

1 **Supplemental Materials**

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3 **Nanopore sequencing.** Long-read genomic sequencing was performed using the third
4 generation Oxford Nanopore MinION Mk1b (Isolates 1-30) and GridION X5 (Isolates 31-40;
5 Oxford, England) sequencing instruments. Genomic DNA was extracted from pure cultures
6 using the DNeasy PowerBiofilm Kit (Qiagen, Hilden, Germany). Each Nanopore sequencing
7 library was prepared using 5 µg of DNA with the 1D ligation kit (SQK-LSK108, Oxford Nanopore
8 Technologies) using R9.4 flowcells (FLO-MIN106). A single isolate was run per flowcell.
9 MinKNOW software was used to collect sequencing data. Three separate analysis pipelines
10 were performed: (1) a real-time Nanopore analysis approach, (2) an assembly-based Nanopore
11 approach, and (3) an Illumina Pilon-corrected approach. The run statistics and the whole
12 genome assembly parameters are summarized in the Supplemental File Run & Assembly
13 Statistics. Sequencing data for this study were deposited in the Sequencing Read Archive
14 (SRA; BioProject number: PRJNA496461), and genome assemblies submitted to NCBI.

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16 **Nanopore real-time approach.** For the real-time analysis approach, the Oxford Nanopore
17 Technology “What’s In My Pot” (WIMP)(1) and Metrichor’s Antimicrobial Resistance Mapping
18 Application (ARMA) (2) real-time analysis tools were run in parallel to both sequencing and
19 base-calling. WIMP uses a customized pipeline applying the Centrifuge metagenomic classifier
20 to the sequencing reads. ARMA aligns sequencing reads to the Comprehensive Antimicrobial
21 Database (CARD)(3), identifying antimicrobial resistance (AMR) gene hits.

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23 **Nanopore assembly-based approach.** For the assembly-based approach, Albacore v2.1.3
24 was used to base-call. Raw data were corrected, trimmed, and assembled using Canu v1.6(4)
25 using default parameters. Genome size was assumed to be 5.3Mb. For isolates that did not
26 assemble under default parameters, either read quality restrictions were relaxed or minimum

27 overlap requirements were shortened as suggested by the software documentation. Assembled
28 contigs were screened for resistance genes using Abricate
29 (<https://github.com/tseemann/abricate>), a tool that uses alignment to search for resistance gene
30 sequences from several database including ResFinder, CARD, ARG-ANNOT, and the NCMI
31 AMR Reference Gene Database. ResFinder results were evaluated in this study(5). In a
32 separate experiment, we were able to build high quality genomes from nanopore electrical data
33 in under 6 hours using a machine with 36 cores and 72GB RAM. Thirteen random blocks of
34 4000 reads were sampled and taken through basecalling, assembly and polishing.
35 Conservatively, it takes at most an hour to reach 52,000 reads on a typical run.

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37 **Illumina sequencing.** WGS was also conducted using Illumina MiSeq short-read sequencing to
38 increase assembly accuracy (Illumina, San Diego, California). A drawback with Illumina
39 sequencing is the turn-around time as sequencing alone requires between 19-24 hours for 300
40 cycles. As the ultimate goal would be to use Nanopore sequencing alone for both resistance
41 gene and chromosomal mutation identification, in the current analysis, the Pilon-corrected
42 assemblies were not meant to supplant or supplement Nanopore sequencing but rather to serve
43 as a reference standard to determine the accuracy of Nanopore sequencing results.

44 Approximately 100-500 ng of gDNA was used to prepare sequencing libraries using the Nextera
45 Flex Kit. The each Illumina library was then sequenced using both v2 2x150 and v3 2x75
46 reagents on an Illumina Miseq. These reads were used to correct the more error prone
47 Nanopore assembly with the Pilon v1.22 software package in conjunction with the short-read
48 aligner Bowtie2 v2.2.6.

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50 **Analysis for Detection of Chromosomal Mutations.** Both Nanopolished Nanopore
51 assemblies and hybrid Illumina-corrected Nanopore assemblies were further evaluated for
52 chromosomal mutations presumed to confer resistance. These included truncations in the outer

53 membrane proteins OmpK35 and OmpK36 as a consequence of premature stop codons
54 resulting in carbapenem resistance and *gyrA* (DNA gyrase; nucleotide positions 247-249 & 259-
55 261) and *parC* (topoisomerase IV; nucleotide positions 238-240 & 250-252) point mutations that
56 encoded amino acid changes resulting in fluoroquinolone resistance. Additionally, mutations in
57 *mgrB*, the negative regulator of the two-component regulatory system that renders polymyxins
58 ineffective were evaluated.

