Optical scatter patterns facilitate rapid differentiation of Enterobacteriaceae on CHROMagar™ Orientation medium

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Summary

Enterobacteriaceae family comprised pathogens and commensals and has a significant impact on food safety and public health. Enterobacteriaceae is often enumerated and presumptively identified on chromogenic media, such as CHROMagar™ Orientation medium based on colony profile; however, classification is highly arbitrary, and some could not be differentiated due to similar chromogen production. Here, we investigated the ability of the laser optical sensor, BARDOT (bacterial rapid detection using optical scattering technology) for rapid screening and differentiation of colonies of the major bacterial genera from Enterobacteriaceae on CHROMagar™ Orientation. A total of 36 strains representing 12 genera and 15 species were used to generate colony scatter image library that comprised 1683 scatter images. This library was used to differentiate mixed cultures of Enterobacteriaceae family – Klebsiella pneumoniae, Enterobacter spp., Citrobacter freundii and Serratia marcescens (KECS group); Proteus mirabilis, Morganella morganii and Providencia rettgeri (PMP group); and non-Enterobacteriaceae family: Pseudomonas aeruginosa, Acinetobacter spp. and Staphylococcus aureus (PAS group) – and data show high accuracy (83–100%) for intra-group classification of colonies in 10–22 h or even before visible production of chromogens. BARDOT successfully differentiated the major genera, including the ones that do not produce visually distinguishable chromogens on CHROMagar™ Orientation, providing a label-free, real-time on-plate colony screening tool for Enterobacteriaceae.

Introduction

Enterobacteriaceae (EB) is the largest family (~ 47 genera and ~ 221 species) in bacterial taxonomy, which comprised enteric pathogens, food- and water-borne pathogens, uropathogens, and commensals (Baylis et al., 2011; Temkin et al., 2014). Enterobacteriaceae is also a major public health concern due to its involvement in community-acquired and nosocomial diseases, and its resistance to β-lactam and carbapenem group of antibiotics (Zurluh et al., 2013; Temkin et al., 2014). In the food industry, EB has been widely used as an indicator microorganism to evaluate microbiological quality and safety, and to assess sanitary and hygienic practices employed during food production, preparation, handling and storage (Stedtfeld et al., 2007; Komacki, 2011; Buchanan and Oni, 2012; Cerna-Cortes et al., 2012; Holvoet et al., 2014; Barco et al., 2015).

Emerging detection and diagnostic technologies, such as those based on biosensors, together with the routine microbiology techniques could expedite detection, diagnosis and therapeutic intervention (Berluti et al., 1989; Liao et al., 2006; Owen et al., 2010; Velusamy et al., 2010; Xu et al., 2012; Clark et al., 2013; Bhunia, 2014; Buchan and Ledeboer, 2014; Hasman et al., 2014). With the new challenges and solutions for modern clinical microbiology, the term ‘culturomics’ has been proposed to emphasize the diversification of culture condition for improved isolation of fastidious microorganisms (Fournier et al., 2013). Furthermore, culturomics could be integrated with the evolving next-generation molecular, immunological, nanobiotechnological and biophysical assays for identification of pathogens in diagnostic laboratories (Fournier et al., 2013).

BBL™ CHROMagar™ Orientation medium (BD, Franklin Lakes, NJ) is a non-selective differential medium that has facilitated rapid identification of colonies of the majority of pathogens in Enterobacteriaceae (Samra et al., 1998; Chaux et al., 2002; D’Souza et al., 2004; Manickam et al., 2013; Payne and Roscoe, 2015). For example,
species of *Klebsiella*, *Enterobacter*, *Citrobacter* and *Serratia* (KECS group) produce metallic/turquoise blue colonies; *Proteus*, *Morganella* and *Providencia* (PMP group) produce translucent colonies with brown halo, while some non-*Enterobacteriaceae*, *Pseudomonas*, *Acinetobacter* and *Staphylococcus* (PAS group) produce cream-coloured colonies (Samra et al., 1998; Chaux et al., 2002; D'Souza et al., 2004; Payne and Roscoe, 2015). However, the major limitation is that the chromogen alone cannot distinguish among the colonies of KECS, PMP and PAS groups. Thus, additional alternative tests are needed for microbial differentiation within a group.

