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Biofilm-related characteristics in clinical isolates of Extraintestinal Pathogenic *E. coli*

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Abstract

The rapid increase in antibiotic resistance is one of the leading global health issues at present time. Bacteria's resistance to antibiotic agents can be a thousand times higher when they are embedded in an extracellular biopolymer matrix, defined as biofilm. The biofilm state is a major driver of persistent infections, offering a challenge in providing adequate treatment. Biofilm producing *E. coli* is a common cause of infections at extraintestinal sites, like the urinary tract or the bloodstream. By investigating the biofilm phenotype of clinical ExPEC isolates and correlating it to other phenotypic and genotypic traits, we aim to generate novel knowledge in this field, and contribute to predict the clinical manifestation, severity, and treatment of such infections.

The biofilm forming capacity of the strains was evaluated both qualitatively and quantitatively. The presence of major biofilm matrix components, curli and cellulose, was investigated by staining with Congo Red and Calcofluor. Additional quantitative measures of number of CFUs contributing to the biofilm, total biomass on pegs and in wells were conducted. Genotypic characterization of the strains ST, *fimH/fumC* allele, serotype, plasmids, virulence factors and resistance genes were conducted by uploading the strains genome to specific webtools. Additionally, the strains motile and haemolytic behaviour was investigated. These traits were analysed for correlation to their biofilm phenotype.

Based on the results from this project, we conclude that total biomass is a better measure of biofilm abundance, compared to number of bacteria in the biofilm. Expression of curli and cellulose is widespread in the included strains, and it may seem that cellulose expression is more related to the ability to produce biofilm, than the expression of curli. We also discovered a variety of biofilm forming capacities across STs and clades. In short, presence of β-lactamases, *pap* genes, *lpfA, usp, iss* and flagella-mediated motility were associated with biofilm formation in this study.

Our results display preliminary trends within biofilm formation in clinical ExPEC isolates, with a potential for further investigation. We suggest that complementation of genotypic analyses and effects of knock out mutants is further investigated with a bigger sample size, to better understand the effects these traits exhibit on biofilm formation.

List of abbreviations

3rd CEP	Third generation cephalosporins
APEC	Avian Pathogenic *E. coli*
CFU	Colony Forming Units
CV	Crystal Violet
E. coli	*Escherichia coli*
ESBL	Extended Spectrum β-lactamase
ExPEC	ExtraIntestinal Pathogenic *E. coli*
IBC	Intracellular Bacterial Communities
LB	Lysogeny Broth
MDR	Multi Drug Resistance
MH	Müeller-Hinton
MIC	Minimum Inhibitory Concetration
MLST	Multi Locus Sequence Typing
NORM	The Norwegian Surveillance System of Antimicrobial Drug Resistance
OD	Optical Density
PBS	Phosphate Buffered Saline
PGA	β-1,6-N-acetyl-D-glucosamine polymer
rdar	Red, dry and rough
rpm	Rounds per minute
SD	Standard Deviation
ST	Sequence Type
UNN	University Hospital of Northern Norway
UPEC	UroPathogenic *E. coli*
UTI	Urinary Tract Infection
WGS	Whole Genome Sequencing

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1 Introduction

The past year we have experienced the devastating consequences of a pandemic. The spread of SARS-CoV-2, the virus causing the COVID-19 disease, has demonstrated how fast pathogenic microorganisms can spread from country to country, continent to continent, until it affects the entire world population. Although not as fast, bacterial strains with increasing resistance towards antibiotics is also spreading around the world. Without drastic action, there is a new pandemic sneaking up on us: antibiotic resistance and a post-antibiotic era where people once again could die of a common infection.

Escherichia coli is a commensal bacterial species that usually is a part of the human gut flora, but some strains can also cause disease, both in and outside of the intestines. The *E. coli* lineages related to foodborne diseases are a major cause of death in developing countries, especially among children (1). *E. coli* is also a leading cause of infections in the urinary tract and the bloodstream. Between 15%-40% of bloodstream infections can be ascribed to *E. coli* (2). The in-hospital mortality rate for bloodstream infections caused by *E. coli* is estimated to be between 5%-30%, much due to the increase in antibiotic resistance (3). According to the 2019 Surveillance Report of Antimicrobial Resistance in Europe, 57.1% of the *E. coli* isolates were resistant to at least one of the antibiotic classes under surveillance (aminopenicillins, carbapenems, third-generation cephalosporines, aminoglycosides and fluoroquinolones) (4). The most common portal of entry for bloodstream isolates of *E. coli* is the urinary tract (3).
E. coli is responsible for 80% of the community acquired urinary tract infections (UTIs), and 40% of the nosocomial UTIs, the latter often following catheterization (5). Recurrent UTIs and catheter related UTIs have been associated with biofilm production (6).

