Original article

Workflow for microbiological diagnosis of bacterial gastroenteritis combining a molecular assay as first-line with reflective stool culture

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Summary

Conventional microbiological methods for bacterial enteric disease diagnosis are time-consuming, labour intensive and provide low sensitivity. The aim of this study was to evaluate the results of a new diagnosis strategy which replaces traditional stool culture with a molecular detection using the BD MAX™ System (BD Life Sciences, Sparks, Maryland, United States) as first-line assay together with reflective culture. A total of 1.590 specimens were prospectively requested for stool culture. The molecular detection included the BD MAX enteric bacterial panel together with the BD MAX extended enteric bacterial panel (BDM GIP) performed simultaneously on the same stool specimen. In 18.8% of specimens (176 of the 936 valid samples) there was one or more than one target positive with the following percent positivity: 9.7%

Campylobacter spp., 5.7% *Salmonella* spp., 1.3% Shiga toxin genes (stx1/stx2), 1.2% *Shigella* spp./enteroinvasive *Escherichia coli* (EIEC), 1% *Yersinia enterocolitica*, 1% *Vibrio* spp. (*V. vulnificus/V. parahaemolyticus/V. cholerae*), 0.3% *Plesiomonas shigelloides*, and 0.2% Enterotoxigenic *E. coli* (ETEC) enterotoxin LT/ST genes. Positive reflective stool culture noted a correlation of 69.5% with the molecular test, missing 23.9% and 15.4% in the cases of *Campylobacter* spp., and *Salmonella* spp., respectively. In conclusion, this clinical study demonstrated very good performance of the BDM GIP. The performance and ease of use may provide advantages to many laboratories, improving the detection of bacterial stool pathogens and time to reporting results.

Key words
bacterial gastroenteritis, molecular detection, BD MAX™ System

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Introduction

Although diarrhoea deaths per year are higher in low income populations, acute infectious gastroenteritis accounts for substantial morbidity, mortality, and cost also in high-income regions.[1] Etiologies of infectious diarrhoea vary and could be bacterial, viral, or parasitic. Bacterial etiology is the second more prevalent after viral, with *Campylobacter* spp. and *Salmonella* spp. being the leading causes of enteritis in developed countries followed by infections with *Aeromonas* spp., *Yersinia* spp., and Shiga toxin-producing *Escherichia coli* (STEC).[2]

The identification of diarrhoeagenic pathogens is important for both the further treatment of patients and public health reports.[3] Diagnosing bacterial enteric disease is particularly problematic as stool culture remains the gold standard despite being a moderately sensitive, time-consuming and labour-intensive method.[4] Commensal microorganisms present in stool may hinder the isolation of possible pathogens, particularly when the pathogens are shed in small amount[3] and some studies have shown a diagnostic yield of stool culture as low as 1.5%.[5]

The BD MAX™ System (BD Life Sciences, Sparks, Maryland, United States) is a fully automated device with a turnaround time of approximately 3 h for up to 24 samples at once with less than five minutes of hands-on time preparation per specimen.[6] In 2014, the BD MAX enteric bacterial panel (EBP) assay for the detection of *Salmonella* spp., *Shigella* spp./enteroinvasive *E. coli* (EIEC), *Campylobacter* spp., and Shiga toxin genes was U.S. FDA cleared, European CE marked, and Health Canada IVD approved.[4] As this panel did not cover the full spectrum of enteric bacterial pathogens, the BD MAX extended enteric bacterial panel (EEBP) assay was launched to be used in conjunction with the BD MAX EBP assay as an optional master mix addition to detect *Yersinia enterocolitica*, entero-toxigenic *Escherichia coli* (ETEC), *Vibrio* spp., and *Plesiomonas shigelloides* simultaneously.[7]

Many reports demonstrate the higher sensitivity of BD MAX EBP assay[4,8-11] or a good correlation of the EEBP assay[7] compared to traditional culture. Molecular panels are also more likely to detect coinfections.[4] Laboratories have demonstrated significant reductions in turnaround time with the use of molecular assays.[12,13] Thus, the BD MAX EBP assay can save technical effort and improve the time of results reporting.[5] However, a few limitations of the BD MAX should be also considered. Since these tests are based on the detection of specific genetic targets, they are unable

to detect pathogens for which a target is missing. Even though the EEBP assay widens the bacterial spectra detected, an important enteric bacterial pathogen such as *Aeromonas* spp. is not included.[14] Another criticism of molecular tests is that they diagnose without strain isolation necessary for determining susceptibility to antimicrobial agents and for epidemiological analyses in an outbreak situation. Anderson and colleagues suggested that subsequent culture of all positive stools could remedy this.[8]

