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Louise Krag, Viktoria Hancock, Bent Aalbæk, Per Klemm. Genotypic and phenotypic characterisation of strains associated with porcine pyelonephritis. *Veterinary Microbiology*, 2009, 134 (3-4), pp.318. 10.1016/j.vetmic.2008.08.025 . hal-00532470

HAL Id: hal-00532470

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Accepted Manuscript

Title: Genotypic and phenotypic characterisation of *Escherichia coli* strains associated with porcine pyelonephritis

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PII: S0378-1135(08)00351-9
DOI: doi:10.1016/j.vetmic.2008.08.025
Reference: VETMIC 4137

To appear in: *VETMIC*

Received date: 12-6-2008
Revised date: 15-8-2008
Accepted date: 18-8-2008

Please cite this article as: Krag, L., Hancock, V., Aalbæk, B., Klemm, P., Genotypic and phenotypic characterisation of *Escherichia coli* strains associated with porcine pyelonephritis, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2008.08.025

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1 **Genotypic and phenotypic characterisation of *Escherichia coli* strains associated**
2 **with porcine pyelonephritis**

3
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14 Running title: Molecular characterisation of porcine pyelonephritis strains.

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20 **Abstract**

21 Urinary tract infection (UTI) is a severe problem in humans as well as in many domestic animals
22 like pigs. The most frequent infectious agent in UTI is uropathogenic *Escherichia coli*. Such strains
23 have been extensively characterised with respect to virulence and fitness factors as well as clonal
24 type when it comes to human isolates. However, relatively little has been done to characterise the
25 corresponding porcine strains. On this background we have analysed 20 porcine pyelonephritis *E.*
26 *coli* strains isolated from infected pig kidneys. The strains were quite distinct from that of human
27 uropathogenic strains with regards to adhesion profile and haemolysin production. Also, the clonal
28 profiles differed from that of human infections since our strains all belonged to the *E. coli* clonal
29 groups A and B1.

30

31

32

33 *Keywords:* Fimbrial adhesins; *Escherichia coli*; Pyelonephritis; Urinary tract infections; Pigs;

34 Siderophores

35 1. Introduction

36 Urinary tract infection (UTI) is a severe problem in humans and domestic animals like
37 pigs. The infection can ascend the urinary tract (UT), and the most severe form of UTI is infection
38 of the kidneys (pyelonephritis). Pyelonephritis is a common cause of severe clinical symptoms and
39 death in breeding sows, and if not treated early and adequately acute pyelonephritis may result in
40 irreversible renal scarring with sequelae of hypertension and chronic renal failure. Consequently,
41 UTI imparts reduced animal welfare and a considerable economic loss due to decreased
42 productivity (Berner, 1981b; Chagnon et al., 1991; Christensen et al., 1995; Bertschinger and
43 Fairbrother, 1999; Mauch and Bilkei, 2004). In previous studies UTI has been observed in 11% to
44 48% of sows in individual herds (Berner, 1981b, a), and UTI is reported as the cause of death in 8%
45 to 15% of spontaneously dead sows (Chagnon et al., 1991; Christensen et al., 1995). In slaughter
46 pigs and slaughtered sows pyelonephritis is observed in 0.018-0.6% (Larsen and Tondering, 1954;
47 Jansen and Nordstoga, 1992) and about 10%, respectively (Berner, 1981a).

48 *Escherichia coli* is the dominating etiological agent in human UTI and is responsible
49 for more than 80% of all UTIs. In pigs *E. coli* is also reported to be a major cause of UTI (Berner,
50 1981b, a; Chagnon et al., 1991; Carr and Walton, 1993; Bertschinger and Fairbrother, 1999; Mauch
51 and Bilkei, 2004). The ability of uropathogenic *E. coli* (UPEC) to cause symptomatic UTI in
52 humans is associated with the expression of a variety of virulence factors such as adhesins (e.g. type
53 1, P and F1C fimbriae), toxins (e.g. haemolysin), siderophores and flagella (Oelschlaeger et al.,
54 2002; Kucheria et al., 2005). The average human produces about two litres of urine per day all of
55 which leaves the body via the urinary tract giving rise to strong hydrodynamic shear forces;
56 arguably the situation in pigs is quite similar. Due to the urine flow fimbriae-mediated adherence to
57 the urinary tract epithelium is generally believed to be important for the bacteria to resist removal
58 by urine flow and to establish in this niche, although it is possible for certain strains to remain in the
59 UT for extended periods of time by virtue of fast growth rates (Roos et al., 2006). Expression of P
60 fimbriae is primarily linked to pyelonephritic strains (Domingue et al., 1985). Type 1 fimbriae are
61 primarily associated with adherence to bladder cells and these organelles are additionally involved
62 in invasion of bacteria into host cells (Connell et al., 1996). F1C fimbriae recognise receptors
63 present in the kidneys, bladder and urethers (Bäckhed et al., 2002).

64 Although strains associated with human UTIs have been extensively characterised
65 very little is known about their porcine equivalents. The aim of this study was to characterise *E. coli*
66 strains associated with porcine pyelonephritis. Twenty strains were investigated for possession of

67 virulence factors such as adhesins (type 1, P and F1C fimbriae), toxins (haemolysin), siderophore
68 production capacity, colicin production, motility and plasmid profile. Furthermore their
69 phylogenetic groups were determined.