Previously, our group has developed a non-invasive laser optical sensor, BARDOT (bacterial rapid detection using optical scattering technology) that can differentiate colonies of bacteria at the genus, species and serovar levels based on colony scatter signatures without using any biological or chemical probes (Bhunia, 2008; 2014). BARDOT is equipped with a red diode laser (635 nm, 1 mW, 1 mm diameter beam), and when the laser shines on the centre of a colony, it instantly generates a forward scatter image captured by a CCD camera (Banada et al., 2007). Such optical sensors generally work on the principles of the physics of diffraction, interference and refraction when the laser beam passes through the centre of individual colony (Bae et al., 2007). The identity of the colony is interrogated upon matching the scatter image with a pre-established scatter image library using the image classifier (Ahmed et al., 2013). We have successfully used this sensor for detection of *Listeria* species, including *L. monocytogenes* (Banada et al., 2007; 2009), *Salmonella enterica* (Singh et al., 2014), Shiga-toxigenic *E. coli* (Tang et al., 2014), *Vibrio* spp. (Huff et al., 2012), *Campylobacter* spp. (He et al., 2015) and *Bacillus* spp. (Kim et al., 2014; Singh et al., 2015a) directly on Petri plates. Recently, we also applied BARDOT to study the streptomycin-induced stress response in *Salmonella enterica* serovar Typhimurium based on differential colony scatter patterns (Singh et al., 2015b). In addition, it was also used as a bioanalytical detection tool to validate performance of a sample processing and enrichment device, a pathogen enrichment device (Hahm et al., 2015).

Here, we investigated whether BARDOT could be used for high-throughput screening and detection of colonies of the members of *Enterobacteriaceae* on a commonly used CHROMagar™ Orientation plate based on scatter pattern. BARDOT-generated scatter pattern could be used to differentiate colonies of *Enterobacteriaceae* and a few non-*Enterobacteriaceae* within the KECS, PMP and PAS groups, which cannot be otherwise differentiated due to production of similar chromogens on CHROMagar™ Orientation medium (Fig. 1). The results of this study will aid clinicians to make rapid diagnosis of pathogens for specific therapeutic intervention at the point of care, and food microbiologists to make a rapid assessment of microbiological safety/quality of the products.

**Results and discussion**

**Chromogenic attributes and scatter image library**

A total of 36 strains of *Enterobacteriaceae* and non-*Enterobacteriaceae* genera representing 12 genera and 15 species were used in this study (Table 1). The scatter pattern of colonies on plates were acquired by BARDOT when the colony diameter reached 1.0 ± 0.2 mm, taking about 10–22 h of incubation for the majority of tested strains with the exception of a few strains that took around 36 h to reach the desired colony size (Table 1). A general *Enterobacteriaceae* (EB) and non-*Enterobacteriaceae* (NE) scatter image library designated EBNE-11, representing 11 genera, 14 species (3 strains per species with the exception of *Morganella* and *Providencia*) (Table 1) was built. The EBNE-11 library consisted of a total of 1683 scatter images representing minimum 50 images per strain obtained from two independent experiments with two technical replicates (n = 4) to account for any strain-to-strain variations.

Since, the BARDOT system works on the principle of forward light scattering, the incident laser should be able to pass through the colonies on agar media, so that the scatter images are captured by a camera placed beneath the agar plate. Opaque colonies with excessive chromogens may hinder laser propagation, thus may not generate scatter patterns. To overcome such limitation, we are now modifying the BARDOT system to capture the backscatters for colony identification.