In order to gain the speed and sensitivity of automated molecular tests, without losing strain isolation for antimicrobial susceptibility testing (AST) and epidemiological purposes, our clinical laboratory implemented a new algorithm which replaces traditional stool culture with a molecular detection as first-line assay followed by the reflective stool culture. We chose the BD MAX system due to its targeted approach as multiplex assays that report bacterial, viral, and parasitic targets simultaneously may generate results that are not requested by clinicians and create complexities in reporting as well as in interpretation.[15] Thus, the aim of this study was to evaluate the results of this new microbiological diagnosis strategy.

Material and methods

A total of 1590 specimens were prospectively requested for stool culture at the General University Hospital Reina Sofia (Murcia, Spain) from January to July 2022. Stool samples were transported to the laboratory unpreserved in a clean container and stored at 2-8°C until their processing. They were evaluated first, according to a laboratory protocol in place, following Spanish Society of Microbiology guidelines,[16] while unformed stool samples were processed, first by molecular methods according to our algorithm.

BD MAX assay

Stools from patients were systematically tested in the BD MAX system between 10 to 17 h from their receipt at the laboratory for the presence of bacteria using both BD MAX Gastrointestinal panels (BDM GIP) in conjunction as a first-line assay for diagnosis.

The samples were tested on the BD MAX System using the BDM GIP according to manufacturer's instructions.

Directed stool culture based on the molecular test (reflective culture)

The stool specimens were inoculated on different culture media depending on the bacterial target detected by the molecular test. The inoculation took place between 13 to 20 h from their receipt at the laboratory and the samples had been stored at 2-8°C until that moment.

Salmonella-Shigella agar (bioMérieux, Marcy-l'Étoile, France) was sowed for the isolation of *Salmonella* spp. and incubated at 37°C at least for 24 h. The specimens were also incubated in a selenite broth (Becton Dickinson, Heidelberg, Germany) for 24 hand then the incubated suspension was inoculated on a *Salmonella-Shigella* agar and incubated at 37°C for at least 24 h.

A Campylobacter selective agar (bioMérieux) was used for the isolation of *Campylobacter* spp. and was incubated in a microaerophilic genbag (bioMérieux) at 42°C. for 48 h.

For isolation of *Yersinia* spp., stool specimen was inoculated on Yersinia agar (bioMérieux) and incubated for 48 h at 30°C.

Specimens positive for *Shigella* spp./EIEC and Shiga toxin genes were inoculated on Salmonella-Shigella agar and MacConkey agar (bioMérieux), and incubated up to 48 h at 37°C.

ETEC enterotoxin genes and *Plesiomonas shigelloides* positive specimens were inoculated on MacConkey agar and incubated up to 48 h at 37°C.

For isolation of *Vibrio* spp., stool specimen was inoculated on blood agar (bioMérieux) and incubated for 48 h at 37°C.

Bacterial identification

Suspected colonies were verified by MALDI-TOF MS (Vitek® MS, bioMérieux). Furthermore, in case of Salmonella isolates, serological testing was conducted with specific antisera (Becton Dickinson, Heidelberg, Germany). When *Shigella* spp./EIEC target was detected, the suspected colonies grown on the culture plate were checked by the use of the VITEK®2 Microbial GN ID testing system (bioMérieux), because reliable differentiation of *Shigella* spp. and *E. coli* by MALDI-TOF MS has not been confirmed.[17]

Statistical analysis

The results of the BD MAX GIP assay and the reflective stool culture were compared to the percentage of positive samples detected by both assays. Demographic statistics were performed with the IBM SPSS version 29.0.0.0. (241).

