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ACS Infectious Diseases

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Rapid Tracing of Resistance Plasmids in a Nosocomial Outbreak Using Optical DNA Mapping

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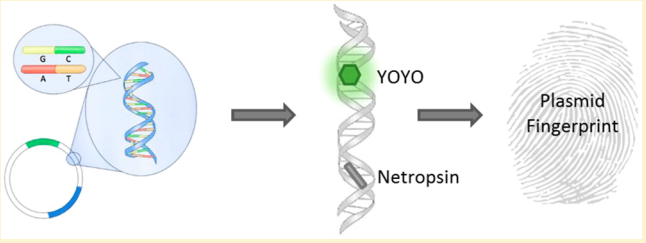
S *Supporting Information*

ABSTRACT: Resistance to life-saving antibiotics increases rapidly worldwide, and multiresistant bacteria have become a global threat to human health. Presently, the most serious threat is the increasing spread of Enterobacteriaceae carrying genes coding for extended spectrum β-lactamases (ESBL) and carbapenemases on highly mobile plasmids. We here demonstrate how optical DNA maps of single plasmids can be used as fingerprints to trace plasmids, for example, during resistance outbreaks. We use the assay to demonstrate a potential transmission route of an ESBL-carrying plasmid between bacterial strains/species and between patients, during a polyclonal outbreak at a neonatal ward at Sahlgrenska University Hospital (Gothenburg, Sweden). Our results demonstrate that optical DNA mapping is an easy and rapid method for detecting the spread of plasmids mediating resistance. With the increasing prevalence of multiresistant bacteria, diagnostic tools that can aid in solving ongoing routes of transmission, in particular in hospital settings, will be of paramount importance.



KEYWORDS: *optical DNA mapping, plasmids, antibiotic resistance, nosocomial outbreak*

Infections with multiresistant bacteria prevent, or complicate, the ability not only to cure infections but also to perform medical treatments where the risks of secondary infections are high, such as surgery, neonatal care, and immunosuppressive therapy.[1] Antibiotic resistance increasingly spreads between bacterial cells and species through mobile genetic elements, such as plasmids.[2,3] With these plasmids in circulation worldwide, we are entering a "post-antibiotic era", where infections that have been treatable for decades again become lethal.[4,5] Knowledge of how, when, and if these plasmids migrate is of utmost importance in forming efficient counter-measures and stopping unwanted chains of transmission, not the least in hospitals.

Standard methods to characterize and identify plasmids include S1-pulsed field gel electrophoresis (S1/PFGE)[6] and polymerase chain reaction (PCR)-based methods.[7] However, S1/PFGE and PCR are increasingly substituted with next-generation sequencing techniques (NGS) that provide base-pair resolution. Still, the dynamic nature of plasmids makes the read assembly of NGS data from fragmented large-size plasmids far from trivial, especially when there is more than one type of plasmid present in the same cell, which demands advanced bioinformatics to resolve the full DNA sequence.[8] Novel sequencing techniques with long reads,[9] such as the platform from Pacific Biosciences, are gaining increased interest for plasmid sequencing but are expensive and suffer from the need of large amounts of sample. In summary, there is a need for rapid, cost-efficient, and simpler methods for the analysis of bacterial plasmids that do not require expert users. In particular, methods that use small amounts of sample, potentially eliminating the step of cultivating bacteria, are of interest to minimize the time from sample collection to diagnosis.

Optical DNA mapping techniques are based on visualizing the sequence of large, intact DNA molecules at the single DNA molecule level. We have developed an optical mapping technique where competitive binding of two small molecules, YOYO-1 and netropsin, to DNA leads to a variation in fluorescence emission intensity along the DNA that reflects the underlying sequence, a DNA "barcode".[10,11] To record the barcode and the size of the plasmids, using standard fluorescence microscopy, the DNA has to be stretched. This is traditionally done on surfaces,[12] but recent advances in nanofabrication have made nanofluidic channels an important alternative.[13,14] Because circular plasmids can be automatically distinguished from linear fragments in the nanochannels, all