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![Fig. 1. Bacterial colony colour and scatter pattern on CHROMagar™ Orientation medium after 10–22 h of growth. Streaked plate image: images of single colonies in rectangular panel depict chromogenic attributes after 24–48 h of incubation; single colony microscopic images indicated colony profile as well as chromogenic attributes after 10–12 h of incubation; black and white images are the scatter pattern of single colony. Colony profile was observed after phase contrast microscopy of ~1 mm diameter colony at 100× magnification. Bacterial cultures were grown in BHI broth at 37°C overnight (14–16 h), serially 10-fold diluted in 100 mM PBS (pH 7.4), and were plated on readymade BBL™ CHROMagar™ Orientation medium (BD) to obtain 50–100 colonies/plate. The scatter patterns of bacterial colony were captured using BARDOT when the colony diameter reached 1.0 ± 0.2 mm in about 10–22 h. Plate picture and its inset were taken at ~24 h of incubation to confirm the chromogenic attributes of cultures on CHROMagar™ Orientation medium. The appearance of expected colour of colony on the CHROMagar™ Orientation medium took minimum 24–48 h, whereas some of the tested species/strains (*Enterobacter* sp. SPB1; *C. freundii* B2643, ATCC 8090, and ATCC 43864; *S. marcescens* BLCC11) did not show the expected colour at 10–22 h when the scatter pattern of colonies (1.0 ± 0.2 mm diameter) were captured.](image-url)
Chromogenic attributes, colony profile, and scatter pattern of *Enterobacteriaceae* and non-*Enterobacteriaceae* species on BBL® CHROMagar™ Medium

1. *E. coli* ATCC 51739
2. *K. pneumoniae* B41958
3. *Enterobacter* sp. SPB1
4. *C. freundii* B2643
5. *S. marcescens* BLCC 11
6. *P. mirabilis* PRI366
7. *P. rettgeri* B14063
8. *P. aeruginosa* ATCC 10145
9. *A. baumannii* ATCC 19606
10. *E. faecium* PRI 524B
11. *S. aureus* ATCC 43300
12. *S. epidermidis* ATCC 14990

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Table 1. List of bacterial cultures used in this study and their characteristics and classification accuracy on CHROMagar™ Orientation medium.

| Bacteria                  | Strain                           | Colony colour on CHROMagar™ Orientation | Incubation time (h) for colonies to reach 1.0 ± 0.2 mm diameter | % Average positive predictive values (PPVs) ± SEM |
|---------------------------|----------------------------------|----------------------------------------|---------------------------------------------------------------|-----------------------------------------------|
| **Enterobacteriaceae**    |                                  |                                        |                                                               |                                               |
| Escherichia coli          | ATCC 51739, ATCC BAA 25922, EDL933 | Pink                                   | 12–17 h                                                       | 83.4 ± 3.5                                      |
| Klebsiella pneumoniae     | B41958, B51036, B41962           | Metallic                               | 12–17 h                                                       | 74.5 ± 4.9                                      |
| Enterobacter spp.         | SPB1, ATCC 13048, ATCC 51697     | Metallic/Turquoise blue                | 10–11 h                                                       | 90.1 ± 1.5                                      |
| Citrobacter freundii      | NRRL B2643, ATCC 8090, ATCC 43864 | Metallic blue                          | 10–17 h                                                       | 81.9 ± 0.1                                      |
| Serratia marcescens       | BLCC 11, ATCC 8100, ATCC 43862   | Metallic blue                          | 12–19 h                                                       | 74.2 ± 1.5                                      |
| Proteus mirabilis         | PRI 366, ATCC 7002, ATCC 35659   | Brown halo                             | 13–21 h                                                       | 77.6 ± 1.2                                      |
| Providencia rettgeri      | B14063, ATCC BAA 2525            | Brown halo                             | 11–36 h                                                       | 92.2 ± 1.0                                      |
| Morganella morganii       | ATCC 25830                       | Translucent, cream                     | 27 h                                                          | Not analysed                                   |
| **Non-Enterobacteriaceae**|                                  |                                        |                                                               |                                               |
| Pseudomonas aeruginosa    | ATCC 10145, ATCC 27853, ATCC 9721| Translucent, cream                     | 14 h                                                          | 94.0 ± 0.6                                      |
| Acinetobacter baumannii/johnsonii | ATCC 19606, B41237, B14920 | Cream                                  | 10–22 h                                                       | 87.7 ± 3.2                                      |
| Enterococcus faecium/aerogenes | PRI 524B, ATCC 700221, PRI522A | Turquoise blue                         | 17–36 h                                                       | Not analysed                                   |
| Staphylococcus aureus     | ATCC 43300, ATCC 10832, NRRL B313| Golden, opaque                         | 13–17 h                                                       | 97.8 ± 0.6                                      |
| Staphylococcus epidermidis| ATCC 14990, ATCC 49461, ATCC 12228| Cream, pinpoint                        | 19–36 h                                                       | 96.6 ± 0.3                                      |