The definition of a biofilm is a community of microorganisms embedded in an extracellular polymeric substance attached to surfaces, or each other (7). The ability of microbes to produce biofilm is a major driver of persistent infections, as it offers increased tolerance to stress, antibiotics and host immunological defences (7, 8). Hence, the biofilm production offers a challenge for the treatment of these types of infections and increased knowledge about the biofilm way of life will hopefully contribute to combat them.

1.1 *Escherichia coli*

E. coli are Gram-negative, rod-shaped bacteria which are extensively examined. *E. coli* is one of the most studied bacteria and is frequently used as a model organism. Its small sized genome makes it relatively easy to implement genetic analysis (9). With a generation time of approximately 20 minutes, one can obtain an isolated colony deriving from the same cell overnight. This rapid growth in several low-cost media, makes *E. coli* favourable as a model organism (9).

Harmless commensal strains of *E. coli* are a part of the human gut microbiota, although some lineages can cause infection both in and outside of the intestines (10). A subset of *E. coli* termed Extraintestinal Pathogenic *E. coli* (ExPEC) is a common cause for infections at extraintestinal sites like the urinary tract, the prostate, or the bloodstream (11). An additional subgroup of ExPEC, called UroPathogenic *E. Coli* (UPEC) is responsible for the majority of ExPEC infections, which are UTIs (12).
UTIs are considered to be one of the most frequent infections worldwide, both community- and hospital-acquired (13). About half of all women will experience at least one UTI in their lives (14). Relapses of UTIs are common, and have previously been related to biofilm formation (6). Uncomplicated, recurrent UTIs are frequent, but a proportion of these infections can evolve to a more severe course of disease. A UPEC infection commonly initiates with infection of the urethra, often originating from the fecal flora. From the urethra, the pathogens can migrate upwards the urinary tract to infect the bladder, kidneys and in severe cases, the systemic circulation (13, 15). Although the majority of UTIs are acute and uncomplicated, the high incidence and possible severity makes it a societal and personal burden, in terms of both economy and morbidity (13). A study from France estimated an annual social cost of UTIs to €58 million (16).

ExPEC is also one of the most frequent causative agents of blood stream infections in high-income countries. The increase in blood stream infections caused by multidrug resistant ExPEC strains, offers a challenge in providing effective antibiotic therapy. This results in increased mortality and longer hospital stays (17). A study conducted at a hospital in Rome, Italy, reported that bloodstream infections caused by multi drug resistant (MDR) *E. coli* often resulted in inadequate initial antibiotic treatment. Subsequently, this resulted in longer hospital stays (+6 days), more costs (+ €4,322.00) and higher 21-day mortality rates (from 5.6% to 40.7%),

compared to the patients receiving adequate initial antibiotic treatment (17). The extensive frequency of ExPEC infections, combined with the dissemination of multidrug resistance (MDR) within ExPEC strains, makes them a challenge within global health care and the battle against antibiotic resistance (15).

1.2 Antibiotic resistance

Since Sir Alexander Fleming first discovered penicillin in 1928, a golden era of antibiotics followed, with the introduction of several new drug classes that showed antibiotic activity (18). However, the last few decades, the discovery of new antibiotic classes have diminished drastically. Meanwhile, misuse of existing antibiotic agents has led to bacteria adapting and developing resistance as a response to increased exposure to antibiotics (18). It is a race between the development of new antibiotics and the development of resistance in bacteria. Antibiotic resistance leads to increased mortality, morbidity, and medical costs in the entire world (19). The European Commission estimated that antibiotic resistance is responsible for 33,000 deaths and a cost of €1.5 billion per year in the European Union (20).