Received: February 2, 2016
Published: March 23, 2016

DOI: 10.1021/acsinfecdis.6b00017
ACS Infect. Dis. 2016, 2, 322−328

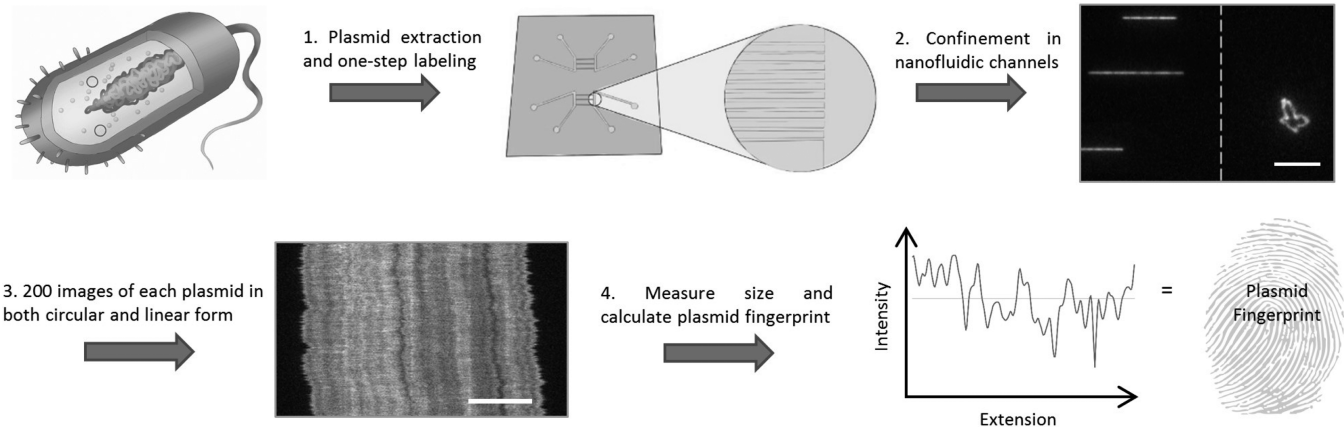


Figure 1. Schematic representation of the optical DNA mapping approach used to characterize plasmids at the single-molecule level by fingerprinting. After extraction of plasmids from the bacterial culture, a single-step competitive binding approach was used to create a sequence-specific pattern, a barcode, along the extension of the molecule. When confined to nanofluidic channels, the extended circular plasmid was linearized by using light irradiation, allowing for visualization of the linear barcode using a fluorescence microscope. Two hundred images of each molecule were recorded and analyzed by automized software to calculate the size of the plasmid and to generate an averaged barcode that serves as a fingerprint of the plasmid. All scale bars correspond to 10 μm.

Table 1. Isolate Characteristics[a]

patient	isolate[b]	species	sequence type (ST)	PFGE−strain type	measured size of detected plasmids (kbp ± SD)
1	P1a	*E. coli*	131[c]	EA[c]	73 ± 7, 152 ± 14
	P1b	*E. coli*	131	EA	74 ± 5, 152 ± 9
2	P2a	*E. coli*	131[c]	EA[c]	75 ± 4, 158 ± 6
	P2b	*E. coli*	73[c]	ED[c]	78 ± 6
	P2c	*E. coli*	131	EA	83 ± 9, 154 ± 4
3	P3a	*E. coli*	1444[c]	EB[c]	79 ± 7, 118 ± 3, 192 ± 11, 240 ± 7
	P3b	*K. pneumoniae*	26	KA	47 ± 4, 88 ± 9, 232 ± 13
4	P4a	*E. coli*	131	EA[c]	89 ± 6, 154 ± 7
	P4b	*K. pneumoniae*	26	KA	90 ± 3, 220 ± 7

[a]Details of the nine isolates that were analyzed for plasmid content, stemming from four patients involved in the nosocomial outbreak. All isolates investigated were shown to carry the ESBL-gene CTX-M-15 by PCR analysis. [b]All isolates were collected in December 2008, when the outbreak was detected, except P1b and P2c, which were collected 5 and 17 months later, respectively. [c]Results reported in a previous study on strain-typing of ESBL-producing bacteria,[18] where isolate P1a = E36047, P2a = E10787, P2b = E36395, P3a = E36046A, and P4a = E36051A.

plasmids that are analyzed are intact.[15,16] Even if the resolution of the method is not at the base-pair level,[11] as for sequencing techniques, transmittable plasmids are generally 30 kb or larger,[17] making them ideal for the competitive binding-based mapping assay.

In this study we demonstrate that plasmid barcodes can be used as fingerprints to follow the spread of plasmids. It is important to note that tracking can be done without any previous sequence information and that after plasmid extraction, the subsequent steps can be finalized in <1 h. The experimental procedure used in this study is summarized in Figure 1 and presented in detail under Methods.

RESULTS AND DISCUSSION

We demonstrate the applicability of the optical DNA mapping assay for investigating the spread of plasmids by confirming transmission routes in a long-standing nosocomial outbreak[18] at a neonatal ward at Sahlgrenska University Hospital in Gothenburg, Sweden. This polyclonal outbreak, affecting more than 20 neonates, mainly included an *Escherichia coli* strain of multilocus sequence type (ST) 131, carrying a gene coding for the β-lactam degrading enzyme CTX-M-15. The ST131 isolates belong to the globally important subclone, O25b-ST131, responsible for a major part of the worldwide spread of CTX-M-15.[19] Some neonates also carried other isolates, including *E. coli* ST73 and ST1444 as well as *Klebsiella pneumoniae* ST26, all producing CTX-M-15.