Results

Demographic data

A total of 1590 specimens were received at the microbiology laboratory for bacterial gastroenteritis dia-

gnosis during the study period. All specimens were collected prospectively. The patients' mean age was 41.4 years (standard deviation, 29.29 years) with a minimum of 14 days and a maximum of 97 years. Approximately 25% of specimens were from children < 12 years of age. A total of 55.1% (876/1590) of specimens belonged to women. Regarding sample origin, stool specimens were collected at the primary care department (1092/1590, 68.6%), from hospitalised patients (254/1590, 16%) and at the emergency room department (207/1590, 13%).

In 2021, in our setting, we performed a total of 2459 traditional stool cultures and found a diagnostic yield of stool culture of 9.1% (224/2459) (data not shown).

Specimens included in the analysis

A total of 619 (39%) specimens were unacceptable and not analysed by BD MAX System due to high consistency. The BDM GIP did not deliver a valid result in 10 (0.6%) cases due to unresolved or inhibitory sample or reagent failure. No technical errors of the BD MAX System were recorded. In 8 out of these 10 specimens, in which there was enough sample left, traditional stool culture was performed for diagnosis, with a no pathogenic bacteria detected result in all cases. In 25 (1.6%) specimens received at the laboratory during the study period, the BD MAX molecular tests could not be performed in 24 cases due to a stock failure and in one case of a hospitalised patient, a different molecular assay was used. So, a total of 936 (58.9%) specimens were included in the statistical analysis (Fig. 1).

Performance of the BD MAX assay

Of 936 specimens, in].3%) cases the internal control did not amplify but one of the tested targets did so and it was accepted as a positive result. In 40 cases (4.3%) the EEBP assay failed and only the EBP assay was completed, leading to four negative results. In 720 cases (76.9%) the 8 samples yielded negative results. In 176 specimens (18.8%) one or more than one pathogens were found as follows: 168 stool samples showed one positive target, 10 specimens showed two positive targets and only 1 stool sample showed

