

1 ***Klebsiella pneumoniae* exhibiting a phenotypic hyper-splitting phenomenon including the**
2 **formation of small colony variants**

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19

20 **Abstract**

21 In this study, we characterized a *Klebsiella pneumoniae* strain in a patient with shrapnel hip
22 injury, which resulted in multiple phenotypic changes, including the formation of a small
23 colony variant (SCV) phenotype. Although already described since the 1960s, there is little
24 knowledge about SCV phenotypes in *Enterobacteriaceae*. The formation of SCVs has been
25 recognized as a bacterial strategy to evade host immune responses and compromise the efficacy
26 of antimicrobial therapies, leading to persistent and recurrent courses of infections. In this case,
27 14 different, clonally identical resisto- and morpho-types were distinguished from the patient's
28 urine and tissue samples. Whole genome sequencing revealed the *K. pneumoniae* high-risk
29 clonal lineage belonging to sequence type 147. Subculturing the SCV colonies consistently
30 resulted in the reappearance of the initial SCV phenotype and three stable normal-sized
31 phenotypes with distinct morphological characteristics. Additionally, an increase in resistance
32 was observed over time in isolates that shared the same colony appearance. Our findings
33 highlight the complexity of bacterial behavior by revealing a case of phenotypic “hyper-
34 splitting” in a *K. pneumoniae* SCV and its potential clinical significance.

35

36 **Introduction**

37 *Klebsiella pneumoniae*, an opportunistic pathogen known for its ability to cause a wide range
38 of nosocomial and community-acquired infections, has emerged as a significant public health
39 threat due to its strain-specific, extensive arsenal of resistance and virulence factors (1, 2).
40 Infections caused by multi-, extensively-, and pandrug-resistant strains result in high mortality
41 due to limited response to antibiotic therapy, which poses an increasing threat (3-5). Apart from
42 classic strains, a hypervirulent *K. pneumoniae* (hvKp) pathotype occurs and is characterized by
43 invasive, often life-threatening and multiple site infection, characteristically in healthy patients
44 from the general population (6). In addition, convergent types that successfully combine
45 resistance and hypervirulence represent a “perfect storm” and have been increasingly reported
46 in recent years (7-9).

47 Beyond typical resistance mechanisms against various antimicrobials, functional resistance
48 mechanisms have been elucidated that lead to antimicrobial treatment failure and foster the
49 development of relapses and persistent infections (10). The formation of a biofilm matrix
50 represents one of these mechanisms that facilitates antibiotic tolerance and the generation of
51 bacterial persister cells (10). Interestingly, it has been demonstrated that a decrease in capsule
52 biosynthesis, which is crucial for hypervirulent phenotypes, leads to increased *in vitro* biofilm
53 formation and intracellular persistence (11). Another non-classical mechanism leading to
54 functional resistance is the formation of the small colony variant (SCV) phenotype. SCVs are
55 subpopulations of bacteria that exhibit slow growth, reduced colony size, and altered
56 phenotypic properties compared to their normal-growing counterparts, making them difficult
57 to detect and treat effectively (12, 13). Their ability to evade the host's immune surveillance
58 and to undermine the effectiveness of antimicrobial interventions by host cell internalization
59 results in intracellular persistence, which contributes significantly to the recurrence and
60 chronicity of the infection (14, 15). Another pivotal attribute facilitating this phenomenon is
61 their capability to modulate metabolic processes and virulence characteristics (16, 17).

62 Hypermutator SCVs characterized by higher mutation frequencies than wild-type strains and
63 isolated especially from cystic fibrosis (CF) patients (18, 19) have also been associated with
64 antibiotic resistance (20, 21) and biofilm formation (22).

65 To date, research has focused on staphylococcal SCVs, while SCVs of Gram-negative bacteria
66 have been investigated in only a few studies and case reports (12). Although the formation of
67 small colonies in *K. pneumoniae* has been noticed during resistance studies against
68 cephalosporins in the mid-1960s (23), this issue has not received sufficient attention and
69 detailed research has not been conducted on this subject. The first clearly defined SCV of *K.*
70 *pneumoniae* (SCV-Kp) in literature was obtained by *in vitro* exposure to gentamicin (24). SCV-
71 Kp were also isolated from a patient treated with aminoglycoside antibiotics (25). Smaller and
72 non-mucoid colonies were obtained as a result of conjugation-induced mutation in the outer
73 membrane protein of a hypervirulent *K. pneumoniae* isolate (26). Another study showed that
74 biofilm-forming *K. pneumoniae* developed heteroresistance to colistin by presenting slow-
75 growing SCV-Kp (27).