70

71 **2. Materials and methods**

72 *2.1 Bacterial strains*

73

74 The *E. coli* strains used in this study were isolated post mortem from 20 sow kidneys
75 with pyelonephritis lesions. The sows were slaughtered at Danish Crown, Skærbæk, Denmark.
76 Bacteria were identified as *E. coli* using standard microbiological techniques and all strains were
77 isolated as monocultures.

78

79 *2.2 Growth media*

80

81 All cultivations were performed in Luria-Bertani (LB), minimal ABT medium [AB
82 minimal medium containing 2.5 mg/l thiamine] (Clark and Maaloe, 1967) or pooled human urine.
83 Human urine was collected from minimum three healthy volunteers who had no history of urinary
84 tract infection or antibiotic use in the prior 2 months. The urine was pooled, filter-sterilised, stored
85 at 4°C and used within the following 1-2 days.

86

87 *2.3 Yeast agglutination after pellicle formation*

88

89 In static liquid medium type 1 fimbriation mediates pellicle formation (Old and
90 Duguid, 1970) and static growth conditions provide an efficient selection pressure for type 1
91 fimbrial expression. The presence of type 1 fimbriae is assayed by the ability of the bacteria to
92 agglutinate yeast cells (*Saccharomyces cerevisiae*) on glass slides. Fifty ml LB or human urine were
93 inoculated with 100 µl of shaken overnight culture and allowed to stand statically for 48 h at 37°C.
94 After incubation, a 100-µl aliquot of the top surface liquid was used for inoculation of a second 50
95 ml static LB or urine culture. After another 48 h of incubation, the cultures were visually inspected
96 for pellicle formation, and for confirmation of positive pellicle formation, samples were extracted
97 from the pellicle and suspensions of bacteria from these cultures were tested for yeast cell
98 agglutination. Ten µl of the culture extracts were mixed with 10 µl 5% yeast cells. The experiment

99 was repeated after the cells had been washed and resuspended in LB containing 50 mM methyl- α -
100 D-mannopyranoside.

101

102

103 2.4 Haemagglutination assay

104

105 The capacity of bacteria to express P fimbriae was assayed by haemagglutination with
106 human type A and pig red blood cells (RBCs). RBCs were washed two times with phosphate-
107 buffered saline (PBS) and 10 μ l of 5% RBCs were mixed with a single bacterial colony (freshly
108 grown on LB plates) suspended in PBS on glass slides. Any strain showing positive
109 haemagglutination was tested again after 30 min incubation with 1% D-mannose to rule out type 1
110 fimbriae and to further support that agglutination was likely mediated by P fimbriae.

111

112 2.5 PCR analysis

113

114 The primers used to amplify all or parts of the *chuA*, *yjaA*, TspE4.C2, *pap*, *fim*, *fyuA*
115 and *focH* genes are listed in Table 1 and Fig. 1. Genomic DNA was prepared by heating samples
116 containing one colony from a fresh LB plate and 5 μ l of sterile milli-Q water to 95°C for 10
117 minutes. The Long PCR Enzyme Mix (#K0189, Fermentas) was used for amplifying large gene
118 fragments.

119 A rapid technique based on triplex PCR using a combination of two genes (*chuA* and
120 *yjaA*) and an anonymous DNA fragment (TspE4.C2) was used for identification of phylogenetic
121 group (Clermont *et al.*, 2000).

122

123 2.6 Plasmid profiling

124

125 For isolation of plasmid DNA (i.e. plasmids of sizes < 40 kb) 1 ml overnight culture
126 was centrifuged at 10000g for 2 min. After removing the supernatant the cells were resuspended in
127 20 μ l TE buffer 5:1 and subsequently 100 μ l of lysis buffer were added. Each tube was turned 10
128 times. After incubation at 56°C for 30 min 100 μ l phenol-chloroform-isoamyl alcohol (25:24:1)
129 were added and the mixture was centrifuged at 10000g for 15 min. Fifteen- μ l samples of plasmid
130 DNA and loading buffer were loaded into wells of a 0.8% agarose gel. The gels were stained with

131 ethidium bromide. The plasmid markers *E. coli* V517 and 39R were used as markers of molecular
132 mass.

133

134 2.7 Growth in urine

135

136 Strains were grown overnight in pooled human urine and 2 μ l were used for
137 inoculation of 200 μ l urine in 96-well flat-bottom microplates. The microplates were incubated
138 shaken at 37°C and optical density at 600 nm (OD_{600nm}) was measured every hour until cells
139 reached stationary phase. Each strain was assayed in at least nine wells distributed over three
140 different plates and in three different batches of urine. The average growth curve of each strain in
141 each batch of urine was used for calculation of growth rates; the four time points resulting in the
142 steepest slope (all with a correlation coefficient above 0.98) were used for each calculation. Growth
143 rates from three independent experiments were then used for calculation of an average generation
144 time for each strain.

145

146 2.8 Motility assay

147

148 The motility ability was tested for all strains. One μ l of overnight urine culture was
149 stabbed into urine plates (human urine) containing 0.3% agar. The distance of migration (the
150 diameter of the ring around the inoculation site) was measured after 16 h of incubation at 37°C. The
151 assay was repeated three times in different batches of urine.

152

153 2.9 Inhibition assay

154

155 The ability for formation of colicin was tested on overnight cultures grown in LB. One
156 hundred μ l of overnight culture of indicator strain MG1655 (Bachmann, 1996) were spread on LB
157 plates and allowed to dry. Aliquots of 1 μ l overnight culture of each strain grown in LB were spot
158 inoculated on the plates. After overnight incubation at 37°C, the plates were inspected for a clearing
159 zone indicative of growth inhibition against the indicator lawn. Each strain was tested
160 independently twice.