Nine representative isolates from four patients were studied with the optical DNA mapping assay, to investigate possible transmission of one or more plasmids between bacterial strains and species as well as bacteria between patients (Table 1). The isolates were obtained from neonates during, as well as after, the 4-month-long outbreak. We analyzed the numbers and sizes of all plasmids in each of the nine isolates as well as their barcodes. For *E. coli* ST131, all isolates contained two plasmids, 73−89 and 151−158 kbp in size, respectively, suggesting that all of the ST131 isolates had similar plasmid content (Figure 2a). Even if the total plasmid content differed between the strains, all nine isolates harbored a plasmid with a size of 73−90 kbp, which hereafter will be referred to as the "shared" plasmid. All barcodes of the shared plasmid displayed similar characteristics (Figure 2b), suggesting that, if the outbreak is plasmid-

DOI: 10.1021/acsinfecdis.6b00017
ACS Infect. Dis. 2016, 2, 322−328

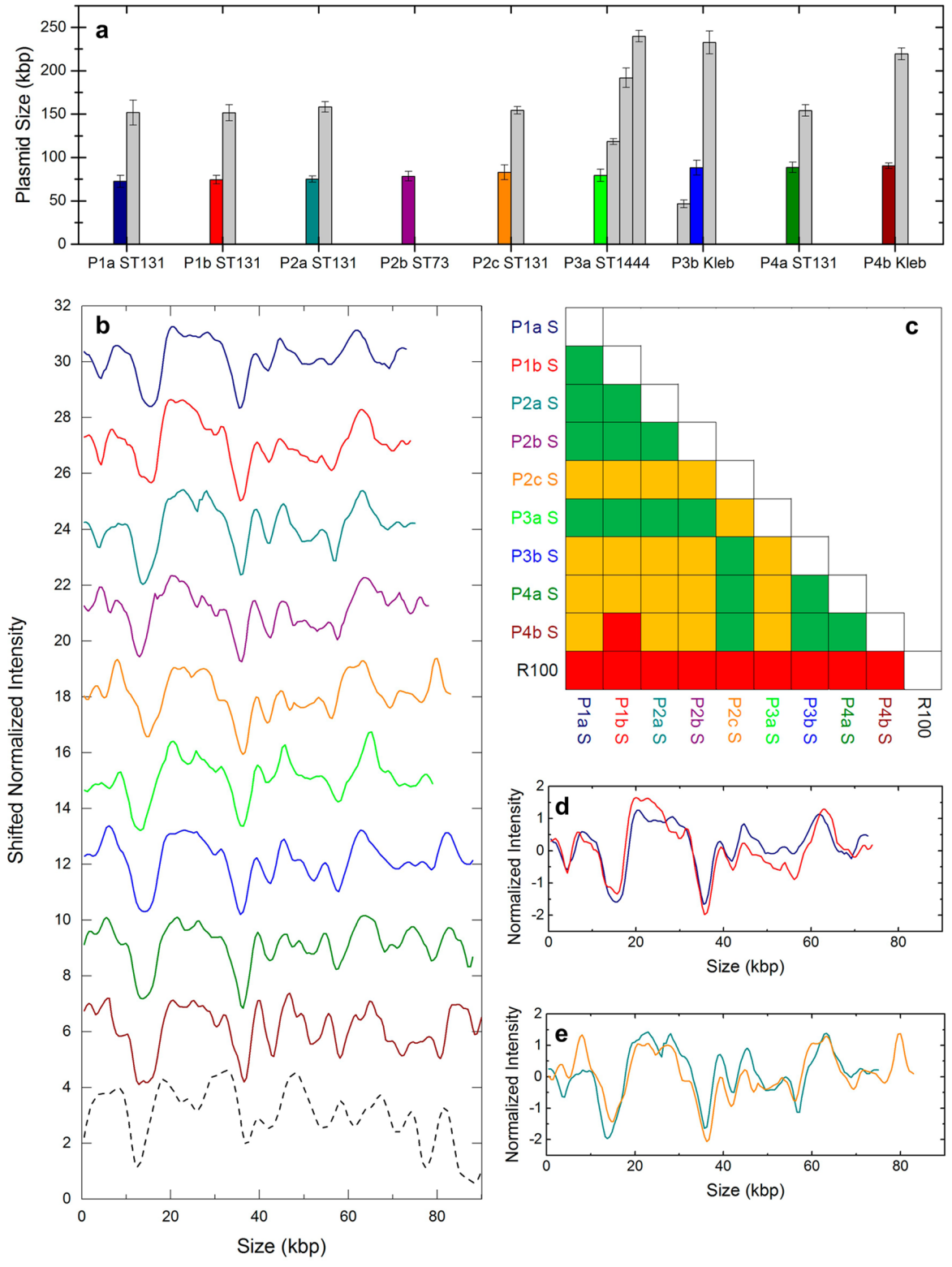


Figure 2. Results from optical DNA mapping of plasmids from the resistance outbreak. (a) Number of plasmids found in each investigated isolate and their corresponding sizes. Each bar represents a detected plasmid. The shared plasmid with a size of 73−90 kbp is represented in nongray bars with color corresponding to their barcodes found in panel b. (b) Consensus barcodes (created from 3 to 10 individual barcodes) of shared plasmids shifted along the *y*-axis and aligned after best possible fit to facilitate visualization. The barcode of plasmid R100[20] (dashed) was included as a control (for additional controls, see Supplementary Tables S1−S4 and Supplementary Figure S4). (c) *p* value matrix showing if the barcode of the shared plasmid (S) in each isolate can be regarded as the same (green), the same but with an insert (yellow), or not the same (red) using a *p* value cutoff <1%. The barcode of R100 was used as a negative control. (d, e) Comparisons of the barocodes of the shared plasmid in isolates P1a (navy) and P1b (red), isolated 5 months apart (d) and in P2a (dark cyan) and P2c (orange) with insert found, isolated 17 months apart (e).