76 Here, we report on *K. pneumoniae* isolates displaying 14 different resisto- and morpho-types
77 obtained from an immunocompetent male patient, who had sustained a traumatic injury caused
78 by shrapnel shell fragments. The isolates comprise an initial, mostly susceptible *K. pneumoniae*
79 isolate with typical morphological characteristics isolated from the patient's urinary specimen.
80 From the urine and tissue samples, 13 additional phenotypes with different combinations of
81 resistance and morphological characteristics including *K. pneumoniae* SCV phenotypes were
82 isolated.

83

84 **Methods**

85 **Patient data.** Sufficient information could not be obtained regarding the period from the
86 patient's first acetabular and femoral head shrapnel-caused war injury in Ukraine in March
87 2022, where he underwent hip prosthesis at an external center before his transfer to our

88 orthopedic service in July 2022. Fracture-related joint infection treatment in our hospital
89 continued through November 2022. The administration of antibiotics during this period
90 included piperacillin/tazobactam from July to October, 2022, trimethoprim/sulfamethoxazole
91 from July to August, 2022, cefiderocol from August to November, 2022, and colistin from
92 October to November, 2022. Daptomycin was introduced into the treatment protocol starting
93 from October 2022 upon detection of *Staphylococcus epidermidis* from tissue samples and
94 central venous catheter tip, and continued until the patient's discharge. Subsequently, a planned
95 course of post-discharge antibiotic suppression therapy with doxycycline for three months was
96 initiated. The first identification of carbapenem-resistant *K. pneumoniae* (CRKP) occurred in
97 July 2022, followed by the initial detection of SCV-Kp in September 2022. Therefore, we
98 decided to aggregate and systematically assess the entirety of *K. pneumoniae* strains isolated
99 from the patient.

100 **Strain identification.** The urine sample obtained from the patient was quantitatively inoculated
101 onto a Columbia agar plate with 5% sheep blood (BD Diagnostics, Heidelberg, Germany) and
102 a MacConkey II-Agar plate (BD Diagnostics) using a 10 µl disposable sterile loop. The plates
103 were then incubated for 48 hours. Tissue samples collected during surgery were inoculated onto
104 Columbia agar plates with 5% sheep blood, MacConkey II-Agar plates, and Mueller Hinton
105 Chocolate agar plates (all from BD Diagnostics). These plates were incubated under
106 capnophilic conditions for up to seven days. The remaining tissue material was inoculated onto
107 Schaedler agar and into BBL Fluid Thioglycollate media (both from BD Diagnostics) and
108 incubated for up to 14 days under anaerobic and capnophilic conditions, respectively.

109 Preliminary characterization of each phenotype was grounded in colony morphology and
110 minimal inhibitory concentration (MIC) results for antibiotics encompassed within the
111 VITEK® 2 AST card specific to *Enterobacterales* (bioMérieux SA, Marcy l'Étoile, France)
112 according to EUCAST criteria. All *K. pneumoniae* strains, isolated from various patient's
113 specimens during the period from July to December 2022, were identified by matrix-assisted

114 laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) utilizing the
115 MALDI Biotyper® sirius system (Bruker Daltonics, Bremen, Germany) with MBT Biotargets
116 96 (Bruker Daltonics). The presence of carbapenemase-encoding genes was verified by a loop-
117 mediated isothermal amplification (LAMP)-based assay (eazyplex®, AmplexDiagnostics,
118 Gars-Bahnhof, Germany).

119 **Characterization of the phenotypes.** Sequential subcultures of all phenotypic variants were
120 carried out on various agar plates (including Columbia agar + 5% sheep blood, MacConkey
121 agar from BD, and CHROMID® CPS® Elite agar from bioMérieux) to observe whether
122 changes in colony morphology occurred and SCVs remained stable, followed by meticulous
123 analysis of generated phenotypic profiles.

124 In order to determine colony sizes, each phenotype was inoculated onto 5% sheep blood agar
125 plates in triplicate on different days. After overnight incubation at $35\pm 1^\circ\text{C}$ in ambient air, the
126 diameters of ten colonies were measured on each plate and mean values were determined.