161

162 2.10 Haemolysin

163

164 Isolated colonies were spot inoculated and production of haemolysin was detected by
165 determining a zone of lysis around each colony on bovine blood agar plates after overnight
166 incubation of the tested strains.

167

168 *2.11 Siderophore production*

169

170 The capacity for siderophore production was tested using blue chrome azurol S (CAS)
171 agar plates prepared by the method of (Schwyn and Neilands, 1987) with some modifications.
172 Thirty point twenty five milligrams CAS (Fluka) was dissolved in 25 ml water and mixed with 5 ml
173 of 1 mM ferric chloride solution (1 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 10 mM HCl). This solution was slowly
174 added to 36.45 g HDTMA (hexadecyltrimethyl ammonium bromide, Sigma) dissolved in 20 ml of
175 water heated to 40°C. The resultant dark blue liquid was autoclaved. Simultaneously, ABT with
176 cas-amino acids and 2% Bacto agar was autoclaved. After cooling the solutions to about 50°C, one
177 volume of the CAS solution was added to 9 volumes of the molten agar, swirled to mix without
178 foaming and immediately poured into sterile Petri plates. The plates were inoculated with 1 μl of
179 overnight ABT culture. The plates were incubated for 16 hours at 37°C. After 2 hours at 4°C the
180 plates were inspected for halo formation and the diameter of the halo was measured.

181

182

183 **3. Results**

184

185 *3.1 Bacterial strains*

186

187 All *E. coli* strains were isolated from individual pig kidneys in monocultures. Eleven
188 strains were isolated from kidneys with acute lesions (Fig. 2A and B), eight strains from kidneys
189 with both chronic and acute lesions (Fig. 2A) and one strain from a kidney with chronic lesions
190 (Table 2). Clinical examination of the sows before slaughtering was not performed.

191

192 *3.2 Adhesin status*

193

194 In human UPEC strains three fimbrial types, i.e. type 1, P and F1C fimbriae, are
195 widespread and linked to urovirulence. In this study we probed the type 1 fimbrial status of our
196 porcine pyelonephritis-associated *E. coli* strains by activity-related phenotypes such as yeast
197 agglutination and pellicle formations as well as PCR amplification of the *fim* gene cluster (Table 2).
198 Fifteen (75%) of the strains were able to express functional type 1 fimbriae. Furthermore, the PCR
199 data show that the whole *fim* gene cluster was present in three (60%) of the five strains which were
200 not able to express functional type 1 fimbriae. All the five strains incapable of agglutinating yeast
201 cells belonged to the same phylogenetic group, i.e. group A.

202 Expression of P fimbriae was monitored by mannose-resistant haemagglutination of
203 human and pig erythrocytes as well as PCR amplification of selected regions of the chromosomally
204 encoded *pap* gene cluster (Fig. 1). Only one strain (5%), i.e. LK82, showed positive agglutination
205 of red blood cells (RBCs), however LK82 was only showing weak haemagglutination of pig RBCs
206 but displayed no agglutination of human RBCs. This strain was the only one in which the whole
207 *pap* cluster was observed. Although only one strain had a profile compatible with functional P
208 fimbriae, 50% of the strains were positive for the *papG* gene, encoding the adhesin (Table 2); of the
209 ten strains positive for *papG*, nine were positive with primers designed for *papG* in J96 (i.e. the
210 PapG variant I) and only one (the same that was positive for all *pap* genes and agglutinated pig
211 RBCs, LK82) was positive with primers homologous to *papG* of the human UPEC strains 536,
212 UTI89, J96 and CFT073 (i.e. PapG/PrsG variants II and III). Interestingly, eight of the nine strains
213 containing the J96-variant of *papG* belonged to the same phylogenetic group, i.e. group A, whereas
214 the LK82 strain belonged to the B1 group. In contrast to the expression of type 1 and P fimbriae
215 none of the pig strains were positive for the presence of the *focH* gene encoding the putative FocH
216 adhesin of F1C fimbriae.

217

218 3.3 Determination of phylogenetic group

219

220 Phylogenetic groups were determined by triplex PCR (Clermont et al., 2000). In this
221 study 10 (50%) strains belonged to group B1 and 10 (50%) strains belonged to group A (Table 2).

222

223 3.4 Plasmid profiling

224

225 The number of plasmids per strain observed in this study varied from zero to four and
226 the size of the plasmids varied from approximately 1.8 kb to 4 kb. The plasmid profile corresponded
227 to the data reported by de Brito et al., 1999. The number of plasmids in the strains showed no
228 correlation with any of the virulence traits investigated in this study. It should be noted however
229 that the plasmid isolation technique used only identifies small to medium sized plasmids (i.e. <40
230 kb) and therefore, the presence of any large conjugative plasmids that might be responsible for
231 some of the virulence characteristics, such as expression of colicins, cannot be ruled out.

232

233 3.5 Growth in urine

234

235 The ability to grow in urine is, arguably, an obvious requirement for UT infectious
236 strains. The growth result in pooled human urine is shown in Fig. 3. The 20 porcine pyelonephritis-
237 associated *E. coli* strains showed slightly different growth characteristics in human urine, displaying
238 generation times of 111-159 min in microtitre plates. This revealed that some of the porcine-related
239 UTI strains grow with similar growth rates as that of the human 83972 UTI reference strain (Roos
240 et al., 2006) which showed a generation time of 115 min in microtitre plates.