a. Total 36 strains representing 12 genera and 15 species were used in this study to capture the scatter pattern (1683 images) on CHROMagar™ Orientation medium. Cultures were procured from ATCC, American Type Culture Collection, Manassas, VA; BLCC, Bhunia Lab Culture Collection, Purdue University, West Lafayette, IN; Presque Isle Cultures, Erie, PA; NRRL, Northern Regional Research Laboratory, Peoria, IL. EDL strain was obtained from CDC, USA. Enterobacter sp. SPB1 is a spinach isolate from our previous study (Singh et al., 2014). b. Scatter patterns of Morganella morganii were not included in the EBNE-11 library since it consisted of only one strain. Scatter images of Enterococcus spp. were also not used in the analysis, since the sensor could not capture adequate numbers of scatter patterns in an automated mode due to the opacity of colonies. A few images for this genera (total 4) were captured in the manual mode (Fig. 1). PPVs are represented as % average PPV ± standard error of mean (SEM) values calculated from the scatter images acquired from two independent experiments with two replicates (n = 4).

After performing image analysis using EBNE-11 library, the accuracy of differentiation, also expressed as positive predictive value (PPV), was estimated to vary from 81.9 ± 0.1% to 97.8 ± 0.6% (Fig. 1, Table 1), while the PPVs for the remaining three species – S. marcescens (74.2%), K. pneumoniae (74.9%) and P. mirabilis (77.6%) – were each very low (Table 1). This variation in PPVs can be attributed to the overlapping scatter features (Zernike moments, ring and spoke patterns; Haralick textures, image texture) among the test strains. In order to improve our overall classification accuracy or to obtain higher PPV for a species within EB, especially among the pathogens within a group that cannot be differentiated based on the colour alone on CHROMagar™ Orientation medium, separate scatter image libraries were built using scatter images from the EBNE-11 library (i) ‘KECS-Library’ representing Klebsiella, Enterobacter, Citrobacter and Serratia; (ii) ‘PMP-Library’ representing Proteus, Morganella and Providencia; and (iii) ‘PAS-Library’ representing Pseudomonas, Acinetobacter and Staphylococcus (Table 2). We have previously shown that appropriate pathogen(s)-specific libraries can be used for specific identification of an organism. For example, Salmonella spp. was detected on XLT-4 agar plate in the presence of background microbiota (E. coli and Enterobacter spp.) from chicken and fresh produce (Singh et al., 2014), and Bacillus spp. was detected on phenol red mannitol plate in the presence of background microbiota (Staphylococcus, Enterococcus, Micrococcus and Serratia genera) from bovine raw milk (Singh et al., 2015a).

Sensitivity, specificity, PPV and negative predictive value (NPV)

A cross-validation experiment was also performed to compute sensitivity, specificity, PPV and NPV (Baldi et al., 2000) for the scatter image libraries of KECS, PMP and PAS groups. The sensitivity describes the probability that our classifier will produce a true result when used on a population of colonies that contain a colony of an Enterobacteriaceae and non-Enterobacteriaceae genera of interest. The sensitivity values for the pathogens belonging to KECS, PMP and PAS groups were very high, falling in the range of 87.1–93.2%, 98.4–100% and 97.8–98.9% respectively (Table 2). The specificity defines the probability that the test will produce a true negative result when used on colonies formed by organisms other than the pathogen of interest. The specificity for the KECS, PMP and PAS groups was in the range of 73.1–97.6%, 99.4–100% and 98.7–99.6% respectively (Table 2). The NPV is the probability that a colony does not represent a pathogen of interest when a negative result is returned. The NPVs for the KECS, PMP and PAS groups were in the range of 88.2–91.7%, 99.3–99.8% and 95.9–99.8% respectively (Table 2). The PPV gives the probability of finding positives when a BARDOT system identifies after...
Table 2. Analysis of scatter pattern on CHROMagar™ Orientation medium of colonies of Enterobacteriaceae family and few non-Enterobacteriaceae species.