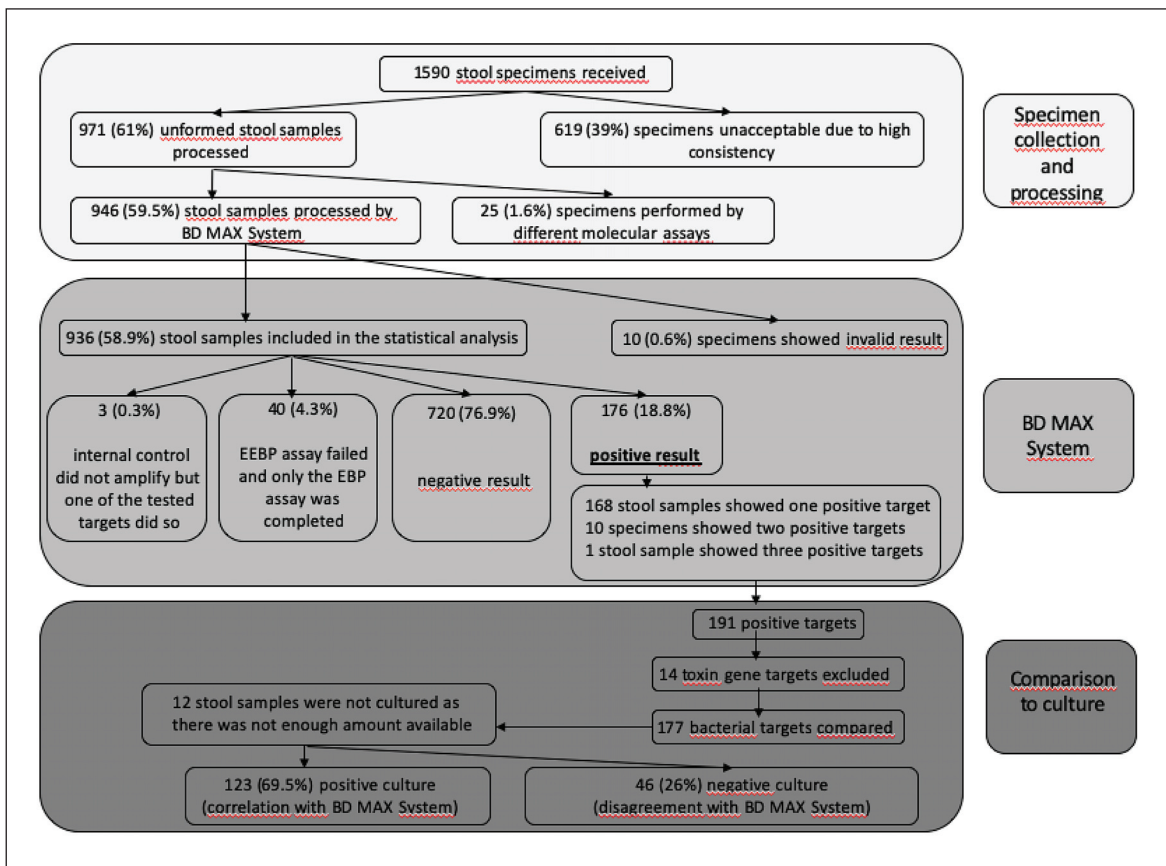

Figure 1 Flow diagram of the study process with the main results.

three positive targets (*Campylobacter* spp., *Plesiomonas shigelloides* and *Vibrio* spp.). Thus, a total of 191 positive targets, from 176 stool specimens, were recorded during the study period (Fig. 1). Based on the molecular method, the global percent positivity rate was 20.4% (191/936) with the following distribution: 9.7% *Campylobacter* spp., 5.7% *Salmonella* spp., 1.3% Shiga toxin genes,1.2% *Shigella* spp./EIEC, 1% *Yersinia enterocolitica*, 1% *Vibrio* spp., 0.3% *Plesiomonas shigelloides*, and 0.2% ETEC enterotoxin LT/ST genes (Table 1).

In those 10 specimens which showed two positive targets, *Campylobacter* spp. was detected in all cases, together with *Salmonella* spp. in four specimens, *Shigella* spp./EIEC in three cases, and, in one case each, Shiga toxin genes, ETEC enterotoxin LT/ST genes and *Vibrio* spp. Thus, *Yersinia enterocolitica* was never found in combination with any other pathogen.

In 5 of the 11 cases of *Shigella* spp./EIEC positive targets, preserved specimens in Cary-Blair transport medium (FecalSwabTM, Copan Group, Brescia, Italy), were sent to the reference national laboratory (Instituto de Salud Carlos III: National Centre for Microbiology, Majadahonda, Spain) where diarrheagenic *Escherichia coli* strains were detected by conventional PCR. In four cases, genes codifying invasive proteins of enteroinvasive *E. coli* (*ipaH*) were found, and, in one case, the gene codifying the virulence plasmid of enteroaggregative *E. coli*, was found (CVD432).

Comparison to subsequent directed stool culture

Of 176 specimens which yielded a positive result by the molecular test, 12 stool samples were not cultured as there was not enough specimen.

Of the 191 positive targets, 14 cases were not compared to culture as a toxin gene was detected (12 Shiga toxin genes and 2 enterotoxin LT/ST genes); in 4 out of the 12 stool samples that could not be cultured, one of these toxin genes was detected. Thus, a total of 177 positive targets detected were subject of the comparative analysis with the directed stool culture results. Positive stool culture showed no disagreement with the molecular test in 123 targets detected, noting a correlation of 69.5%. So, there was no isolation of pathogenic bacteria in the directed stool culture of the remaining 46 (26%) positive targets detected by the molecular method (Fig. 1). In the case of *Plesiomonas shigelloides* and *Vibrio* spp., none of the molecular targets detected by the molecular method were recovered in culture in all cases in which the stool specimen was available. In case of *Yersinia enterocolitica*, the reflective stool culture failed to recover 4 out of 10 positive targets detected during the study period. In the case of *Shigella* spp./EIEC target, the reflective stool culture did not isolate any of those two bacteria in 3 out of 9 specimens that could be cultured. Regarding the most prevalent pathogenic enteric bacteria, *Salmonella* spp. could not be recovered on the stool culture in 8 of the 52 (15.4%) cases con-

Table 1 Targets detected using BD MAX enteric bacterial and extended enteric bacterial assays.