241

242 3.6 Motility

243

244 Motility is regarded as an important fitness factor in the urinary tract. Motility test of
245 the strains on low-agar urine plates revealed that the majority of the strains (70%) were motile
246 (Table 2). Four (20%) of the strains were highly motile while seven (35%) strains showed low
247 motility in urine. Although the three most motile strains belonged to phylogenetic group A, and four
248 of the six non-motile strains belonged to group B1 there was no significant correlation between
249 motility and phylogenetic group (two-tailed *t* test; $P = 0.09$). Interestingly, the five strains that did
250 not express functional type 1 fimbriae, i.e. LK13, LK67, LK85, LK86 and LK87 (and which all
251 belonged to phylogenetic group A), were also all *fyuA* negative, showed small halos in the
252 siderophore production test on CAS plates and did not produce colicins or haemolysin; however,
253 four of these five strains belonged to the top five most motile strains in the study, indicating
254 motility as a probable fitness factor for these strains.

255

256 3.7 Colicin formation

257

258 In order to determine the capacity for colicin production the porcine pyelonephritis
259 associated *E. coli* strains were tested on LB plates against MG1655 as indicator strain. The test
260 showed inhibition zones of similar size in eight (40%) of the tested strains (Table 2), six of these
261 strains (75%) belonged to phylogenetic group B1. The frequency of colicin-producing strains (40%)
262 corresponded well to the only known pig study testing colicin production capacity of porcine UPEC
263 (de Brito et al., 1999).

264

265 3.8 Haemolysin

266

267 Haemolysin is regarded as an important virulence factor and many human UPEC are
268 known to secrete haemolysin (Raksha *et al.*, 2003). Surprisingly, only one of the porcine
269 pyelonephritis-associated *E. coli* strains, LK82, was haemolytic (Table 2).

270

271 3.9 Siderophore production

272

273 Iron is essential for bacterial growth. To counter iron-limiting conditions in urine
274 bacteria use various highly efficient mechanisms of iron acquisition. A typical high-affinity iron-
275 uptake system consists of a low-molecular-mass Fe³⁺-chelating compound, known as a siderophore,
276 combined with its cognate membrane-located receptor (Martinez *et al.*, 1990). Occurrence of the
277 *fyuA* gene encoding the 71 kDa outer-membrane protein FyuA (ferric yersiniabactin uptake
278 receptor), which acts as a receptor for Fe-Ybt siderophore uptake was probed by PCR amplification
279 of the corresponding gene. Seven (35%) of the porcine pyelonephritis strains had the *fyuA* gene on
280 their chromosome. The capacity of the *E. coli* strains for siderophore production was also assayed
281 on blue chrome azurol S (CAS) agar plates. After 16 hours of incubation all strains were able to
282 make a variable sized halo on the plates (Table 2 and Fig. 4). The presence of *fyuA* corresponded
283 well to the measured siderophore production and all but one strain that contained the *fyuA* displayed
284 large halos (Fig. 4). Interestingly, the ability to take up iron as indicated by the orange halos was
285 significantly higher for the strains belonging to phylogenetic group B1 than those of group A (two-
286 tailed *t* test, $P < 0.05$, $P = 0.016$). Furthermore, five of the seven strains containing the *fyuA* gene
287 belonged to group B1. The two strains containing *fyuA* and belonging to group A, i.e. LK15 and
288 LK16, were the only two strains in group A displaying larger halos; notwithstanding these two

289 strains, the correlation between phylogenetic group and siderophore production turned out to be
290 highly significant, i.e. $P < 0.001$ (two-tailed t test). Interestingly, the two group A strains, LK15 and
291 LK16, containing the *fyuA* gene, were also the only two strains in group A which showed positive
292 colicin production. In actual fact, a highly significant correlation between siderophore production
293 and colicin production was found (two-tailed t test, $P < 0.0001$), with only one strain (LK57)
294 displaying a large halo without showing colicin production, but which was positive for the *fyuA*
295 gene.

296

297

298 4. Discussion

299 In contrast to porcine UPEC strains human UPEC strains are well characterised. Since
300 the anatomy and physiology of the porcine and human urinary tracts are quite similar one might
301 expect that porcine UPEC strains would be similar to human UPEC strains with respect to virulence
302 and fitness factors. The urinary tract is a high-flow environment and tissue surfaces in the urinary
303 tract are submitted to strong hydrodynamic shear forces. Accordingly, it is generally believed that
304 adherence factors are important for bacterial establishment in this environmental niche. The ability
305 of human UPEC strains to cause disease has been shown to be enhanced by adhesins (Klemm and
306 Schembri, 2000; Oelschlaeger et al., 2002). Adherence to the UT epithelium enables the bacterium
307 to resist removal by flow shear forces and to establish infection. The three primary fimbrial
308 adhesive organelles associated with urovirulence in humans are P, type 1 and F1C fimbriae that are
309 present in high proportions in human UPEC strains. P fimbriae recognise the α -D-galactopyranosyl-
310 (1,-4)- β -D-galactopyranoside receptor epitope in glycolipids abundant in kidney tissue and on
311 erythrocytes (Leffler and Svanborg-Eden, 1981). Type 1 fimbriae confer binding to α -D-
312 mannosylated proteins, such as uroplakins, which are abundant on bladder surfaces (Connell et al.,
313 1996). Finally F1C fimbriae recognise galactosylceramide targets present on epithelial cells in the
314 kidneys, ureters and bladders (reviewed in Klemm et al., 2007). These fimbriae recognise their
315 receptor targets by virtue of tip located adhesins, namely PapG, FimH and FocH (reviewed in
316 Klemm and Schembri, 2000).