| Bacteria                | Strains tested (n) | % Average scores ± SEM | Negative predictive value (NPV) | Positive predictive value (PPV) |
|-------------------------|--------------------|-------------------------|---------------------------------|---------------------------------|
|                         |                    | Sensitivity | Specificity |                  |                                |                                |                                |                                |                                |
| Enterobacteriaceae      | KECS-group         | Klebsiella pneumoniae 3 | 87.1 ± 0.1 | 73.1 ± 0.1 | 90.1 ± 0.5 | 88.2 ± 2.2 |                                |                                |
|                         |                    | Enterobacter spp. 3    | 93.2 ± 0.8 | 97.6 ± 0.3 | 88.2 ± 1.0 | 93.8 ± 1.0 |                                |                                |
|                         |                    | Citrobacter freundii 3 | 90.1 ± 0.8 | 97.4 ± 0.1 | 88.4 ± 1.2 | 93.2 ± 0.3 |                                |                                |
|                         |                    | Serratia marcescens 3  | 88.7 ± 1.6 | 94.3 ± 0.2 | 91.7 ± 1.1 | 83.6 ± 0.5 |                                |                                |
|                         | PMP-group          | Proteus mirabilis 3    | 99.7 ± 0.1 | 99.4 ± 0.1 | 99.8 ± 0.1 | 98.9 ± 0.3 |                                |                                |
|                         |                    | Morganella morganii 1  | 100.0 ± 0  | 100.0 ± 0  | 99.3 ± 0.1 | 100.0 ± 0  |                                |                                |
|                         |                    | Providencia rettgeri 2 | 98.4 ± 0   | 99.8 ± 0   | 99.4 ± 0.1 | 99.8 ± 0.2 |                                |                                |
|                         | Non-Enterobacteriaceae | Pseudomonas aeruginosa 3 | 98.6 ± 0   | 99.2 ± 0.0 | 96.2 ± 1.5 | 98.7 ± 0.1 |                                |                                |
|                         |                    | Acinetobacter baumannii 3 | 97.8 ± 0  | 98.7 ± 0.0 | 98.9 ± 0.0 | 93.2 ± 3.0 |                                |                                |
|                         |                    | Staphylococcus aureus 3 | 98.9 ± 0   | 99.6 ± 0.0 | 95.9 ± 1.4 | 99.7 ± 0.2 |                                |                                |

a. KECS group (Klebsiella, Enterobacter, Citrobacter and Serratia spp.) library consisted of 505 scatter images; PMP group (Proteus, Morganella and Providencia spp.) of 266 scatter images; and PAS group (Pseudomonas, Acinetobacter and Staphylococcus spp.) of 690 scatter images. b. Percentage average scores ± standard error of mean (SEM) values were calculated from the analysis of the colony scatter patterns acquired from two independent experiments with two replicates (n = 4).

A match with the scatter images from a library. The PPVs for the KECS, PMP and PAS groups were high and were in the range of 83.6–93.8%, 98.9–100% and 93.2–99.7% respectively (Table 2). The PPV and NPV presented in Table 2 were applied to classify each bacterial species within a group (KECS, PMP or PAS). The scatter image libraries for the KECS, PMP and PAS were used independently, and the resulting NPV and PPV do not imply for the inter-group differentiation. These data demonstrate that using the three scatter image libraries can potentially differentiate colonies of Enterobacteriaceae and non-Enterobacteriaceae genera, especially the species of Klebsiella, Enterobacter, Citrobacter, Proteus, Morganella and Providencia on CHROMagar™ Orientation medium with high accuracy. The ability of group-specific scatter image libraries to identify true positive and true negatives is described in more detail in the next section.