DOI: 10.1021/acsinfecdis.6b00017
ACS Infect. Dis. 2016, 2, 322−328

borne, this plasmid is the underlying cause. In both *K. pneumoniae* isolates, P3b and P4b, as well as in the ST131 *E. coli* isolates, P2c and P4a, the shared plasmid is slightly, but significantly, larger, suggesting that an insertion of 10−15 kb in size has occurred. The inserts all occur within the same region of the plasmid, suggesting that this region contains sequences that allow for insertion of genes (Figure 2b). This possibility of obtaining local sequence information is a strength of the optical DNA mapping assay, compared to, for example, S1/PFGE.

To quantify the similarity of the barcodes, we introduce a *p* value, where we use theoretical barcodes based on all sequenced plasmids in the RefSeq database as reference (see Methods and Supplementary Methods). Because some of the plasmids in the outbreak isolates were suggested to contain an insert, we compared the barcodes in two ways—by stretching the two barcodes to the same length (Q1) and by allowing the smaller barcode to be a part of the larger one (Q2)—and calculated *p* values for both scenarios. If the *p* value is <1% for Q1, the compared plasmids are considered the same (green in Figure 2c). If the *p* value for Q1 is ≥1% but <1% for Q2, they are considered the same but with an insertion (yellow in Figure 2c), and if they do not fulfill either of these two criteria, they are considered not the same (red in Figure 2c). All possible combinations between the five shared plasmids without an insert (from isolates P1a, P1b, P2a, P2b, and P3a), as well as all possible combinations of the four shared plasmids with inserts (from isolates P2c, P3b, P4a, and P4b), had a *p* value well below the threshold of 1% when stretched to the same length (Q1). Furthermore, when plasmids from the two groups were compared, all combinations (except P1b vs P4b, *p* value = 1.36%) obtained a Q2 *p* value <1% (all *p* values and additional controls can be found in Supplementary Tables S1−S4 and Supplementary Figure S4).

The similarity of the barcodes from the shared plasmids strengthens the hypothesis that this plasmid was the underlying cause of the outbreak, propagating the spread of the ESBL-gene. It also strongly indicates that there has occurred a transfer of the shared plasmid between different strains and species, resulting in the polyclonal nature of the outbreak. The possibility that the newborn children had been colonized with multiple isolates carrying the shared plasmid *before* being struck by the outbreak isolates is less likely, considering the extreme rarity of ESBL-producing bacteria in western Sweden at the time.[21] Access to isolates emanating from the same patient, but collected 5 (P1a and P1b) and 17 (P2a and P2c) months apart, respectively, provided the opportunity to conclude that for patient 1, no changes are visible over 5 months (Figure 2c,d), whereas in isolate P2c, the shared plasmid has obtained an extra piece of DNA that was not present in isolate P2a 17 months earlier (Figure 2c,e).

To strengthen our observations from the barcodes, PCR was used to detect the presence of the CTX-M-15 gene after isolating the plasmids from the chromosomal DNA. The results showed that all nine plasmid isolates were CTX-M-15 positive, including isolate P2b, where only one plasmid was detected, supporting our findings that the resistance gene is present on the shared plasmid. Conjugative plasmids require a size of approximately 25 kbp to harbor the necessary genes,[17] and described ESBL-carrying plasmids are generally in the size range of 50−200 kbp.[22] Both of these requirements are in accordance with our findings of a 73−90 kbp plasmid being responsible for the outbreak.

The optical mapping assay has thus allowed us to (i) monitor plasmid content in patients over time, as for the shared plasmid detected in ST131 isolates collected 5 (P1) and 17 months apart (P2); (ii) find the shared plasmid in three different *E. coli* strains and one *K. pneumoniae* strain, indicating plasmid transfer between bacterial strains and species as a potential route of resistance transmission during the outbreak; (iii) find the shared plasmid in all isolates from all patients, suggesting that the outbreak indeed was plasmid-borne; and (iv) find and locate inserts, 10−15 kbp in size, in four of the shared plasmids.