317 In the present study the majority (75%) of the tested porcine pyelonephritogenic *E.*
318 *coli* strains expressed type 1 fimbriae corresponding well to human UPEC strains. Meanwhile, in
319 previous studies of porcine *E. coli* strains type 1 fimbriae was reported in 41% (yeast agglutination
320 and inhibition of agglutination by D-mannose) and 16% (mannose-sensitive haemagglutination) of

321 the strains, respectively (Carr and Walton, 1993; de Brito et al., 1999). However, these authors did
322 not pre-select for the type 1 expression by static growth as done in this work. Surprisingly, only one
323 of the porcine *E. coli* strains in this study may express functional P fimbriae (as monitored by
324 mannose-resistant agglutination of pig erythrocytes; however, since no agglutination of human
325 RBCs was observed this indicates an atypical adherence profile for P fimbriae) and only that strain
326 seemed to have the whole *pap* gene cluster (monitored by the presence of the whole *papB-G* gene
327 fragment as well as *papIB* and *papC*) on the chromosome. However, 10 strains (50%) had the *papG*
328 gene on the chromosome, which probably represent the remains of strongly eroded *pap* gene
329 clusters. The expression of P fimbriae in porcine pyelonephritis-associated *E. coli* strains have
330 never, as far as we know, been studied before, but a previous study of *E. coli* strains isolated from
331 the porcine urinary tract did not observe P fimbriated strains (Carr and Walton, 1992; Carr and
332 Walton, 1993). However, another study of 31 urine isolates reported the expression of P fimbriae as
333 identified by haemagglutination of pig and human erythrocytes as well as the presence of *papC*;
334 although 24 and 22 of the 31 strains agglutinated human and pig RBCs, respectively, only 5 of 17
335 *papC*⁺ strains exhibited haemagglutination of both erythrocytes indicative of P fimbriae while three
336 *papC*⁺ strains agglutinated pig RBCs but not human RBCs, similar to our pig isolate LK82,
337 suggesting the presence of fimbriae different from P fimbria (de Brito *et al.*, 1999). Finally, none of
338 the 20 porcine strains in our study had the *focH* locus, whereas FIC fimbriae are expressed in 14 to
339 30% of human UPEC isolates (reviewed in Klemm and Schembri, 2000; Klemm et al., 2007).

340 In addition to adhesins we also investigated the presence of a number of other
341 virulence and fitness factors linked to urovirulence in human strains. Haemolysin is important for
342 iron acquisition. The number of haemolysin producing strains among the tested strains was
343 expected to be high as many human pyelonephritis-associated UPEC are haemolytic but
344 surprisingly only one strain (5%) turned out to be haemolytic. In previous studies of porcine UPEC
345 the number of haemolytic strains varied from 0 to 25.8% (Carr and Walton, 1993; de Brito et al.,
346 1999). Analysis for the presence of the ferric yersiniabactin high affinity uptake system was
347 monitored by PCR of the receptor-encoding gene, *fyuA*, and revealed that 35% of the strains had
348 *fyuA*. In studies of human UPEC isolates, the presence of *fyuA* has been reported to be linked with
349 urovirulence (Schubert et al., 2002; Hancock et al., 2008). A high proportion (82-99%) of human
350 UPEC strains carries the *fyuA* gene (Takahashi et al., 2006; Houdouin et al., 2007). Although
351 flagella are not essential for virulence of human UPEC it has been demonstrated that flagella
352 contribute to the fitness of such strains (Wright *et al.*, 2005), and in the present study the majority of

353 the porcine UPEC were motile; specifically, the most motile strains were the ones displaying the
354 smallest number of other virulence traits, indicating that flagella might play a role for the virulence
355 of porcine UPEC.

356 The virulence factor profile in our 20 porcine pyelonephritis strains differed
357 significantly from that of human UPEC strains. Notably the presence of P and F1C fimbriae was
358 significantly lower as was haemolysin. In this context it is interesting to note that UPEC strains
359 from dogs and cats have virulence factor profiles that essentially match human UPEC strains
360 (Johnson et al., 2001; Johnson et al., 2008).

361 Phylogenetic analysis of our porcine pyelonephritis strains showed that all strains
362 belonged to clonal groups A (50%) and B1 (50%). In contrast, human studies have shown that
363 virulent extraintestinal strains mainly belong to groups B2 and D (Clermont *et al.*, 2000).
364 Interestingly, the phylogenetic profile of canine UPEC isolates is very similar to that of human
365 UPEC and demonstrates a preponderance of the B2 and D clones (Johnson et al., 2001; Johnson et
366 al., 2008). This is surprising in light of the fact that evolutionarily pigs are closer to humans than
367 dogs. Strains belonging to clonal groups A and B1 are characterised by extensive antibiotic
368 resistance and typically exhibit multiple drug resistance profiles (Johnson et al., 2005; Johnson and
369 Russo, 2005). It is therefore tempting to speculate that the high frequency of multi-drug resistant
370 clones in clonal groups A and B1 to which our porcine pyelonephritis strains belong might be a
371 reflection of indiscriminate use of antibiotics in pig farms (Ungemach et al., 2006).

372

373

374 **Acknowledgements**

375

376 This work was supported by grants from the Danish Medical Research Council (grant no. 271-07-
377 0291) and Lundbeckfonden (grant no. R17-A1603).