Antibiotic resistance is widespread in *E. coli*, both Intestinal Pathogenic *E. coli* and ExPEC (10). Especially alarming, is the rapid emergence of resistance towards third generation cephalosporins (3rd CEP) and fluoroquinolones among ExPEC isolates, as these are used as last resort antibiotics (21). Resistance towards fluoroquinolones is mainly due to mutations in *gyrA* and *parC* genes (22). These mutations alter the fluoroquinolone target and thereby decreases its bactericidal activity. Resistance towards 3rd CEP is mainly due to the production of extended-spectrum-β-lactamases (ESBL). β-lactamases are enzymes that cleave the characteristic β-lactam ring of certain antibiotic agents, like e.g. penicillin and cephalosporins (10). Some bacteria produce β-lactamases specific for penicillin, some for cephalosporins, and some that targets both, thereby termed ESBL (23). In 2019, The Norwegian Surveillance System of Antimicrobial Drug Resistance (NORM) (24), reported that 7.1% of *E. coli* blood culture isolates produced ESBL, while the corresponding number for UPEC isolates was 3.0%. Among the ESBL producers, 65.8% of the blood culture isolates and 64.6% of the urinary tract isolates were additionally resistant towards ciprofloxacin, a fluoroquinolone agent.

Norwegian guidelines recommend nitrofurantoin, mecillinam or trimethoprim as first-line treatment against uncomplicated UTIs (25). Because of emerging resistance to fluoroquinolones, like ciprofloxacin, this agent is recommended as a last resort option (25).

According to the 2019 NORM report (24), only 0.9% of UPEC strains are resistant towards nitrofurantoin, while 3.9% are resistant to mecillinam, 23.8% towards trimethoprim and 8.3% towards ciprofloxacin. The antibiotic resistance is widespread also among sepsis isolates. In Norway, sepsis originating from the urinary tract is generally treated with ampicillin and gentamicin, alternatively cefotaxime (a 3rd CEP) or ciprofloxacin (26). Among the *E. coli* blood culture isolates collected by NORM in 2019 (24), 43.3% showed resistance towards ampicillin, 5.9% towards gentamicin, 7.2% towards cefotaxime and 11.3% against ciprofloxacin. It is commonly referred to the antibiotic resistance pattern of pathogens in a planktonic state, but bacteria embedded in a biofilm matrix can be a thousand times more resistant to antibiotic agents than their planktonic form (27). This drastic tolerance to antibiotics has made it an increasing field of interest over the last decades (28).

The level of antibiotic resistance in a biofilm depends on both the species-antibiotic relationship and the stage of biofilm development (6). There are several mechanisms for this phenomenon. Some antibiotics are unable to penetrate the biofilm matrix, or they penetrate too slowly relatively to the antibiotic lifetime (6, 7, 11). Alternatively, bacterial cells undergo a much slower growth rate in a biofilm than in their planktonic state. The slow growth rate and reduced metabolic activity, make them less susceptible to antibiotics that target growing bacteria, like penicillin and other β-lactam antibiotics (6, 7, 11). The presence of such metabolically inactive cells, called persister cells, allows for biofilm regrowth when the exposure of antibiotics is discontinued, like illustrated in Figure 1.1 (7, 28).