127 **Antimicrobial susceptibility testing.** In addition to the initial VITEK® 2 AST, the MICs of a
128 standardized set of antibiotics were determined by the broth microdilution (BMD) method using
129 cation-adjusted Mueller–Hinton broth (CAMHB; Micronaut-S 96-well microtiter plates,
130 Merlin, Bornheim-Hersel, Germany), and for cefiderocol using iron-depleted CAMHB
131 (UMIC®, Merlin, Bornheim-Hersel, Germany), as recommended by ISO 20776-1, the
132 European Committee on Antimicrobial Susceptibility Testing (EUCAST), and the Clinical and
133 Laboratory Standards Institute (CLSI) guidelines (28-30). The results were observed following
134 18 ± 2 hours of incubation at $35\pm 1^\circ\text{C}$ in ambient air. All tests were conducted in triplicate on
135 different days, and median MIC values were computed for analysis. *Escherichia coli* ATCC
136 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 700603, and *Pseudomonas aeruginosa*
137 ATCC 27853 were used as quality control (QC) strains, and their results were within the QC
138 range throughout the study. EUCAST Clinical Breakpoint Tables v. 13.1 were used for MIC
139 interpretation (31).

140 **DNA isolation and sequencing.** After overnight growth on blood agar plates at 37 °C, ten
141 colonies were randomly selected and suspended in 1.5 mL tubes (Carl Roth, Karlsruhe,
142 Germany) with 1 mL of phosphate buffered saline. Total DNA was extracted using the
143 MasterPure DNA Purification kit for Blood, v. 2 (Lucigen, Middleton, WI, USA) according to
144 the manufacturer's instructions. Quantification of isolated DNA was performed with the Qubit
145 4 fluorometer and the dsDNA HS Assay kit (Thermo Fisher Scientific, Waltham, MA, USA).
146 DNA was sent to SeqCenter (Pittsburgh, PA, USA), where sample library preparation using the
147 Illumina DNA Prep kit and IDT 10bp UDI indices was performed. Subsequently, libraries were
148 sequenced on an Illumina NextSeq 2000, producing 2x151bp reads. Demultiplexing, quality
149 control and adapter trimming at the sequencing center was performed with bcl-convert v. 3.9.3
150 ([https://support-](https://support-docs.illumina.com/SW/BCL_Convert/Content/SW/FrontPages/BCL_Convert.htm)
151 [docs.illumina.com/SW/BCL_Convert/Content/SW/FrontPages/BCL_Convert.htm](https://support-docs.illumina.com/SW/BCL_Convert/Content/SW/FrontPages/BCL_Convert.htm)).

152 **Assembly and genomic characterization.** We employed a custom assembly and polishing
153 pipeline to assemble raw sequencing reads to contigs. This pipeline consists of four parts,
154 namely trimming (BBduk from BBTools v. 38.98 [<https://sourceforge.net/projects/bbmap/>],
155 quality control (FastQC v. 0.11.9 [<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>]), assembly (shovill v. 1.1.0
156 [<https://github.com/tseemann/shovill>]) with SPAdes v. 3.15.5 (32), and polishing (BWA-
157 MEM2 v. 2.2.1 (33), Polypolish v. 0.5.0 (34)).

159 Genotyping was performed with Kleborate v. 2.2.0 (35) and Kaptive (36, 37).

160 **Confirmation of clonality.** Trimmed sequencing reads of all isolates were mapped against
161 isolate 1-A with snippy v. 4.6.0 (<https://github.com/tseemann/snippy>) and the SNP distance
162 matrix calculated with snp-dists v. 0.8.2 (<https://github.com/tseemann/snp-dists>).

163

164 **Results**

165 Overall, 14 distinct phenotypes were determined (Table 1). From the urine, two phenotypes (1-
166 A and 1-B) exhibiting a normal colony size and glistening surface but differing in the color of
167 their colonies displaying whitish or grey colonies, were isolated. All other phenotypes (n = 12)
168 were isolated from tissue specimens. Strains numbered 1-A, 2-A, 3-A, 4-B, 5-B, numbered 1-
169 B, 2-B, 3-B, 4-C, 5-C, and numbered 4-D, 5-D, displayed identical morphological attributes
170 each, distinguished by whitish, glistening, and smooth (Figure 1-A), grey, glistening, and
171 smooth (Figure 1-B), and grey, dry, and rough colonies (Figure 1-C), respectively. These strains
172 revealed a normal colony size of 2.4 mm on average (range, 1 – 5.5 mm). The isolates displaying
173 the SCV phenotype, numbered 4-A and 5-A, exhibited similar morphological characteristics,
174 and colony sizes were smaller than 0.5 mm (Figure 1-D). No discernible variation in terms of
175 colony clustering was observed among the various agar plates.