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472

Table 1

473

Primers used to amplify genes from the genome of porcine *E. coli* strains

| Gene | Primer | Sequence | PCR product length (bp) |
|---------------|--------|--|-------------------------|
| <i>chuA</i> | P763 | 5'-GACGAACCAACGGTCAGGAT-3' | 279 |
| | P764 | 5'-TGCCGCCAGTACCAAAGACA-3' | |
| <i>yjaA</i> | P765 | 5'-TGAAGTGTCCAGGAGACGCTG-3' | 211 |
| | P766 | 5'-ATGGAGAATGCGTTCCTCAAC-3' | |
| TspE4.C2 | P767 | 5'-GAGTAATGTCTGGGGCATTCA-3' | 152 |
| | P768 | 5'-CGCGCCAACAAAGTATTACG-3' | |
| <i>papB-G</i> | P528 | 5'-CACTCCCCTAAGGAGGTCTGCGAG-3' | ~9000 |
| | P539 | 5'-AATCCATTGCAGTCCACATAGC-3' | |
| <i>papG</i> | P474 | 5'-GCGGGGATATCGGCGGGGATTTCCAGACC-3' | 1096 and 1200 |
| | P513 | 5'-GCGCGGGATCCCATTTATGGCAATATCATGAGCAGC-3' | |
| | P475 | 5'-TGGTGACGGATCCTGACATTG-3' | |
| <i>papB</i> | P231 | 5'-GGGGAACCATGGCGCATCATGAAGTC-3' | 310 |
| | P232 | 5'-CTCCGGAATTCCATAATTTAGTCAAATG-3' | |
| <i>papIB</i> | P512 | 5'-GCGGGGAATTCTAAGTTGTGGAAGAACAGCTCTGCC-3' | 734 |
| | P529 | 5'-AAATGCATTTTCAGACATAGACC-3' | |
| <i>papC</i> | PLK1 | 5'-GACGGCTGTACTGCAGGGTGTGGCG-3' | 328 |
| | PLK2 | 5'-ATATCCTTTCTGCAGGGATGCAATA-3' | |
| <i>focH</i> | P297 | 5'-ATGCGTAAATATTATCCCCTC-3' | 603 |
| | P298 | 5'-GCCACCTGTCTGGATATAGAC-3' | |
| <i>fimB-H</i> | P590 | 5'-AAAAAATCTAGACAAGCTAACTCAGTGAGCGTGGTG-3' | 9614 |
| | P591 | 5'-AAAAAAGAGCTCGCGTACCTGCATTAGCAATGCCC-3' | |
| <i>fyuA</i> | P713 | 5'-GCGCCAAGCTTGTCTGACCGTTATCGCC-3' | ~2200 |
| | P714 | 5'-GCGCCGGATCCCGACTTTGCTGAGTTACTGG-3' | |

474

1 Table 2
 2 Characteristics of 20 porcine pyelonephritis-associated *E. coli* strains

| Strain | Corresponding kidney lesion ^a | Agglutination of | | PCR | | | | Phylogenetic group | <i>fyuA</i> | Siderophore production Size of halo (mm) ^f | Haemolysin production | Motility (mm) | Colicin production Inhibition MG1655 (mm) | No. of plasmids |
|--------|--|---|--------------------------|----------------------------|----------------------------|-------------|-------------|--------------------|-------------|--|-----------------------|---------------|--|-----------------|
| | | Yeast cells after pellicle formation ^b | Blood cells ^c | <i>fimB-H</i> ^d | <i>papB-G</i> ^e | <i>papG</i> | <i>focH</i> | | | | | | | |
| LK4 | A | + | - | - | - | + | - | B1 | + | 15 | - | - | - | |
| LK7 | A | + | - | - | - | - | - | B1 | - | 28 | - | - | 1.5 | 2 |
| LK8 | A | + | - | - | - | - | - | B1 | - | 29 | - | - | 1.75 | 2 |
| LK13 | A | - | - | - | - | + | - | A | - | 12 | - | 63 | - | 3 |
| LK15 | A | + | - | - | - | + | - | A | + | 26 | - | 13 | 2 | 2 |
| LK16 | CA | + | - | - | - | - | - | A | + | 28 | - | - | 2 | 0 |
| LK53 | CA | + | - | - | - | - | - | B1 | - | 29 | - | 11 | 1 | 4 |
| LK54 | A | + | - | - | - | - | - | B1 | - | 30 | - | 23 | 1.75 | 4 |
| LK57 | CA | + | - | - | - | - | - | B1 | + | 30 | - | - | - | 1 |
| LK63 | C | + | - | - | - | - | - | B1 | - | 12 | - | 44 | - | 0 |
| LK65 | CA | + | - | - | - | + | - | A | - | 16 | - | 17 | - | |
| LK67 | A | - | - | - | - | + | - | A | - | 11 | - | 31 | - | 0 |
| LK68 | A | + | - | - | - | + | - | A | - | 12 | - | 15 | - | 0 |
| LK69 | CA | + | - | - | - | + | - | A | - | 12 | - | 19 | - | |
| LK75 | A | + | - | - | - | - | - | B1 | + | 25 | - | 12 | 1.75 | 0 |
| LK76 | A | + | - | - | - | - | - | B1 | + | 26 | - | 10 | 1.75 | 0 |
| LK82 | CA | + | -/+ | - | + | + | - | B1 | + | 17 | + | 27 | - | 2 |
| LK85 | A | - | - | + | - | + | - | A | - | 16 | - | >90 | - | 0 |
| LK86 | CA | - | - | + | - | + | - | A | - | 17 | - | >90 | - | 0 |
| LK87 | CA | - | - | + | - | - | - | A | - | 15 | - | - | - | 1 |