Target	Number of positive target detections (n=191)	Percentage of positive specimens (n=936)
Campylobacter (jejuni /coli)	91 (47.6%)	9.7%
Salmonella spp.	53 (27.7%)	5.7%
Shiga toxin genes (stx1/stx2)	12 (6.3%)	1.3%
Shigella spp./ enteroinvasive *E. coli* (EIEC)	11 (5.8%)	1.2%
Yersinia enterocolitica	10 (5.2%)	1.0%
Vibrio spp. (*V. vulnificus/ V. parahaemolyticus/V. cholerae)*	9 (4.7%)	1.0%
Plesiomonas shigelloides	3 (1.6%)	0.3%
Enterotoxigenic *E. coli (*ETEC) enterotoxin LT/ST genes	2 (1.0%)	0.2%
Total	191 (100 %)	20.4%

sidered. In case of *Campylobacter* spp., stool culture was possible in 88 of the positive targets, without isolation in 21 (21/88, 23.9%) cases. Correlation between molecular test and reflective stool culture for each target is shown in Fig. 2. The percent positivity rate of enteric bacterial pathogens found by the BD MAX system was 20.4% (191/936) (Table 1) while according to culture it was 13.1% (123/936).

In the 8 cases in which *Salmonella* spp. was not isolated by the reflective stool culture, the cycle threshold (Ct) in the BD MAX System was higher than 30, with a mean of 34.9 (range: 30.9-37.9). Out of the 21 cases in which *Campylobacter* spp. was not recovered on the reflective stool culture, the Ct in the BD MAX System was higher than 30 in only 5 cases with a mean of 33.4 (range: 30.1-37.5).

Discussion

To our knowledge, this is the first prospective clinical study which assays both BD MAX bacterial panels simultaneously and compares the results with the reflective culture.

In the study of Knabl and colleagues, the number of cases in which the analysis with the BD MAX EBP assay did not give a valid result initially was higher than ours (8% and 0.6% respectively).[9] They described that failures of the molecular test appeared more often in stool with a higher consistency than in soft or liquid stool specimens. So, our lower percentage could be due to our selection of unformed stool samples. A failure in the testing of 0.6% from a number of 1557 stool specimens that were tested by the FilmArray® Gastrointestinal Panel was reported,[18] and this finding is in more accordance with our results. However, we found a percentage of 4.3 of specimens in which the EEBP assay yielded an invalid result due to inappropriate sample or reagent failure. No invalid results are described in the multisite evaluation of the EEBP assay.[7] Further studies should evaluate the higher percentage of invalid results with the EEBP assay compared to the EBP assay when they are performed at the same time.

The stratification based on the patients' age and the origin of the specimens is highly similar to that recently described in another prospective study in our country.[19]

While assessing the targeted techniques, the current study also evaluated the bacterial pathogens causing acute gastroenteritis in our population; the findings revealed that almost half of the cases were attributed to *Campylobacter* spp. (47.6%) and a quarter (27.7%) to *Salmonella* spp. This was followed by Shiga toxin genes (6.3%), *Shigella*/EIEC (5.8%), *Yersinia enterocolitica* (5.2%), *Vibrio* spp. (4.7%), *P. shigel-*