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60 Coding sequences encompassing chromosomal mutations of interest were extracted from the
61 genome of the *K. pneumoniae* ATCC 13883 strain and used to align with genomic assemblies
62 using minimap2 (6). Code written in C++ was developed for parsing alignment data and
63 mutations, evaluating their impact on amino acid translation based on resulting codon
64 changes(7). Substitutions causing synonymous codons were reported as such, and frameshift
65 mutations were followed downstream to report premature stop codons. Additionally, the
66 program analyzed DNA sequences surrounding mutation sites looking for homopolymers or
67 methylation motifs specific for *K. pneumoniae* as indicators of potential sequencing issues
68 rather than mutations in the genome.

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70 **Predicted correlations between resistance genes/mutations and AST results.** Predictions
71 of resistance were performed without reference to phenotypic data. The correlation of resistance
72 genes and sequence variants with anticipated AST results for the evaluated antibiotics was
73 determined based on reference gene databases and the published literature(8-26). For
74 associations for which ambiguity existed, the following rules were established *a priori*:

- 75 • SHV 2be extended-spectrum β -lactamase enzymes were assumed to inactivate
76 ceftriaxone and cefepime if they contained G238S or E240K mutations(19, 27)
- 77 • The relative contributions of porins OmpK35 and/or OmpK36 truncations to β -lactam
78 resistance have not been well established(13, 28-32). There appears to be consensus

79 that when premature stop codons are present for both, in conjunction with ESBLs or
80 AmpC cephalosporinases, carbapenem resistance is likely.

81 • Aminoglycoside modifying enzymes (AMEs)- including aminoglycoside N-
82 acetyltransferases [AACs], aminoglycoside O-nucleotidyltransferases [ANTs], or
83 aminoglycoside O-phosphotransferases [APHs] are anticipated to confer resistance to
84 gentamicin and tobramycin (17). Correlations between the number of different enzymes
85 produced and the association with aminoglycoside resistance are not well defined. After
86 exploratory analysis (Table 1), we predicted that when four or more AMEs were present,
87 gentamicin and tobramycin resistance was likely.

88 • The AME *aac(6')Ib-cr* was also evaluated for its contribution to ciprofloxacin
89 resistance(17).

90 • Ribosomal RNA methyltransferases (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, or *rmtE*) are
91 expected to confer resistance to amikacin, gentamicin, and tobramycin (18).

92 • Plasmid-encoded quinolone resistance genes *qnrB*, *qnrS*, *aac(6')Ib-cr* and *oqxAB*
93 (chromosomally encoded) were predicted to cause low-level fluoroquinolone resistance,
94 but not frank resistance (16, 33, 34).

95 Single *gyrA* or *parC* mutations were not predicted to cause fluoroquinolone resistance but two-
96 step *gyrA* mutations or the presence of both *gyrA* and *parC* mutations translating to amino acid
97 changes were expected to result in fluoroquinolone resistance (33, 34).