The fact that optical mapping is performed at the single DNA molecule level and from picograms of DNA opens up the possibility to overcome one of the main hurdles in identifying resistant bacteria; the need for cultivation before analysis. For cultivation-free analysis, plasmids can potentially be isolated directly in clinical samples or even in fully integrated lab-on-a-chip-like devices. Without the need for cultivation, significant reduction in diagnostic times would be achieved. This reduction would be advantageous in many clinical situations, not the least for tracking the routes of transmission in an outbreak situation. Furthermore, the method is simple, performed in a single step, and does not require expensive chemicals or complex protocols because it is based on two commercially available molecules. Although the nanofluidic channels used in this particular study are made via E-beam lithography, other groups have demonstrated the possibility to decrease the cost to $3 per chip by casting them in plastic.[23,24] The data analysis is based on an automated software that has been specifically developed for the competitive binding assay. Finally, we stress that Wei et al. recently demonstrated that single DNA molecules can be visualized using a small and cheap microscope mounted on a smartphone.[25] The optical mapping method used here is perfectly suited for this format because the barcodes are based on variations in a strong emission signal, rather than emission from individual fluorophores. The paper by Wei et al. also suggests that the computations performed here are readily transferred to the smartphone format. We therefore propose that the assay presented here can be transferred to point-of-care situations, where it is not possible to use traditional techniques and where a rapid, low-cost solution is preferred.

In conclusion, we have demonstrated a one-step optical DNA mapping assay that can be used to easily and rapidly identify and trace plasmids. The assay works without any previous sequence information and can be used to complement and/or replace existing techniques. In particular, we foresee that our assay can be used as a time-saving first step in identifying samples that later can be fully characterized, for example, phenotypically or with sequencing methods. By measuring the size and creating a fingerprint barcode of the detected plasmids, we could for this particular outbreak clarify previously unsolved, but suspected, routes of transmission and demonstrate that the shared plasmid had moved between strains and species that were transmitted between patients in this nosocomial outbreak. The study clearly demonstrates that in an outbreak situation, possible plasmid migration, and not only transmission of strains, needs to be investigated to elucidate suspected chains of transmission when mobile genetic elements propagating resistance are involved. With the alarming increase of multiresistance mediated by highly mobile plasmids, there will be an upcoming demand of fast and easy methods, such as the assay presented here, to keep track of elements conferring resistance in clinical settings.

DOI: 10.1021/acsinfecdis.6b00017
ACS Infect. Dis. 2016, 2, 322−328

METHODS

Bacterial Isolates and Patients. Seven ESBL-producing *E. coli* and two ESBL-producing *K. pneumoniae* isolates were isolated from four patients (Table 1), aged 1−19 months (median age 3 months). These patients were part of a polyclonal outbreak of ESBL-producing Enterobacteriaceae that occurred in a neonatal postsurgery ward at Sahlgrenska University Hospital, Gothenburg, Sweden, during September−December 2008.[18] Patients three (P3) and four (P4) were colonized with ESBL-producing *K. pneumoniae* simultaneously with ESBL-producing *E. coli*. Two *E. coli* isolates from patients one (P1) and two (P2) were received during the follow-up period 5 and 17 months after the outbreak was detected, respectively.

Identification of Isolates and Phenotypic Detection of ESBL Resistance. All isolates were detected at the Clinical Bacteriology Laboratory, Sahlgrenska University Hospital, and routinely stored at −70 °C with epidemiological data documented. The isolates were identified according to routine practice at the laboratory using conventional biochemical tests and later confirmed by MALDI-TOF for species identification. Antibiotic susceptibility was determined according to the Swedish Reference Group for Antibiotics (SRGA), using the disc diffusion method in practice at the time (http://www.nordicast.org). Cephalosporin-resistant isolates were screened for the ESBL phenotype, using the double-disc diffusion test.[26]

DNA Extraction. DNA from all frozen samples was obtained by plating on blood agar medium and incubation overnight. Two to five bacterial colonies were suspended in 100 μL of Tris−EDTA (TE) buffer, heated to boiling for 15 min at 95 °C, and centrifuged at 18000*g* for 5 min. Cell supernatants, containing the bacterial DNA, were separated from pelleted cell debris and used for the DNA analyses.

Genotypic Detection of ESBL Genes. All isolates that were characterized phenotypically as ESBL-positive were confirmed using a multiplex PCR assay for detecting β-lactamase genes as previously described.[18] This was done both on the total DNA content and after plasmid extraction. Isolates with the CTX-M gene were analyzed for CTX-M phylo-groups, using a real-time TaqMan multiplex PCR assay.[27] Isolates belonging to the CTX-M phylogroup 1 were amplified and sequenced to confirm the presence of CTX-M-15 as previously described.[28] The raw trace files were edited, and contig sequences were generated using the BioNumerics version 6.6 software (Applied Maths NV, Sint-Martens-Latem, Belgium).

Pulsed-Field Gel Electrophoresis (PFGE). All isolates were subtyped by PFGE profiling, following extraction of genomic DNA and digestion with *Xba*I restriction enzyme[29] as described previously.[30] DNA band patterns were analyzed, using the BioNumerics software version 6.6 with the Dice coefficient for calculating pairwise similarities and the UPGMA algorithm for constructing dendrograms of estimated relatedness. Strains were designated to be indistinguishable if their electrophoresis profile similarities were ≥90%.[31]

Multilocus Sequence Typing (MLST). MLST was performed, according to the method of the *E. coli* MLST database Web site.[32] Seven housekeeping genes, *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*, were targeted for PCR amplification and sequencing. The sequences were submitted to the MLST database for *E. coli*, and the respective sequence types were determined (http://mlst.ucc.ie/mlst/dbs/Ecoli). MLST of the *K. pneumonia* isolates were performed as described by Diancourt et al.,[33] detecting sequences of the seven conserved housekeeping genes *rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB*, and *tonB*. PCRs were carried out using the primers and conditions detailed on the Institute Pasteur MLST database (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html).