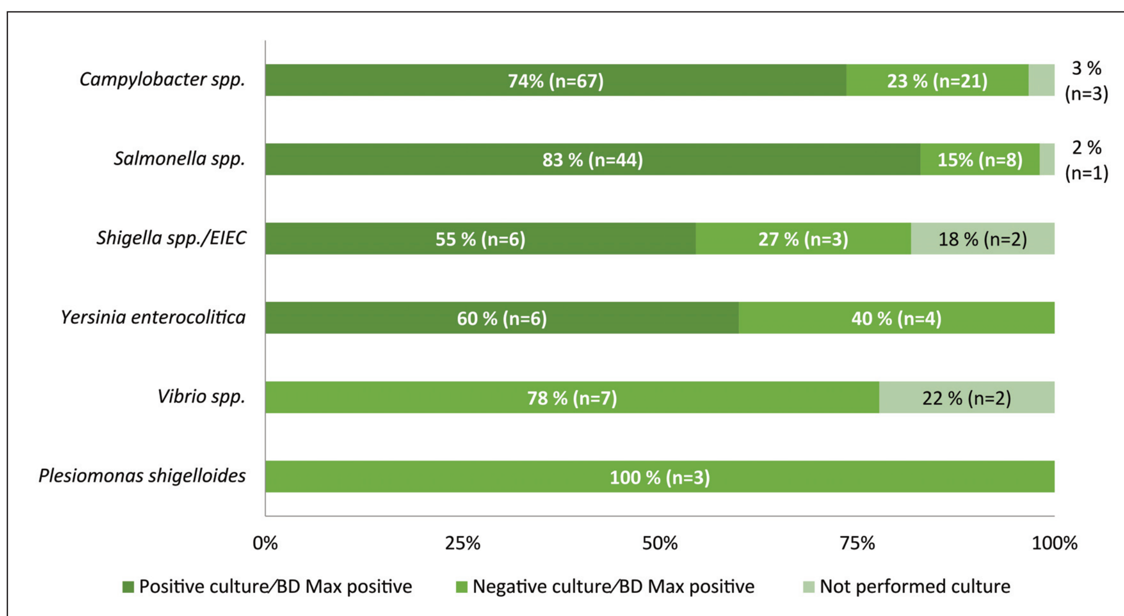

Figure 2 Correlation between BD MAX detection and stool culture result for each target based on the number of positive targets subject of the comparative analysis.

loides (1.6%) and ETEC toxin genes (1%). Our findings are in agreement with those published by Koo and colleagues regarding the Singaporean population, the prevalence of co-infections by two organisms (1.0%) and also their majority of single-pathogen positive samples.[11]

The correlation rate between the BD MAX and the subsequent stool culture reached 69.5%. The BD MAX had a higher overall detection rate (20.4%) compared to the use of bacterial culture alone (13.1%). Our findings show that the use of the culture method alone would miss 46 (26%) out of 177 positive targets, including *Campylobacter* (n=21), *Salmonella* (n=8), *Shigella*/EIEC (n=3), *Yersinia enterocolitica* (n=4), *Vibrio* spp. (n=7) and *Plesiomonas shigelloides* (n=3). This could possibly be due to non-viable organisms or low copy numbers present on the stool specimens that failed to grow on culture.

Regarding *Campylobacter* spp., Gueduet and colleagues suggested that a BD MAX positive result for *Campylobacter* with Ct >30 should have a special comment until the stool culture was available as it could represent a false negative.[20] However, we only had 5 of the 21 disagreements between BD MAX positive and culture negative for *Campylobacter* whose Ct was above 30. Buchan and colleagues also reported that 7/13 (53.4%) specimens positive for *Campylobacter* spp. by the Pro-Gastro SSCS PCR and negative by culture were not confirmed by an alternate PCR method.[21] Buss and colleagues noted 5/24 (21%) samples false positive for *Campylobacter* by FilmArray® Gastrointestinal panel that were not confirmed by alternate PCR, and Coste and colleagues reported confirming 9/15 (60%) false-positive results by using an alternate PCR and EIA methods.[18,22]

Only 15.4% of the positive targets for *Salmonella* spp., were not recovered by the reflective stool culture. A possible explanation for these results is the greater sensitivity of the detection of *Salmonella* spp. by stool culture compared to other enteric pathogens, because of the enrichment in selenite broth.[13,23] All the 8 positive targets, which were not recovered on culture, showed Cts>30.

Our prevalence of diarrhoeagenic *Escherichia coli* is in accordance with previous findings.[7,24] When a microorganism is in low numbers in stool specimens, storage conditions may dilute the target below the limit of detection for stool culture.[4] This fact connected to the results observed in the reference laboratory where none of the 5 stool specimens which were sent recovered *E. coli* on culture and only the characterisation of diarrheagenic *Escherichia coli* strains by conventional PCR was performed.

In the systematic review and meta-analysis conducted by Riahi and colleagues, the pooled prevalence of *Y.enterocolitica* in cases of gastroenteritis was estimated as 1.97% (1.32-2.74%) in the culture method and 2.41% (1.07-4.22%) in the molecular method which is in agreement with our culture and molecular results.[25]

Stool culture in addition to the molecular method has been suggested as a solution by some authors in cases that *Aeromonas* spp. possibility needs to be excluded.[11]

Economical parameters of a molecular assay implementation have not been calculated in this study. However, it should be noted that the economical beneficial effects come from the reduced workload, savings in time-consuming isolation procedures and the increased diagnosis of defined pathogens which should be notified.

To our knowledge, no data on implementation of this assay in a routine workflow is available by now. However, we acknowledge some of the study's limitations, such as the fact that we do not display the results for solving discrepancies between the molecular method and culture, as future studies should be conducted to demonstrate which actions are more efficient and cost-effective.