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104 **References**

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30	<i>bla_{SHV}</i> [45]; <i>bla_{TEM}</i> [38]; <i>bla_{OXA}</i> [6]; <i>bla_{KPC}</i> [9]; <i>aac</i> [2]; <i>aadA</i> [5];	<i>oqxA</i> ; <i>oqxB</i> ; <i>qnrS1</i> ; two-step <i>gyrA</i> mutation; <i>parC</i> mutation; <i>phoP</i> mutation; OmpK36 truncation; <i>aph(3')-Ia</i> ; <i>sul1</i> ; <i>sul3</i> ; <i>dfrA12</i>	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Green	Green	Green	Red	Green	Red	Green	Green	Green	Red	
31	<i>bla_{CTX-M}</i> [19]; <i>bla_{TEM}</i> [22]; <i>bla_{SHV}</i> [36]; <i>qnr</i> [11]	<i>oqxA</i> ; <i>oqxB</i> ; OmpK36 truncation; <i>aph(6)-Id</i> ; <i>aph(3'')-Ib</i> ; <i>sul2</i> ; <i>dfrA14</i>	Green	Green	Red	Red	Red	Red	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Red
32	<i>bla_{OXA}</i> [3]; <i>bla_{SHV}</i> [60]; <i>bla_{CTX-M}</i> [23]; <i>bla_{TEM}</i> [20]; <i>qnr</i> [12]; <i>aac</i> [2]	<i>oqxA</i> ; <i>oqxB</i> ; <i>tet(A)</i> ; <i>aph(6)-Id</i> ; <i>aph(3'')-Ib</i> ; <i>sul2</i> ; <i>dfrA14</i> ; OmpK36 truncation	Green	Red	Red	Red	Red	Red	Green	Green	Green	Green	Green	Green	Red	Green	Red	Green	Green	Red	Green	Green	Red
33	<i>bla_{SHV}</i> [25](<i>bla_{LEN}</i> [25]); <i>oqxA</i> [1]; <i>oqxB</i> [1]; <i>sul2</i> [1]; <i>dfrA14</i> [1]	OmpK36 truncation; <i>tet(D)</i>	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Red	Red	Red
34	<i>bla_{SHV}</i> [23]; <i>oqxA</i> [1]; <i>oqxB</i> [1]	OmpK36 truncation	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
35	<i>bla_{SHV}</i> [24]; <i>oqxA</i> [1]; <i>oqxB</i> [1]	OmpK36 truncation	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
36	<i>bla_{SHV}</i> [22]; <i>oqxA</i> [1]; <i>oqxB</i> [1]	<i>ant(2'')-Ia</i> ; OmpK36 truncation	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
37	<i>bla_{TEM}</i> [41]; <i>bla_{KPC}</i> [8]; <i>bla_{SHV}</i> [85]; <i>ant</i> [1]	<i>qnrS1</i> ; OmpK36 truncation	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Green	Green	Green	Red	Green	Red	Green	Green	Green	Green	
38	<i>bla_{OKP}</i> [9]; <i>bla_{TEM}</i> [12]; <i>bla_{SHV}</i> [1]; <i>oqxA</i> ; <i>oqxB</i> ; <i>sul</i> ; <i>dfr</i>	<i>tet(D)</i> ; OmpK36 truncation	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Red	Red	Red

Supplemental Table 2: Anticipated antibiotic susceptibility results for 40 *Klebsiella pneumoniae* isolates based on resistance determinants detected by Nanopore sequencing technology with assembly; first column for each antibiotic indicates presumed activity and second column for each antibiotic indicates *in vitro* activity based on broth microdilution results with green indicating susceptible and red indicating intermediate or resistant; number on bottom represents percent agreement between presumed and *in vitro* activity

#	Resistance determinants identified with Nanopore sequencing with assembly	Missing resistance determinants based on pilon-corrected Illumina data	Piperacillin-tazobactam	Ceftriaxone	Cefepime	Ertapenem	Meropenem	Amikacin	Gentamicin	Ciprofloxacin	Colistin	Doxycycline	TMP-SMX
1	<i>bla</i> _{SHV-1} ; <i>bla</i> _{CTX-M-15} ; <i>bla</i> _{OXA-1} ; <i>bla</i> _{KPC-3} ; OmpK36 truncation; <i>qnrS1</i> ; <i>oqxA</i> ; <i>oqxB</i> ; two-step <i>gyrA</i> mutations; <i>aac(6')-Ib-cr</i> ; <i>aadA2</i> ; <i>sul1</i> ; <i>dfrA12</i>	None	Red	Red	Red	Red	Red	Green	Green	Red	Green	Red	Red
2	<i>bla</i> _{SHV-11} ; <i>bla</i> _{CTX-M-15} ; <i>bla</i> _{OXA-1} ; <i>bla</i> _{TEM-1} ; <i>bla</i> _{KPC-2} ; OmpK35 and OmpK36 truncation; <i>qnrB1</i> ; <i>oqxA</i> ; <i>oqxB</i> ; one-step <i>gyrA</i> mutation; <i>parC</i> mutation; <i>tet(B)</i> ; <i>aadA2</i> ; <i>aph(3')-Ia</i> ; <i>aac(3)-IIId</i> ; <i>aac(6')-Ib-cr</i> ; <i>aadA2</i> ; <i>aac(3)-IIa</i> ; <i>aph(6'')-Id</i> ; <i>aaA1</i> ; <i>aac(6')-Ib</i> ; <i>sul1</i> ; <i>sul2</i> ; <i>dfrA12</i> ; <i>dfrA30</i>	None	Red	Red	Red	Red	Red	Green	Red	Red	Green	Red	Red
3	<i>bla</i> _{SHV-11} ; <i>oqxA</i> ; <i>oqxB</i> ; OmpK36 truncation	None	Green	Red	Green	Green	Red	Green	Green	Green	Green	Red	Green
4	<i>bla</i> _{SHV-11} ; <i>bla</i> _{TEM-1} ; <i>bla</i> _{KPC-3} ; OmpK36 truncation; <i>oqxA</i> ; <i>oqxB</i> ; one-step <i>gyrA</i>	None	Red	Red	Red	Red	Red	Green	Green	Red	Green	Green	Green