Plasmid Extraction. Isolates were grown on blood agar plates at 37 °C overnight. Plasmid DNA was prepared from overnight culture with the Qiagen Mini kit for *E. coli* isolates and Midi kit for *K. pneumoniae* (Qiagen, Germany) according to the manufacturer's description for low-copy plasmids. Eluted DNA was precipitated with isopropanol, washed with 70% ethanol, and resuspended in TE buffer, pH 8.0.

Sample Preparation. To obtain the size and sequence-specific patterns of the plasmids during the nanofluidic experiments, DNA was stained with YOYO-1 (Invitrogen) in a molar ratio of 1:5 of the total number of bp and with netropsin (Sigma-Aldrich) in a molar ratio of 150:1 with respect to YOYO-1. In addition to the DNA from the plasmid extraction, λ-DNA (48502 bp, New England Biolabs) was included in each experiment as an internal standard for size determination. First, samples were mixed and left during 20 min in high ionic strength (5× TBE (Tris−borate−EDTA, Medicago, diluted with mQ from 10× tablets)) to facilitate rapid equilibration.[10] Following this, the samples were diluted to approximately 0.05× TBE using MQ, resulting in a final DNA concentration of 0.1 μM (bp) (=52 pg/μL) plasmid DNA and 0.1 μM (bp) λ-DNA. To suppress excessive photonicking, β-mercaptoethanol (BME, Sigma-Aldrich) was added in 2% (v/v).

Experimental Procedure. Nanofluidic channels were fabricated in fused silica as described elsewhere,[14] using conventional techniques, with dimensions of 100 × 150 nm^2 and a length of 500 μm. To acces the nanochannels, four inlets were fabricated in the chip, connected two and two by microchannels, which in turn are spanned by nanochannels. To achieve uniform conditions to the sample solution, the channels were prewetted with 0.05× TBE together with 2% (v/v) BME before the sample was loaded. Pressure-driven flow of nitrogen gas was used to force the DNA inside the nanochannels. Because intact, circular plasmids can be detected by eye in the microchannels, fragments of plasmids or remaining chromosomal DNA were discarded, and only circular plasmids were imaged, ensuring that the entire DNA sequence of the plasmid was analyzed. To obtain the intensity pattern across the extension (the barcode), plasmids were linearized by spontaneous photonicking caused by intense irradiation with light. Imaging was performed using an inverted microscope (Zeiss AxioObserver.Z1) with a 100× oil immersion objective (Zeiss, NA = 1.46). To measure the size and obtain the barcodes, a series of 200 images with an exposure time of 100 ms was obtained using an EMCCD camera (Photometrix Evolve).

Data Analysis. The size of the plasmid was obtained from its circular form inside the nanochannel.[16] By using a conversion factor of 1.8[15] as well as measuring the average extension of the linear reference λ-DNA, the number of base pairs in the plasmid could be approximated. Barcodes were obtained from the linearized form of the plasmid by aligning and averaging up to 200 frames, using the application-specific Matlab software.[10] All barcodes from plasmids of similar size within an isolate were aligned and compared (using the Pearson correlation coefficient) to evaluate that they all displayed

DOI: 10.1021/acsinfecdis.6b00017
ACS Infect. Dis. 2016, 2, 322−328

similar features. The individual barcodes of the same size were then merged to create consensus barcodes, which later were used to compare the barcodes of plasmids from different isolates (see Supplementary Methods for details).

The similarity of two barcodes is quantified by a p value, which in turn is based on a best Pearson correlation coefficient.[10] The p value utilizes randomized barcodes (based on information on all sequenced plasmids in the RefSeq database) as reference and turns a particular best correlation coefficient into a quantity insensitive to barcode length (see Supplementary Methods for details). For answering question (Q1) we stretch (if the barcodes differ <20% in length) the two barcodes to the same length and calculate the correlation coefficient by "sliding" one barcode across the other (and possibly flipping). For answering question (Q2) we need to identify a partial match of the two barcodes. To that end, besides the sliding and flipping, the shorter barcode is circularly shifted to all possible shifts before the correlation coefficient is applied (see Supplementary Methods). We also allow a small degree of stretching of the barcodes. By construction, the p value is in the range 0−1. A small p value indicates a significant similarity between two plasmid barcodes, and we deem two barcodes significantly similar (with or without inserts) if the p value score is smaller than a set threshold (here =0.01).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsinfecdis.6b00017.