176 Initially, largely antibiotic-susceptible *K. pneumoniae* phenotypes exhibiting whitish and grey
177 colony morphologies on Columbia agar plates were isolated from the urine sample. Following
178 antibiotic treatment, MDR *K. pneumoniae* strains displaying the normal colony size were
179 isolated from tissue samples, again characterized by subsequent whitish or grey colony
180 formations. Subsequently, SCVs of *K. pneumoniae* were isolated from tissue samples.
181 Subcultivation of different SCV colonies consistently yielded a division into four distinct
182 colony morphotypes including one SCV phenotype that resembled the initial SCV, along with
183 three normal-sized phenotypes distinguished by variations in colony color and visual attributes.
184 While normal-sized phenotypes exhibited stability following each round of re-cultivation, SCV
185 isolates displayed instability and recurrently diverged into the four phenotypes described above.
186 We have designated the emergence of these multiple phenotypes as “hyper-splitting”. Despite
187 minor variations in MIC values, these “hyper-splitting” phenotypes exhibited multidrug
188 resistance (Table 1).

189 Except for isolates 1-A and 1-B, all isolates were resistant to the tested carbapenems. Initially,
190 during routine diagnosis, isolate 2-B was found to be carbapenem-resistant by VITEK[®] 2 AST,

191 and to harbor *bla*_{OXA-48} gene by LAMP. After subcultivation of this isolate for MIC
192 determination, this resistance disappeared and the isolate became susceptible to all tested beta-
193 lactam antibiotics except piperacillin. Only isolates 1-A and 1-B were susceptible to
194 piperacillin, and only isolate 4-B was not resistant to the cephalosporins tested. Interestingly,
195 only isolates 4-A and 5-A, which demonstrated the SCV phenotype, were resistant to amikacin
196 and trimethoprim-sulfamethoxazole. Another remarkable finding was the observed increase in
197 the MIC values of cefiderocol and trimethoprim-sulfamethoxazole over time (Table 1).

198 Whole-genome sequence (WGS) analysis revealed that all isolates belonged to sequence type
199 (ST) 147. Lipopolysaccharide antigen (O) loci were O1/O2v1 and capsule biosynthesis (KL)
200 loci were KL64 for all isolates except isolate 4-D, which could not be assigned, as it missed
201 most genes of this locus. Isolates 1-A, 1-B and 2-B showed lower Kleborate resistance score
202 than the other isolates (resistance: 0 vs. 2). The resistance score of 0 indicates that the isolate(s)
203 did not carry any genes for extended-spectrum beta-lactamases (ESBL) or carbapenemases and
204 a score of 2 correlated with the presence of carbapenemase genes without colistin resistance
205 genes (35). In accordance with the resistance scores, we detected several beta-lactamase genes,
206 such as *bla*_{SHV-11}, *bla*_{TEM-1} and *bla*_{OXA-9}, ESBL genes, such as *bla*_{CTX-M-15} and *bla*_{OXA-1}, and the
207 carbapenemase genes *bla*_{NDM-1} and *bla*_{OXA-48}. *bla*_{SHV-11} was found in all isolates whereas *bla*_{TEM-}
208 ₁ and *bla*_{OXA-9} were present in all isolates except 1-A and 1-B. However, *bla*_{CTX-M-15} was not
209 found in isolate 4-A. Genes associated with sulphonamide (*sul1*) and chloramphenicol (*catB3*)
210 resistance were also detected in all isolates except 1-A, 1-B and 2-B (Table S1). Note that we
211 did not detect any common cefiderocol resistance genes.

212 The isolates exhibited clonality as emphasized by the low number of SNPs among them (Table
213 S1). Especially isolates from the same time point showed no difference in the core genome
214 alignment (5,360,988 bp) with the exception of 2-A and 2-B (six SNPs) and 5-D (one additional
215 SNP compared to 5-A–C). The largest distance with 17 SNPs was between 2-A and 5-D (Table
216 S1).

217

218 **Discussion**

219 When evaluating the results, we can roughly identify three distinct outcomes. The first
220 significant observation concerns the emergence of resistance development chronologically
221 within a *K. pneumoniae* strain, originating from a patient subjected to continuous, uninterrupted
222 antibiotic intervention. This scenario promptly elicits contemplation of the subject concerning
223 within-host adaptive evolution of bacteria. In fact, in-host resistance evolution, either due to
224 plasmid mediation or chromosome mutations, has been observed even shortly after the initiation
225 of antimicrobial treatment (38).

226 The second notable observation in our study is the occurrence of SCVs from patient specimens
227 following the detection of normal-sized morphotypes. SCVs demonstrate remarkable abilities
228 to invade and persist within host cells, thus evading the surveillance mechanisms of the immune
229 system (39). The existence of SCVs, mostly observed in *Staphylococcus* spp., has been
230 documented since the onset of the 20th century and has gained increasing attention due to its
231 potential implications for both clinical and basic research (12, 40). Regarding the SCVs of
232 Gram-negative bacteria, studies have particularly focused on *Burkholderia* and *Pseudomonas*
233 spp. isolated from CF patients (18, 41, 42). However, there are only sparse data on the
234 occurrence of SCV in *Klebsiella* spp. (23-27).