3 ^aA: acute, C: chronic and CA: chronic and acute kidney lesions

4 ^bResults from bacteria grown in LB. After growth in human urine all strains showed negative results

5 ^cMannose-resistant haemagglutination. Strain LK82 showed weak positive agglutination with pig RBCs but not with human RBCs

6 ^dPCR performed on strains that did not agglutinate yeast cells after pellicle formation

7 ^eThe results display presence/absence of *papB* and *papC* for all strains. PCR of *papIB* and the whole *papB-G* gene fragment was performed on
 8 12 and 3 of the 20 strains (including LK82), respectively, with the same results as displayed

9 ^fThe capacity for siderophore production was indicated by the ability of the strains to make orange halos on blue chrome azurol S (CAS) agar
 10 after incubation at 37°C for 16 h

1 **Figure captions**

2

3 Fig. 1. The three fimbrial gene clusters (the *fim*, *pap* and *foc* clusters) encoding the UPEC-
4 associated type 1, P and F1C fimbriae. The arrows indicate approximate positions for the primers of
5 the three gene clusters listed in Table 1. The genes are drawn to scale but the primers are enlarged
6 for better resolution.

7

8 Fig. 2. (A) A kidney with both acute and chronic primarily polar distributed lesions. (B) Cut surface
9 of a kidney. Acute lesions are present in both cortex and medulla.

10

11 Fig. 3. Growth of 20 porcine pyelonephritis-associated *E. coli* strains in human urine. (A) The final
12 OD and (B) the generation time of the 20 strains as means of three independent experiments each in
13 triplicates. The error bars indicate standard deviations.

14

15 Fig. 4. Siderophore production by strains LK85, LK75 and LK76 indicated by orange halo
16 formation on blue chrome azurol S agar plates. The two strains LK75 and LK76 both contain the
17 *fyuA* gene while LK85 does not.