Also, this is a single-site study. Additionally, a correct molecular diagnosis was assumed, but formally, we could not settle the percentage of probable false positives with the BD MAX system as we have not used a reference method.

In summary, this clinical study demonstrated a very good performance of the BDM GIP assay. Many laboratories might take advantage of this strategy improving the detection of bacterial enteritis pathogens as well as the reporting time, and enhancing the spectrum of epidemiological studies.

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Statements and Declarations

The study has not been presented previously as an abstract in any congress or symposium.

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Conflicts of interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

M.J. Munoz-Davila contributed to the study conception and design. Material preparation, data collection and analysis were performed by M. Piqueras, C. Candel-Pérez and M.J. Munoz-Davila. The first draft of the manuscript was written by M.J. Munoz-Davila and several contributions were made by C. Candel-Pérez who revised it critically. All authors read and approved the final manuscript.

Disclaimer

BD Life Sciences had no influence on the design of this study, data evaluation, and interpretation and was not involved in manuscript preparation.

Ethical approval/informed consent

This study was performed in line with the principles of the Declaration of Helsinki and approved by the Research Ethics Committee of General University Hospital Reina Sofia (Murcia, Spain). All data were anonymized before analysis.

Data Availability

The datasets generated during and/or analysed during the current study are not publicly available due to adhere to the tenets of the Declaration of Helsinki but are available from the corresponding author on reasonable request.

Περίληψη

Workflow for microbiological diagnosis of bacterial gastroenteritis combining a molecular assay as first-line with reflective stool culture

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Οι συμβατικές μικροβιολογικές μέθοδοι για τη διάγνωση της βακτηριακής γαστρεντερίτιδας είναι χρονοβόρες, κοπιώδεις και παρέχουν χαμηλή ευαισθησία. Ο στόχος αυτής της μελέτης ήταν να αξιολογήσει τα αποτελέσματα μιας νέας στρατηγικής διάγνωσης που αντικαθιστά την κλασική καλλιέργεια κοπράνων με μοριακή ανίχνευση χρησιμοποιώντας το σύστημα BD MAX™ (BD Life Sciences, Sparks, Maryland, United States) ως μεθοδολογία πρώτης γραμμής μαζί με την καλλιέργεια. Συνολικά 1.590 δείγματα κοπράνων μελετήθηκαν για με καλλιέργεια. Η μοριακή ανίχνευση περιελάμβανε το εντερικό βακτηριακό πάνελ BD MAX μαζί με το εκτεταμένο εντερικό βακτηριακό πάνελ BD MAX (BDM GIP) που έγινε ταυτόχρονα στο ίδιο δείγμα κοπράνων. Στο 18,8% των δειγμάτων (176/936) ανιχνεύθηκε ένας ή περισσότεροι από έναν στόχοι θετικοί με την ακόλουθη ποσοστιαία θετικότητα: 9,7% *Campylobacter* spp., 5,7% *Salmonella* spp., 1,3% γονίδια Shiga toxin (stx1/stx2), 1,2% *Shigella* spp./εντεροδιεισδυτική *Escherichia coli* (EIEC), 1% *Yersinia enterocolitica*, 1% *Vibrio* spp. (*V. vulnificus/V. parahaemolyticus/V. cholerae*), 0,3% *Plesiomonas shigelloides* και 0,2% γονίδια εντεροτοξίνης *E. coli* (ECET) LT/ST. Η καλλιέργεια κοπράνων έδωσε συμφωνία αποτελεσμάτων σε 69,5% των δειγμάτων με το μοριακό τεστ, με αρνητικό αποτέλεσμα σε 23,9% και 15,4% των περιπτώσεων *Campylobacter* spp. και *Salmonella* spp., αντίστοιχα. Συμπερασματικά, η κλινική μελέτη έδειξε καλή απόδοση του BDM GIP. Η απόδοση και η ευκολία χρήσης μπορεί να προσφέρουν πλεονεκτήματα σε πολλά εργαστήρια, βελτιώνοντας την ανίχνευση βακτηριακών παθογόνων σε δείγματα κοπράνων και μειώνοντας το χρόνο αναφοράς των αποτελεσμάτων.

Λέξεις κλειδιά
βακτηριακή γαστρεντερίτιδα, μοριακή ανίχνευση, BD MAX™ System

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