235 Basically, SCVs have been determined as a subpopulation characterized by their distinct
236 phenotypic properties, such as atypical colony morphologies including the reduced colony size
237 (43). Their decreased growth rate is thought to contribute to their inherent resistance, given that
238 the decelerated growth dynamics potentially hinder the effectiveness of antibiotics geared
239 towards rapidly proliferating cell populations (44). Furthermore, this phenomenon concurrently
240 signifies decreased metabolic activity, which may engender modifications in cell wall
241 permeability, drug uptake, or the modulation of efflux pump expression (45).

242 For electron transport chain-defective staphylococcal SCVs, lower efficacy of aminoglycosides
243 known to be taken up through electrical potential across the cytoplasmic membrane ($\Delta\Psi$) was
244 demonstrated, which is attributable to low $\Delta\Psi$ (46). These alterations could collectively
245 contribute to enhancing resistance patterns. In this study, we observed an increase in the MIC
246 values of amikacin, cefiderocol, and trimethoprim-sulfamethoxazole in the isolates recovered
247 over time. This MIC increase was especially pronounced for amikacin in SCV phenotypes.
248 Moreover, most antibiotics penetrate into host cells poorly, so the concentrations required to
249 kill intracellularly persistent SCVs cannot be achieved (12).

250 SCVs, known for their inducible formation through *in vitro* processes involving various agents,
251 including antibiotics (23), have exhibited a propensity for increased persistence and adaptability
252 when confronted with challenging environments (47). An enhanced ability to form biofilms on
253 biotic and abiotic surfaces has been shown for SCVs of different bacterial species (41, 48-51).
254 The substantial implication of SCVs extends to their involvement in biofilm development, as
255 biofilms effectively shield bacteria from harsh host environments, thereby complicating the
256 elucidation of drug resistance mechanisms within biofilm structures (52). Biofilms not only
257 confer protection against host immune defenses but also serve as reservoirs for persistent
258 infections and recurrent episodes (53). The impact of SCV phenotype on biofilm formation in
259 *Klebsiella* remains to be elucidated in further studies.

260 Furthermore, the emergence of SCVs could plausibly be due to selection pressure from
261 antibiotic regimens or other host-associated factors, e.g., host cationic peptides. Consistent with
262 the case that was the subject of our study, the higher frequency of SCVs in isolates from chronic
263 and recurrent infections compared to acute infections suggests a potential role for these variants
264 in evading host immune responses and antimicrobial treatments (12). In the context of our
265 study, the emergence of SCVs after the initiation of cefiderocol treatment while already
266 undergoing antibiotic therapy could be construed as a form of *in vivo* or *in host* induction.

267 The third noteworthy finding from our study underscores the inherent instability of SCVs. This
268 dynamic interplay between stable and unstable SCVs is still poorly understood and its
269 elucidation may contribute to a deeper understanding of their role in infection in general and
270 persistence phenomena in particular (54). Despite comprehensive explorations largely focusing
271 on staphylococci, a lack of investigations concerning *Klebsiella* spp. persists, and requires
272 attention.

273 The observed instability among SCVs, combined with distinct antibiotic susceptibility profiles
274 across phenotypes, increases the significance of investigating SCV plasticity (43). Stable SCVs
275 represent a long-term adaptation strategy, whereas their unstable counterparts may arise as
276 stress-induced variants that result from rapid adaptation to fluctuating environments (14, 55,
277 56). This inherent instability potentially serves as a mechanism for evading host immune
278 responses and circumventing antibiotic interventions (55). Furthermore, the involvement of
279 epigenetic modifications, including alterations in DNA methylation patterns, could
280 significantly influence SCV stability (57). In addition, regulatory systems, such as two-
281 component systems and quorum sensing, play a crucial role in SCV formation by modulating
282 bacterial behavior and adaptation. Disruption or dysregulation of these systems could lead to
283 the emergence of SCVs with altered phenotypic properties (58). Due to instability, slow-
284 growing SCVs may generate mutants that exhibit a faster growth rate than usual (59). In
285 instances of reversion to the wild type, rapidly growing mutant revertants may demonstrate
286 either the loss or preservation of antibiotic resistance (59).