Supplemental Table 3: Anticipated antibiotic susceptibility results for 40 *Klebsiella pneumoniae* isolates based on resistance determinants detected by Nanopore sequencing technology with assembly and Pilon-corrected Illumina data; first column for each antibiotic indicates presumed activity and second column for each antibiotic indicates *in vitro* activity based on broth microdilution results with green indicating susceptible and red indicating non-susceptible; number on bottom represents percent agreement between presumed and *in vitro* activity

#	Resistance mechanisms identified	Piperacillin-tazobactam	Ceftriaxone	Cefepime	Ertapenem	Meropenem	Amikacin	Gentamicin	Ciprofloxacin	Colistin	Doxycycline	TMP-SMX
1	<i>bla</i> _{SHV-1} ; <i>bla</i> _{CTX-M-15} ; <i>bla</i> _{OXA-1} ; <i>bla</i> _{KPC-3} ; OmpK36 truncation; <i>qnrS1</i> ; <i>oqxA</i> ; <i>oqxB</i> ; two-step <i>gyrA</i> mutations; <i>aac(6')-Ib-cr</i> ; <i>aadA2</i> ; <i>sul1</i> ; <i>dfrA12</i>	Red	Red	Red	Red	Red	Green	Green	Red	Red	Green	Red
2	<i>bla</i> _{SHV-11} ; <i>bla</i> _{CTX-M-15} ; <i>bla</i> _{OXA-1} ; <i>bla</i> _{TEM-1} ; <i>bla</i> _{KPC-2} ; OmpK35 and OmpK36 truncation; <i>qnrB1</i> ; <i>oqxA</i> ; <i>oqxB</i> ; one-step <i>gyrA</i> mutation; <i>parC</i> mutation; <i>tet(B)</i> ; <i>aadA2</i> ; <i>aph(3')-Ia</i> ; <i>aac(3)-IIa</i> ; <i>aac(6')-Ib-cr</i> ; <i>aadA2</i> ; <i>aac(3)-IIa</i> ; <i>aph(6'')-Id</i> ; <i>aaA1</i> ; <i>aac(6')-Ib</i> ; <i>sul1</i> ; <i>sul2</i> ; <i>dfrA12</i> ; <i>dfrA30</i>	Red	Red	Red	Red	Red	Green	Red	Red	Red	Green	Red
3	<i>bla</i> _{SHV-11} ; <i>oqxA</i> ; <i>oqxB</i> ; OmpK36 truncation	Green	Red	Green	Red	Green	Green	Green	Green	Green	Red	Green
4	<i>bla</i> _{SHV-11} ; <i>bla</i> _{TEM-1} ; <i>bla</i> _{KPC-3} ; OmpK36 truncation; <i>oqxA</i> ; <i>oqxB</i> ; one-step <i>gyrA</i> mutation; <i>parC</i> mutation; <i>aadA2</i> ; <i>sul1</i>	Red	Red	Red	Red	Red	Green	Green	Red	Red	Green	Green
5	<i>bla</i> _{SHV-11} ; OmpK36 truncation; <i>oqxA</i> ; <i>oqxB</i>	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
6	<i>bla</i> _{SHV-11} ; <i>bla</i> _{TEM-1} ; <i>bla</i> _{OXA-9} ; <i>bla</i> _{KPC-3} ; <i>aac(6')-Ib-cr</i> ; <i>oqxA</i> ; <i>oqxB</i> ; two-step <i>gyrA</i> mutations; <i>parC</i> mutation; OmpK35 and OmpK36 truncation; <i>aph(3'')-Ib</i> ; <i>aph(6)-Id</i> ; <i>aadA1</i> ; <i>aac(6)-Ib</i> ; <i>sul2</i> ; <i>dfrA14</i>	Red	Red	Red	Red	Red	Green	Green	Red	Red	Green	Red
7	<i>bla</i> _{OXA-2} ; <i>bla</i> _{SHV-11} ; <i>bla</i> _{SHV-30} ; <i>bla</i> _{SHV-12} ; <i>oqxA</i> ; <i>oqxB</i> ; <i>aac(6')-Ib-cr</i> ; two-step <i>gyrA</i> mutations; <i>parC</i> mutation; OmpK35 and OmpK36 truncation; <i>aadA2</i> ; <i>aacA4</i> ; <i>sul1</i> ; <i>dfrA12</i> ; <i>fosA</i>	Red	Red	Red	Red	Red	Green	Green	Red	Red	Green	Red
8	<i>bla</i> _{OXA-1} ; <i>bla</i> _{OXA-47} ; <i>bla</i> _{TEM-1} ; <i>bla</i> _{CTX-M-15} ; <i>bla</i> _{SHV-28} ; OmpK36 truncation; <i>aac(6')</i>	Green	Red	Red	Red	Green	Red	Red	Red	Red	Red	Red