Detailed description of the data analysis; tables and figures showing all determined p values, including extra controls (PDF)

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Author Contributions

N.K., C.Å., and F.W. conceived the idea of the project. V.M. performed and planned the nanofluidics experiments and analyzed all of the data supervised by L.K.N. and F.W. C.P., P.C.T.P., S.Q., and T.A. developed the analysis methods with input from V.M. and F.W. C.P., P.C.T.P., and S.Q. wrote the analysis software supervised by T.A. N.K. prepared the plasmid samples and the genetics to characterize the strains and their ESBL-genes. J.F. fabricated the nanofluidic devices. V.M. and F.W. wrote the manuscript with help from N.K., L.K.N., T.A., and C.Å. C.P., T.A., V.M., and F.W. wrote the Supporting Information, C.P. and T.A. the theory part, and V.M. and F.W. the experimental part. All authors discussed the results and commented on the manuscript. F.W. coordinated the project.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This project is funded by grants to F.W. from the Chalmers Areas of Advance in Nanoscience and Nanotechnology, the Adlerbertska Foundation, the EU Horizon 2020 program (Grant 634890, BeyondSeq), the EuroNanoMed2 project NanoDiaBac, and Torsten Söderbergs Stiftelse. T.A. acknowledges funding from the Swedish Research Council (Grant 2014-4305). N.K. and C.Å. acknowledge funding from Västra Götalandsregionen and Sahlgrenska University hospital. We acknowledge Professor Linus Sandegren for constructive comments on the manuscript.

■ REFERENCES

(1) Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K., Wertheim, H. F., Sumpradit, N., Vlieghe, E., Hara, G. L., Gould, I. M., Goossens, H., Greko, C., So, A. D., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A. Q., Qamar, F. N., Mir, F., Kariuki, S., Bhutta, Z. A., Coates, A., Bergstrom, R., Wright, G. D., Brown, E. D., and Cars, O. (2013) Antibiotic resistance − the need for global solutions. *Lancet Infect. Dis. 13*, 1057−1098.

(2) Svara, F., and Rankin, D. J. (2011) The evolution of plasmid-carried antibiotic resistance. *BMC Evol. Biol. 11*, 130.

(3) Carattoli, A. (2013) Plasmids and the spread of resistance. *Int. J. Med. Microbiol. 303*, 298−304.

(4) World Health Organization. (2015) *Worldwide Country Situation Analysis: Response to Antimicrobial Resistance*.

(5) Mathers, A. J., Peirano, G., and Pitout, J. D. (2015) The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. *Clin. Microbiol. Rev. 28*, 565−591.

(6) Barton, B. M., Harding, G. P., and Zuccarelli, A. J. (1995) A general method for detecting and sizing large plasmids. *Anal. Biochem. 226*, 235−240.

(7) Carattoli, A. (2011) Plasmids in Gram negatives: molecular typing of resistance plasmids. *Int. J. Med. Microbiol. 301*, 654−658.

(8) Brolund, A., and Sandegren, L. (2016) Characterization of ESBL disseminating plasmids. *Infect. Dis. (London, England) 48*, 18−25.

(9) Heather, J. M., and Chain, B. (2016) The sequence of sequencers: the history of sequencing DNA. *Genomics 107*, 1−8.

(10) Nilsson, A. N., Emilsson, G., Nyberg, L. K., Noble, C., Stadler, L. S., Fritzsche, J., Moore, E. R., Tegenfeldt, J. O., Ambjornsson, T., and Westerlund, F. (2014) Competitive binding-based optical DNA mapping for fast identification of bacteria − multi-ligand transfer matrix theory and experimental applications on *Escherichia coli*. *Nucleic Acids Res. 42*, e118.

(11) Nyberg, L. K., Persson, F., Berg, J., Bergstrom, J., Fransson, E., Olsson, L., Persson, M., Stalnacke, A., Wigenius, J., Tegenfeldt, J. O., and Westerlund, F. (2012) A single-step competitive binding assay for mapping of single DNA molecules. *Biochem. Biophys. Res. Commun. 417*, 404−408.

(12) Neely, R. K., Deen, J., and Hofkens, J. (2011) Optical mapping of DNA: single-molecule-based methods for mapping genomes. *Biopolymers 95*, 298−311.

(13) Levy, S. L., and Craighead, H. G. (2010) DNA manipulation, sorting, and mapping in nanofluidic systems. *Chem. Soc. Rev. 39*, 1133−1152.

(14) Persson, F., and Tegenfeldt, J. O. (2010) DNA in nanochannels − directly visualizing genomic information. *Chem. Soc. Rev. 39*, 985−999.

(15) Alizadehheidari, M., Werner, E., Noble, C., Reiter-Schad, M., Nyberg, L. K., Fritzsche, J., Mehlig, B., Tegenfeldt, J. O., Ambjörnsson, T., Persson, F., and Westerlund, F. (2015) Nanoconfined circular and linear DNA: equilibrium conformations and unfolding kinetics. *Macromolecules 48*, 871−878.

(16) Frykholm, K., Nyberg, L. K., Lagerstedt, E., Noble, C., Fritzsche, J., Karami, N., Ambjornsson, T., Sandegren, L., and Westerlund, F. (2015) Fast size-determination of intact bacterial plasmids using nanofluidic channels. *Lab Chip 15*, 2739−2743.