287 A high mutation rate might favor the emergence of SCVs (20) and also explain the emergence
288 of antibiotic resistance as a result of antibiotic selective pressure and the adaptation of
289 hypermutable strains in patients, especially CF patients (19). CF-like chronic infections have
290 been shown to specifically contribute to the development of bacterial mutations (60).
291 Hypermutation could result in a subpopulation of bacteria that temporarily does not grow, thus
292 leading to persistence (61). Additionally, an increase in the prevalence of mutator bacterial

293 strains with deficient DNA mismatch repair (MMR) system has been detected in CF patients,
294 who are used as a reservoir for mutation (62). To our best knowledge, we were unable to identify
295 any instance in the available literature wherein a solitary SCV colony has given rise to four
296 distinct colonies exhibiting disparate morphologies. Accordingly, we suggest the designation
297 "phenotypic hyper-splitting" for this distinctive phenomenon.

298 We described in this study unprecedented phenotypic attributes and primarily focused on *in*
299 *vitro* experiments. Therefore, the clinical relevance of our findings necessitates validation
300 through animal models and clinical sample analyses. In this context, macrophage and neutrophil
301 assays would be valuable for assessing both the extent of immune response and the presence of
302 persistent cells. Moreover, the determination of the auxotrophism (13, 17) of *K. pneumoniae*
303 SCVs and of the molecular mechanisms that drive SCV formation and the resulting antibiotic
304 resistance in this species require further investigation. Integrating a comprehensive range of
305 approaches encompassing genomics, transcriptomics, and proteomics, the utilization of
306 experimental evolutionary models can yield valuable insights into the genetic determinants and
307 regulatory networks orchestrating SCV phenotypes.

308 The genomic analysis conducted in this study has revealed clonality among all 14 isolates.
309 Further exploration is warranted to uncover the intricate molecular mechanisms underlying
310 phenotypic hyper-splitting and to elucidate the potential pathogenic implications of this
311 phenomenon. To better understand the formation of the SCV phenotype especially in Gram-
312 negative pathogens, efforts need to be intensified (i) to improve the detection and
313 characterization of SCVs recovered from clinical samples and (ii) to elucidate their clinical
314 impact.

315

316 **Data availability**

317 The data for this study have been deposited in the European Nucleotide Archive (ENA) at
318 EMBL-EBI under accession number PRJEB71325
319 (<https://www.ebi.ac.uk/ena/browser/view/PRJEB71325>).

320

321 **Supplemental material**

322 **Table S1.** Core SNP distance matrix. The complete core genome alignment (gaps and
323 ambiguous bases removed) contained 5,360,988 bp. The reference sequence for alignment was
324 1-A.

325

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333 the Federal Ministry of Education and Research (BMBF, Germany) to KSc entitled “Disarming
334 pathogens as a different strategy to fight antimicrobial-resistant Gram-negatives” (01KI2015).