Scatter pattern-based multi-pathogen differentiation and detection

Next we examined if these libraries could be used to differentiate colonies of different bacterial genera within the KECS, PAS and PMP group separately as mixed culture. The overnight (14–16 h) grown cultures were mixed (1:1:1:1) in PBS, serially diluted, plated on CHROMagar™ Orientation medium, and incubated for 10–22 h or until the colonies reached to the desired diameter of 1.0 ± 0.2 mm. Plates were then screened by BARDOT, and scatter images were matched against the scatter image libraries for KECS, PMP and PAS groups. The representative scatter image of each species of KECS (Fig. 2A), PMP (Fig. 2B) and PAS group (Fig. 2C) revealed differential scatter features on CHROMagar™ Orientation medium. Furthermore, analysis of the scatter pattern by the surface plot profile using IMAGEJ also revealed qualitative differences in the scatter pattern of Enterobacteriaceae in respective groups (Fig. 2A–C). The scatter pattern-based dendrogram was also constructed from cross-validation matrix of scatter images of KECS, PMP and PAS groups as described in our previous study (Singh et al., 2015a). The bacterial species within the libraries of KECS, PMP and PAS groups showed divergent branching in the dendrogram. Such divergent branching confirmed differentiating scatter patterns among the bacterial genera within KECS, PMP and PAS groups, which could not be distinguished based on the colour alone on the CHROMagar™ Orientation medium (Fig. 2A–C). BARDOT also facilitated rapid identification of E. coli within 12–17 h (Fig. 1, Table 1), compared with colour-based detection on CHROMagar™ Orientation medium where the turnaround time was approximately 20–32 h (Manickam et al., 2013).

We also tested the robustness of the group-specific libraries (KECS, PMP and PAS) by matching the scatter pattern of strains that were not included in the group-specific libraries (Table S1). A total of 991 scatter patterns from individual strains or mixed strains were matched separately with the group-specific libraries. The scatter pattern of colonies of Proteus vulgaris PRI 365, P. vulgaris ATCC 33420 and P. mirabilis ATCC 25933 that were not
Included in the library revealed 96%, 98% and 97% classification accuracy (PPV), respectively, with the *Proteus* genus when matched against the PMP-Library. As anticipated, the classification accuracy for the same strains against the KECS-Library was only 58% or less. However, the *Proteus* species also showed a high PPV (≥ 89%) when matched against the PAS-Library (Table S1). This could be attributed to the shared scatter features between the strains of *Proteus* and *Pseudomonas* species tested. The colony scatter patterns of *Staphylococcus aureus* B41012 and *Pseudomonas fluorescens* ATCC 13525, matched against the PAS-Library individually or in a mixture, also revealed high classification accuracy (> 96%).

For cross-validation purposes, we also matched colony scatter patterns of *Citrobacter freundii* B2643 and *Klebsiella pneumonia* ATCC 51036 (members of the KECS group) as true negatives against the PMP and PAS libraries. As expected, this analysis revealed a very low PPV (≤ 57%). Likewise, *Proteus* species also revealed a low PPV with the KECS (≤ 58%), but high PPV (≥ 85%) when matched against the PAS-Library (Table S1). Thus