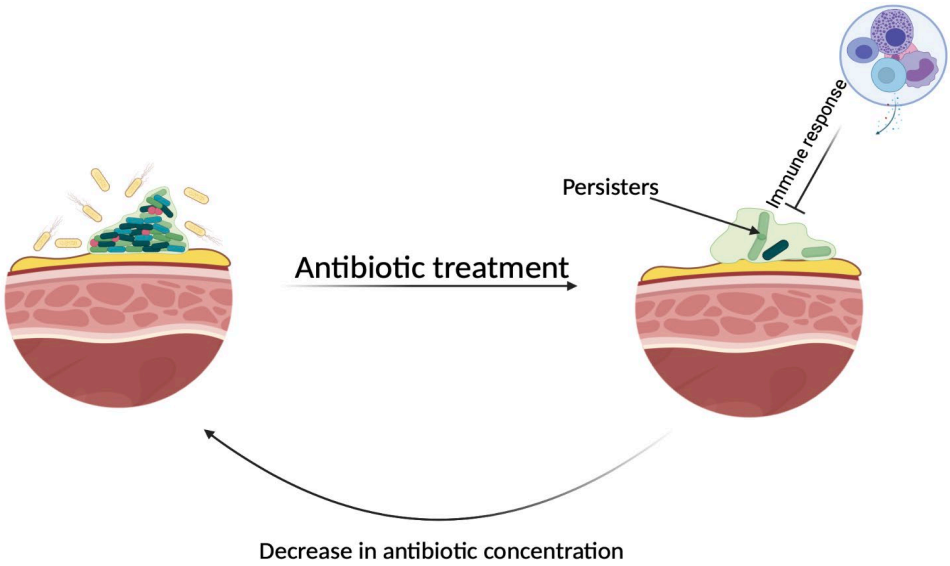

Figure 1.1. Model of antibiotic resistance in biofilms based on persister cells. Based on (28). Antibiotic therapy kills most of planktonic and biofilm-encapsuled cells. The immune system kills surviving planktonic cells, but the persisters in the biofilm are protected by the biofilm matrix, allowing for regrowth and recurrence of infection. Figure created in BioRender.com.

The antibiotics can also be inactivated because of environmental changes in the biofilm like for example pH (6). Additionally, the increased transfer of resistance genes within the bacterial community also contributes to the increased resistance in biofilm (7). The increased resistance to antibiotics and host immunological defences due to biofilm formation, largely contributes to the initiation and persistence of ExPEC infections, which is caused by a wide spectre of *E. coli* lineages.

1.3 *E. coli* lineages

ExPEC comprises many *E. coli* lineages with a variety of genotypic and phenotypic traits. Their common characteristic is the acquisition and expression of virulence-associated genes, allowing them to invade extraintestinal sites and cause infection (10). The study of these genetic characteristics in relation to the infectious organism, may contribute to prediction of the clinical manifestation, occurrence, and severity of the infectious disease (12).

E. coli can be classified in multiple ways, both phenotypic and genotypic. Most of *E. coli* strains used in the current study have been previously classified into subdivisions based on information from whole genome sequencing (WGS). From this data we have information on the serotypes, sequence types (ST) and clades (29). Division into serotypes was originally based on phenotypic tests to determine O, H and K antigens presented on the outside of the bacterial cell, with a focus on O- and H- antigen for epidemiological surveillance (30).

Multi Locus Sequence Typing (MLST) is another common way to classify *E. coli* lineages. MLST is a phylogenetic way to characterize different isolates within the same species. This is done by sequencing a number of house-keeping genes on the chromosome. House-keeping genes are genes required for basic cellular function. They are expressed in all cells of a species, but different isolates can have distinct alleles of these genes. The isolate is assigned a sequence type (ST), based on the allelic profile (31).

ExPEC comprises many STs, although a limited number of these are major contributors to human infections (32).
An ST can partially contribute to predict an isolate's likely virulence, pathogenicity, and resistance pattern (33). In this study, three different STs were included, each presented below. The STs are selected because they are representative of very successful STs in human infection.

Additionally, the strains are selected based on their resistance towards tetracycline and their production of β-lactamases, to investigate if this affects the ability to produce biofilm. Tetracycline belongs to a group of structurally related compounds, which exhibit a bacteriostatic effect by binding to the bacterial ribosomes and inhibiting the protein synthesis. Tetracycline is a broad-spectrum antibiotic, and tetracycline-resistance is widespread in *E. coli* (34). The β-lactamase genes present in the included strains, belong to class A (*bla*$_{TEM-1B}$, *bla*$_{CTX-M14}$, *bla*$_{CTX-M15}$ and *bla*$_{CTX-M27}$) or class D (*bla*$_{OXA-1}$) (35).

ST131

E. coli ST131 is one of the most common ExPEC lineages and a major cause of ExPEC infections, like UTIs and blood stream infections, worldwide (10). It is further divided into subgroups, called clades. This is based on clade specific single nucleotide polymorphism, *fimH* alleles and corrected for phylogenetic clade membership (29). ST131 mainly consists of the clades A, B and C with further subdivision of clade C into C1 and C2, among other (10, 32).

ST131 is considered a very successful lineage, as it is associated with MDR and being responsible for a globally increased resistance to fluoroquinolones and 3rd CEP (22). The importance of these antibiotic agents in treatment of serious infections, makes this a global health issue (21).

The increase in resistance towards 3rd CEP in *E. coli* ST131, is due to the production of ESBL (21). Among the ESBL-producing *E. coli* from the 2019 NORM report, 56.7% belonged to ST131 (24). ST131 is included in this project as it is one of the most common STs causing extra intestinal infections, and as it is associated with the global emergence of MDR.