335

336 **Conflict of interest**

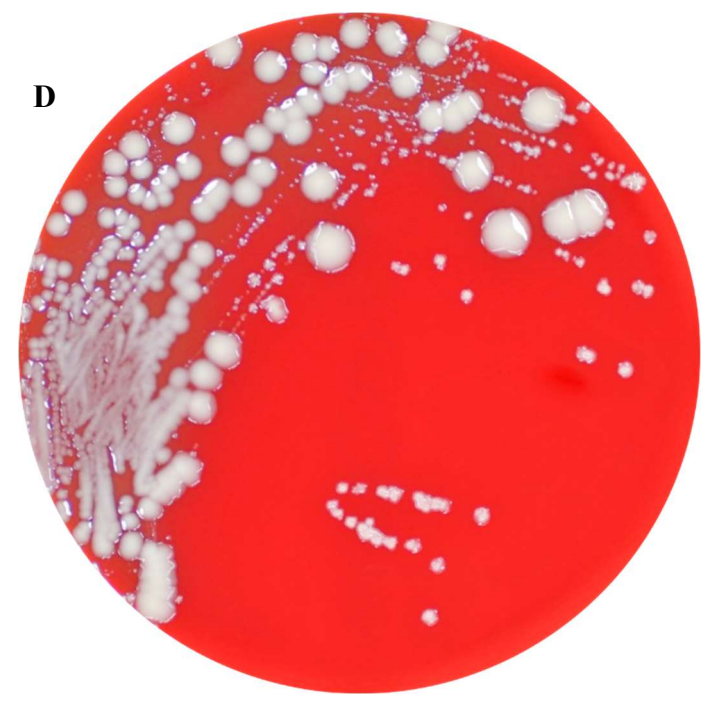
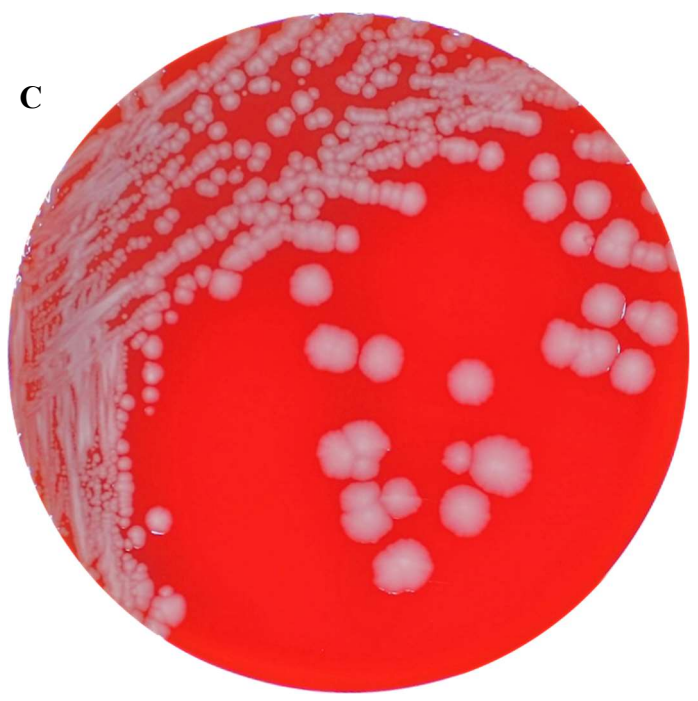
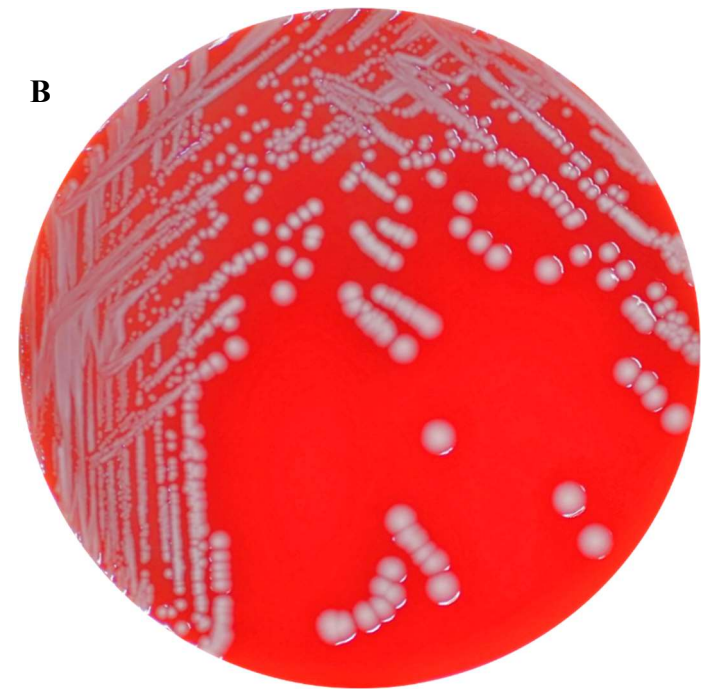
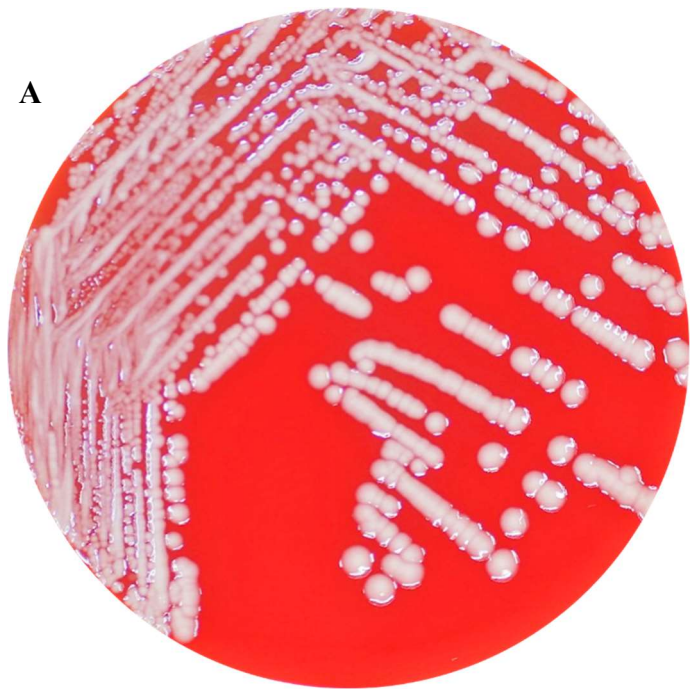
337 The authors declare no conflict of interest.