Supplemental Table 4: Average time to detection of resistance genes based on the number of reads detected in minutes

Gene ¹	One copy (minutes)	Ten copies (minutes)	Forty copies (minutes)	Fifty copies (minutes)	One hundred copies (minutes)
β-lactam genes					
Non-ESBL TEM enzymes	0.39 minutes (IQR 0.14-2)	13 minutes (IQR 8-16)	66 minutes (IQR 54-74)	78 minutes (IQR 71-99)	155 minutes (IQR 136-174)
Non-ESBL OXA enzymes	0.91 minutes (IQR 0.6-4.8)	20 minutes (IQR 14-22)	75 minutes (IQR 55-83)	92 minutes (IQR 66-99)	163 minutes (IQR 125-177)
Non-ESBL SHV enzymes	0.6 minutes (IQR 0.1-1.1)	13 minutes (IQR 9-17)	57 minutes (IQR 48-69)	73 minutes (IQR 60-87)	146 minutes (IQR 129-170)
ESBL SHV enzymes	1.1 minutes (IQR 0.03-1.9)	17 copies (IQR 12-17)	57 minutes (IQR 52-63)	74 minutes (IQR 73-75)	125 minutes (IQR 106-139)
ESBL CTX-M-type enzymes	0.5 minutes (IQR 0.14-1.4)	11 minutes (IQR 10-14)	50 minutes (IQR 40-66)	64 minutes (IQR 46-86)	138 minutes (IQR 102-164)
ESBL OXA enzymes	0.58 minutes (IQR 0.1-1.3)	16 minutes (IQR 0.1-0.2)	61 minutes (IQR 55-71)	75 minutes (IQR 72-90)	165 minutes (IQR 148-184)
KPC carbapenemases	0.33 minutes (IQR 0.1-1.1)	12 minutes (IQR 7-14)	57 minutes (IQR 49-71)	74 minutes (IQR 66-90)	150 minutes (IQR 131-169)
NDM carbapenemases	0.7 minutes (IQR 0.01-0.9)	14 minutes (IQR 3-26)	50 minutes (IQR 23-116)	64 minutes (IQR 35-173)	153 minutes (IQR 85-489)
Aminoglycoside inactivating enzymes					
Aminoglycoside modifying enzymes	1.0 minute (IQR 0.3-4)	15 minutes (IQR 9-24)	66 minutes (IQR 50-80)	82 minutes (IQR 61-99)	151 minutes (IQR 130-172)
Ribosomal RNA methyltransferase mutations	0.6 minutes (IQR 0.3-0.9)	12 minutes (IQR 8-15)	55 minutes (IQR 41-69)	70 minutes (IQR 58-81)	132 minutes (IQR 116-148)
Tetracycline resistance genes					
<i>tet(A), tet(B), tet(D), tet(G)</i>	0.9 minutes (IQR 0.4-1.8)	11 minutes (IQR 7-18)	59 minutes (IQR 38-73)	73 minutes (IQR 54-89)	133 minutes (IQR 116-166)
Trimethoprim/sulfamethoxazole related genes					
<i>dfrA</i> genes	0.2 minutes (IQR 0.02-0.5)	14 minutes (IQR 7-16)	68 minutes (IQR 53-75)	85 minutes (IQR 69-92)	164 minutes (IQR 136-180)
<i>sul</i> genes	0.5 minutes (IQR 0.27-1.3)	13 minutes (IQR 7-18)	57 minutes (IQR 45-76)	80 minutes (IQR 65-96)	166 minutes (IQR 131-173)

¹Only including genes detected in at least three isolates; ESBL: extended-spectrum beta-lactamase