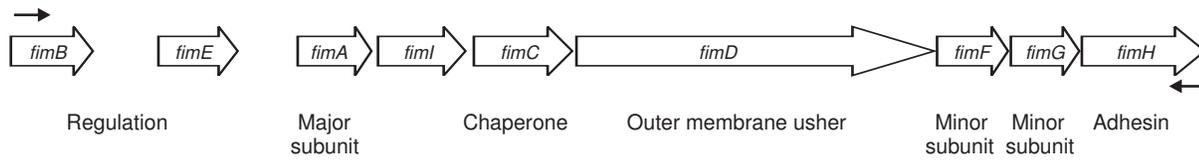
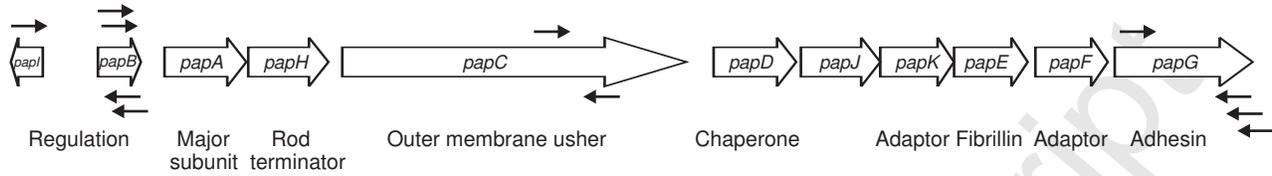
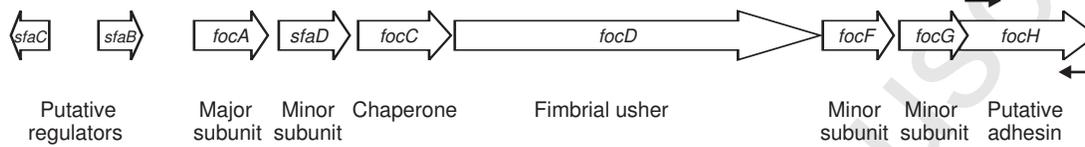
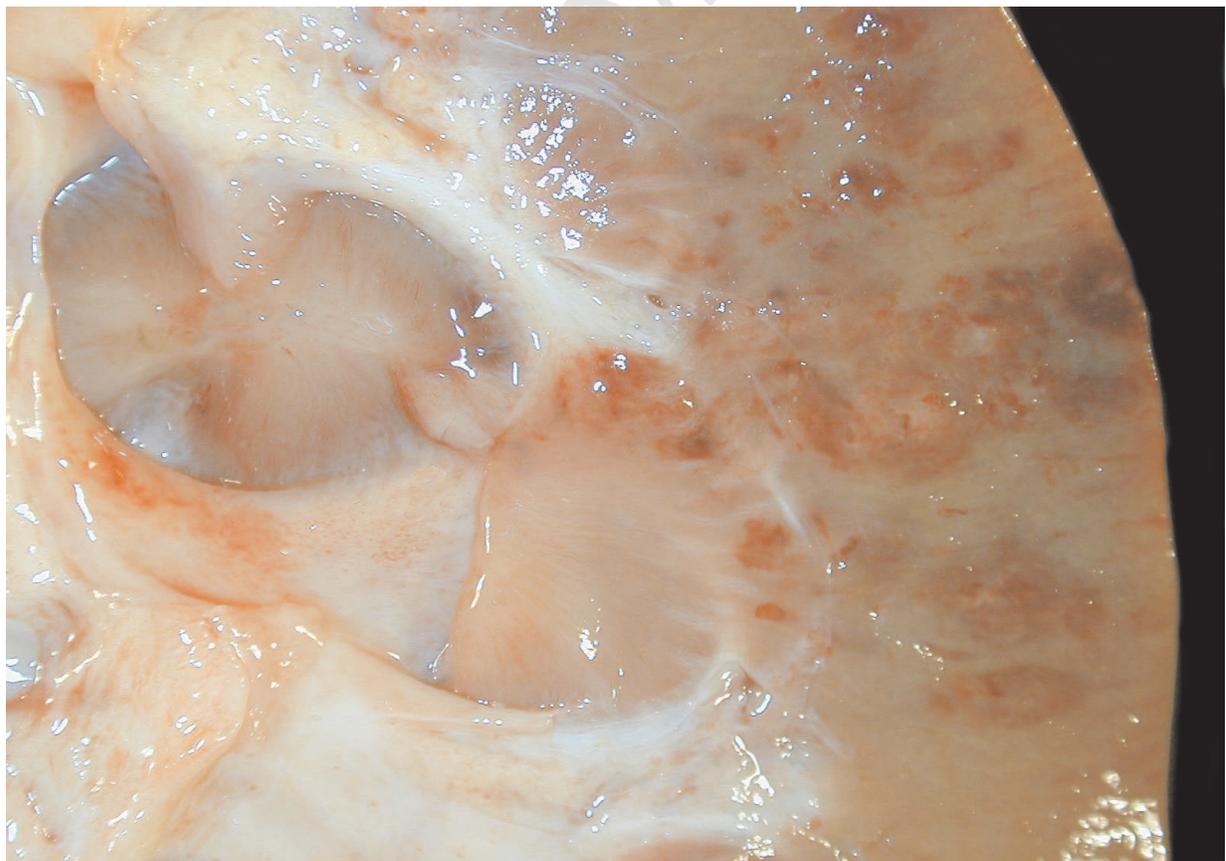
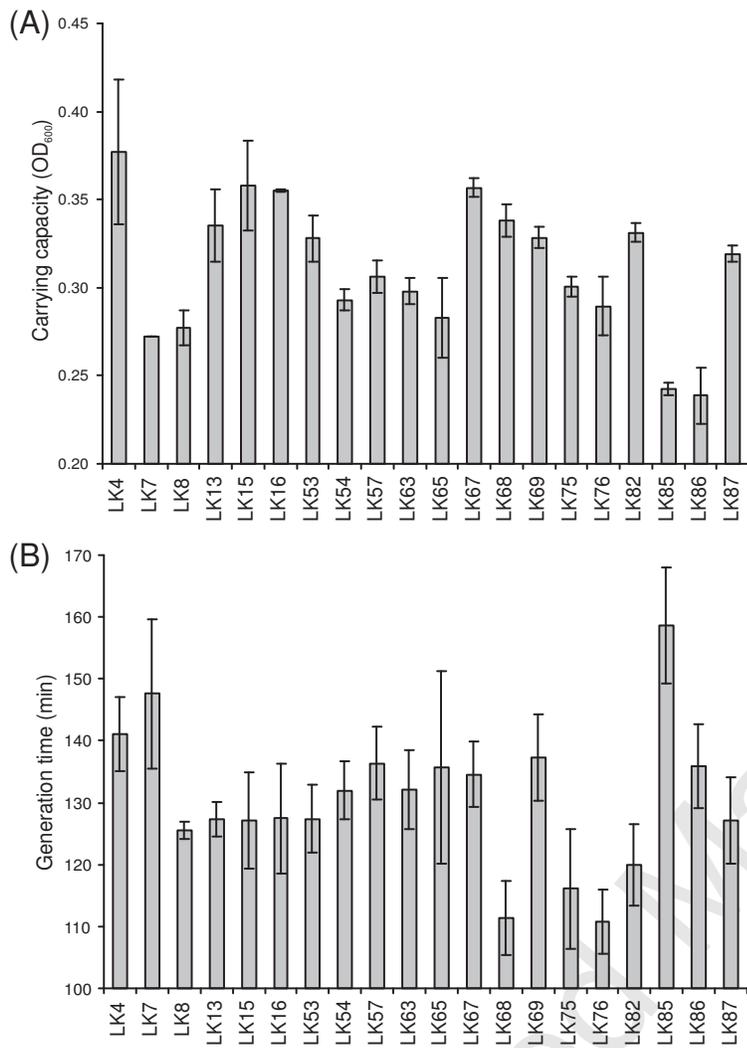
The *fim* clusterThe *pap* clusterThe *foc* cluster

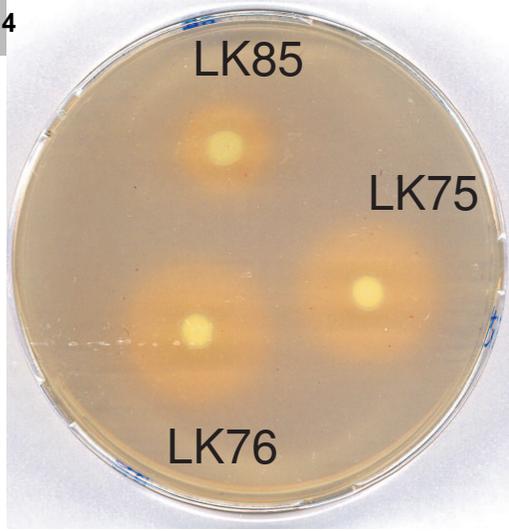
Figure 2 (A)



(B)







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