338

Table 1. Colony morphology and antimicrobial susceptibility characteristics of the 14 phenotypes of the *Klebsiella pneumoniae* strain

14 phenotypes of the <i>K. pneumoniae</i> strain					Median minimum inhibitory concentrations (MICs), µg/ml ^a																	
Isolate number	Specimen	Date	Colony morphology		PIP	TZP	TEM	FDC	CTX	CAZ	CZA	C/T	IPM	MEM	CIP	LVX	AMK	TGC	CHL	CST	FOF	SXT
			Color	Surface																		
1-A	Urine	Jul 22	Whitish	Glistening, smooth	≤8	≤4	≤32	≤0,03	≤1	≤1	≤1	≤1	≤1	≤0,125	>2	>2	≤4	0,5	16	≤1	>128	≤1
1-B			Grey	Glistening, smooth	≤8	≤4	≤32	≤0,03	≤1	≤1	≤1	≤1	≤1	≤0,125	>2	>2	≤4	0,5	16	≤1	>128	≤1
2-A	Tissue	Jul 22	Whitish	Glistening, smooth	>16	>64	>128	1	>2	>128	>16	>8	>8	128	>2	>2	8	0,5	>16	≤1	>128	≤1
2-B			Grey	Glistening, smooth	>16	8	≤32	0,06	≤1	≤1	≤1	≤1	≤1	≤0,125	>2	>2	8	≤0,25	≤8	≤1	>128	≤1
3-A	Tissue	Aug 22	Whitish	Glistening, smooth	>16	>64	>128	1	>2	>128	>16	>8	>8	128	>2	>2	8	≤0,25	>16	≤1	>128	≤1
3-B			Grey	Glistening, smooth	>16	>64	>128	2	>2	>128	>16	>8	>8	128	>2	>2	8	≤0,25	>16	≤1	>128	≤1
4-A	Tissue	Sep 22	Small colony variant		>16	>64	>128	0,25	>2	>128	>16	>8	>8	128	>2	>2	32	0,5	>16	≤1	>128	>4
4-B			Whitish	Glistening, smooth	>16	64	>128	0,125	2	≤1	≤1	≤1	8	16	>2	>2	8	0,5	>16	≤1	>128	2
4-C			Grey	Glistening, smooth	>16	>64	>128	1	>2	>128	>16	>8	>8	128	>2	>2	8	0,5	>16	≤1	>128	4
4-D			Grey	Dry, rough	>16	>64	>128	2	>2	>128	>16	>8	>8	64	>2	>2	8	0,5	>16	≤1	>128	≤1
5-A	Tissue	Sep 22	Small colony variant		>16	>64	>128	0,25	>2	>128	>16	>8	>8	>128	>2	>2	32	0,5	>16	≤1	>128	>4
5-B			Whitish	Glistening, smooth	>16	>64	>128	2	>2	>128	>16	>8	>8	128	>2	>2	8	0,5	>16	≤1	>128	4
5-C			Grey	Glistening, smooth	>16	>64	>128	2	>2	>128	>16	>8	>8	128	>2	>2	8	0,5	>16	≤1	>128	4
5-D			Grey	Dry, rough	>16	>64	>128	1	>2	>128	>16	>8	>8	64	>2	>2	8	0,5	>16	≤1	>128	4

^aAbbreviations of antibacterial agents: PIP: piperacillin, TZP: piperacillin-tazobactam, TEM: temocillin, FDC: cefiderocol, CTX: cefotaxime, CAZ: ceftazidime, CZA: ceftazidime-avibactam, C/T: ceftolozane-tazobactam, IPM: imipenem, MEM: meropenem, CIP: ciprofloxacin, LVX: levofloxacin, AMK: amikacin, TGC: tigecycline, CHL: chloramphenicol, CST: colistin, FOF: fosfomicin, SXT: trimethoprim-sulfamethoxazole



342 **Figure legends**

343 **FIG 1** Columbia blood agar plates showing the different colonial morphotypes of the *K.*
344 *pneumoniae* isolates comprising regular sized colonies (wild-type) with glistening whitish
345 (Figure 1-A) and grey (Figure 1-B), and dry and rough grey colonies (Figure 1-C), respectively,
346 as well as tiny grey and whitish colonies displaying the SCV phenotype (Figure 1-D). Figure
347 1-D also shows the hyper-splitting phenomenon of the SCV phenotype into the colony
348 morphotypes shown in figures 1-A–C.

349

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