Fig. 2. Scatter pattern-based analysis of the *Enterobacteriaceae* and non-*Enterobacteriaceae* species as a mixed culture on the CHROMagar™ Orientation medium. The Petri dish on the left-hand side shows the mixed culture colonies for the respective groups; circular images are the representative scatter pattern of single colony; three-dimensional circular plots are the surface plots of the scatter images. The overnight (14–16 h) grown bacterial cultures (10^8–10^9 CFU ml^{-1}) were mixed (1:1:1:1) in PBS, serially diluted, plated on CHROMagar™ Orientation medium, and incubated for 10–22 h or until the colonies reached a diameter of 1.0 ± 0.2 mm. Plates were then screened by BARDOT, and scatter images were matched against the image libraries for KECS, PMP and PAS groups separately. Representative scatter images of (A) (KECS group): *Klebsiella*, *Enterobacter*, *Citrobacter* and *Serratia*; (B) PMP group: *Proteus*, *Morganella* and *Providencia*; and non-*Enterobacteriaceae* (C) (PAS group): *Pseudomonas*, *Acinetobacter* and *Staphylococcus*. Representative surface plots depicting a three-dimensional display of the intensities of pixels in a greyscale or pseudo colour image (non-RGB images) to qualitatively visualize the differences in scatter pattern of colonies of different bacteria after circular selection of the entire scatter image using NIH IMAGE software and are plotted below each scatter pattern (Schneider et al., 2012). For greyscale values, x and y axis range were 678–992 pixels and z axis (intensity scale) was set at 0–255. Phenogram (dendrogram) was generated from the scatter patterns of three strains of each pathogen except for Providencia rettgeri (two strains) and *M. morganii* (one strain), using BioNJ algorithm (Dereeper et al., 2008). Dendrogram was constructed after analysing Newick output file in tree viewer (TreeDyn). All analyses were performed using open-access algorithm and programmes available at www.phylogeny.fr. Scale bar represents distance as percentage dissimilarity.
we conclude that a PPV above 90% is considered true positive, while a PPV below 90% may have the possibility of producing false-positive result.

The food industry routinely monitors Enterobacteriaceae and/or the coliforms, a subgroup of Enterobacteriaceae, as indicator organism to assess good manufacturing practice and sanitary conditions employed during food processing (Kornacki, 2011; Buchanan and Oni, 2012; Barco et al., 2015). The ability of coliforms to ferment lactose is used as a marker to identify and enumerate them during conventional culturing; however, some non-coliforms such as Aeromonas spp., can ferment lactose and produce false results (Baylis et al., 2011). Among the coliforms, Enterobacter, Klebsiella, Citrobacter and Escherichia (particularly E. coli) are important, and BARDOT showed high PPV (93.2–93.8%) for Enterobacter spp. and Citrobacter freundii, and slightly lower PPV for Klebsiella pneumoniae and Serratia marcescens (83.6–88.2%). These data indicate that BARDOT would be useful for analysis of both Enterobacteriaceae and coliforms on CHROMagar™ Orientation medium (Table 2).

The laser optical sensor applied in this study is an emerging detection technology that offers a rapid, label-free, non-invasive, real-time and on-plate screening of EB on CHROMagar™ Orientation medium as a companion tool. This sensor does not destroy the integrity of the colonies; thus, they can be used for further molecular and serological characterizations. To the best of our knowledge, this is the first report where a laser sensor assisted differentiation of genera within the KECS, PMP and PAS group colonies with PPVs of > 90%, when the CHROMagar™ Orientation medium alone could not differentiate bacteria due to similar chromogenic properties. This technology has the potential to facilitate high-throughput screening of colonies of Enterobacteriaceae for both clinical and food safety application, and could assist in making informed decision and reducing workload of laboratories in public health and food industry while detecting and differentiating multiple pathogens at the same time.

Conventional culturing method is considered a gold standard and is integral to all official detection methods (Swaminathan and Feng, 1994; FDA, 2001; Bruins et al., 2004; USDA-FSIS, 2015). Moreover, a brief culturing (enrichment) is recommended for all commercially available rapid methods. This is because culturing increases target microorganism numbers, thus essential for addressing ‘zero tolerance’ policy for many food-borne pathogens, where a single viable cell per 25 g of analytical unit can be detected with high accuracy (Swaminathan and Feng, 1994; Hahm et al., 2015). BARDOT fits well with the culturing method and can be used as a screening tool to isolate suspect colonies for further verification by polymerase chain reaction, next-generation sequencing and mass-spectrometry (Bhunia, 2014). Therefore, it has the potential to be an indispensable tool in the food testing and public health laboratories.

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Supporting information
Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:
Table S1. Analysing robustness of the group-specific libraries with the scatter pattern of test strains.