number of stool specimens being submitted for testing though a significant proportion of positive stools are not yielding an isolate to enable strain characterisation for public health surveillance. Should pathology laboratories reduce or terminate concurrent and reflex culture, the number of specimens without an isolate for further characterisation will continue to increase. Until new techniques such as amplicon or whole genome metagenomics (direct sequencing of clinical specimens without need for isolates) are introduced, culture (reflex or concurrent) of enteric pathogens should remain a priority to enable appropriate public health surveillance for detection of outbreaks and monitoring of trends.

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