(17) Smillie, C., Garcillan-Barcia, M. P., Francia, M. V., Rocha, E. P., and de la Cruz, F. (2010) Mobility of plasmids. *Microbiol. Mol. Biol. Rev.: MMBR 74*, 434−452.

(18) Karami, N., Helldal, L., Welinder-Olsson, C., Åhrén, C., and Moore, E. R. B. (2013) Sub-typing of extended-spectrum-β-lactamase-producing isolates from a nosocomial outbreak: application of a 10-loci generic *Escherichia coli* multi-locus variable number tandem repeat analysis. *PLoS One 8*, e83030.

DOI: 10.1021/acsinfecdis.6b00017
ACS Infect. Dis. 2016, 2, 322−328

(19) Rogers, B. A., Sidjabat, H. E., and Paterson, D. L. (2011) *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. *J. Antimicrob. Chemother. 66*, 1−14.
(20) Silver, L., Chandler, M., de la Tour, E. B., and Caro, L. (1977) Origin and direction of replication of the drug resistance plasmid R100.1 and of a resistance transfer factor derivative in synchronized cultures. *J. Bacteriol. 131*, 929−942.
(21) Helldal, L., Karami, N., Floren, K., Welinder-Olsson, C., Moore, E. R., and Ahren, C. (2013) Shift of CTX-M genotypes has determined the increased prevalence of extended-spectrum beta-lactamase-producing *Escherichia coli* in south-western Sweden. *Clin. Microbiol. Infect. 19*, E87−90.
(22) Brolund, A., and Sandegren, L. (2016) Characterization of ESBL disseminating plasmids. *Infect. Dis. (London, England) 48*, 18−25.
(23) Utko, P., Persson, F., Kristensen, A., and Larsen, N. B. (2011) Injection molded nanofluidic chips: fabrication method and functional tests using single-molecule DNA experiments. *Lab Chip 11*, 303−308.
(24) Peter Friis, Ø., Joanna, L.-J., Jonas Nyvold, P., Niels, T., Anders, K., Henrik, F., Asli, S., Rodolphe, M., and Rafael, T. (2015) Optical mapping of single-molecule human DNA in disposable, mass-produced all-polymer devices. *J. Micromech. Microeng. 25*, 105002.
(25) Wei, Q., Luo, W., Chiang, S., Kappel, T., Mejia, C., Tseng, D., Chan, R. Y., Yan, E., Qi, H., Shabbir, F., Ozkan, H., Feng, S., and Ozcan, A. (2014) Imaging and sizing of single DNA molecules on a mobile phone. *ACS Nano 8*, 12725−12733.
(26) Legrand, P., Fournier, G., Bure, A., Jarlier, V., Nicolas, M. H., Decre, D., Duval, J., and Philippon, A. (1989) Detection of extended broad-spectrum beta-lactamases in Enterobacteriaceae in four French hospitals. *Eur. J. Clin. Microbiol. Infect. Dis. 8*, 527−529.
(27) Birkett, C. I., Ludlam, H. A., Woodford, N., Brown, D. F., Brown, N. M., Roberts, M. T., Milner, N., and Curran, M. D. (2007) Real-time TaqMan PCR for rapid detection and typing of genes encoding CTX-M extended-spectrum beta-lactamases. *J. Med. Microbiol. 56*, 52−55.
(28) Hussein, A. I., Ahmed, A. M., Sato, M., and Shimamoto, T. (2009) Characterization of integrons and antimicrobial resistance genes in clinical isolates of Gram-negative bacteria from Palestinian hospitals. *Microbiol. Immunol. 53*, 595−602.
(29) Gautom, R. K. (1997) Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other Gram-negative organisms in 1 day. *J. Clin. Microbiol. 35*, 2977−2980.
(30) Welinder-Olsson, C., Kjellin, E., Badenfors, M., and Kaijser, B. (2000) Improved microbiological techniques using the polymerase chain reaction and pulsed-field gel electrophoresis for diagnosis and follow-up of enterohaemorrhagic *Escherichia coli* infection. *Eur. J. Clin. Microbiol. Infect. Dis. 19*, 843−851.
(31) van Belkum, A., Tassios, P. T., Dijkshoorn, L., Haeggman, S., Cookson, B., Fry, N. K., Fussing, V., Green, J., Feil, E., Gerner-Smidt, P., Brisse, S., and Struelens, M. (2007) Guidelines for the validation and application of typing methods for use in bacterial epidemiology. *Clin. Microbiol. Infect. 13* (Suppl. 3), 1−46.
(32) Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L. H., Karch, H., Reeves, P. R., Maiden, M. C., Ochman, H., and Achtman, M. (2006) Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol. Microbiol. 60*, 1136−1151.
(33) Diancourt, L., Passet, V., Verhoef, J., Grimont, P. A., and Brisse, S. (2005) Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J. Clin. Microbiol. 43*, 4178−4182.

DOI: 10.1021/acsinfecdis.6b00017
ACS Infect. Dis. 2016, 2, 322−328