ST648

ST648 is a nascent lineage, with a potential to follow ST131's success (36). It is among the top ten most abundant *E. coli* STs causing extraintestinal infections (32), despite the observation that it commonly lacks virulence factors associated with UTI, like adhesion-factor genes such as the *pap*-operon (36). Similar to ST131, it is associated with MDR, including resistance towards fluoroquinolones and 3rd CEP (33). Its resistance towards 3rd CEP is ascribed to the carriage of plasmids encoding the CTX-M-15 type of ESBL. These ESBL-plasmids seem to enhance virulence in both ST131 and ST648, contributing to their successful global spread (37). Biofilm is an important survival strategy for this ST (36). The association with increased biofilm formation is the reason for the inclusion of ST648 in this study.

ST73

ST73 is a common ST among ExPEC isolates, associated with both UTIs and blood stream infections (38). It is found to be overrepresented in UTI samples compared with faecal samples (39). It was one of the most predominant STs in the two ECO SENS studies, performed in 1999-2000 (40) and 2007-2008 (41). The ECO SENS project is a survey of the prevalence and antibiotic susceptibility of urinary tract pathogens in Europe (40). A consecutive study done in 2011 by Bengtsson *et al.,* collected *E. coli* isolates from the two ECO SENS studies, originating from Greece, Portugal, Sweden, and UK. The selection of strains was based on their susceptibility towards most clinically relevant antibiotics. They found that most of the strains were also plasmid-free. This implies that the alarming rise in ST73 strains carrying MDR-plasmids are recent (38). Alhashash *et al.* suggested that the increase in MDR ST73 strains is not due to the spread of one dominant clone like for ST131, as the strains carry a diverse set of resistance plasmids (42).

ST characteristic and pathogenicity is partially related to the acquisition and expression of various virulence-associated genes and the ability to produce biofilm. Below I will review some central ExPEC virulence factors.

1.4 ExPEC virulence

Virulence is clinically defined as a microorganisms ability to cause disease in the host (43). Hence, virulence factors are factors that are associated with a pathogen's virulence, its potential to cause disease and to avoid the hosts defence mechanisms (44). The prevalence of a virulence factor in clinical isolates should be examined, in order to assess its clinical relevance (45). With an increased dissemination of antibiotic resistance, virulence factors are potential targets for development of new therapeutic drugs (13). A summary of central ExPEC virulence factors with a focus on those involved in biofilm formation is presented in Table 1.1.

Table 1.1. Central ExPEC virulence factors classified by their function. The classification is based on (15) and (12). Underlined virulence factors are suggested to be important for biofilm formation.

Category	Gene	Protein	Reference
Adhesins	*pap*	P fimbriae	(12).
	fim	Type 1 fimbriae	
	afa	Afimbrial adhesin	
	lpfA	Long polar fimbriae	
	sfa	S fimbriae	
	foc	F1C fimbriae	
	csg	Curli (biofilm component)	
Toxin	*hlyA*	α-haemolysin	(12).
	cnf1	Cytotoxic necrotizing factor 1	
	usp	Uropathogenic-specific protein	
Nutrition	*fyuA, iuc, iroN, ireA*	Siderophores	(12).
	chuA	Heme receptor	
Immune evasion/Survival	*kpsMT II/III*	Group II/III capsular polysaccharide synthesis	(12).
	iss	Increased serum survival	
	traT	Serum-survival associated	
Other	*fliC*	Flagellin variant	(15).
Biofilm matrix	*csg operon*	Curli	
	bcs operon	Cellulose	
	pga operon	β-1,6-N-acetyl-D-glucosamine polymer (PGA)	

Although not usually listed as a virulence factor, the central role of biofilm in colonization and infection makes it highly relevant in the pathogenesis and virulence of ExPEC strains. Hence,

biofilm along with haemolysins and capsules, two central ExPEC virulence factors, will be further elaborated in the next sections.

1.4.1 Biofilm as a virulence factor

Bacteria can exist in two modes of growth; planktonic, or in a community embedded in an extracellular biopolymer matrix, called biofilm. The planktonic state is essential for rapid proliferation, whereas the biofilm state is important for persistence (7). When bacteria live in a biofilm, they will express genes necessary for adhesion and biofilm matrix production and therefore exhibit an altered phenotype compared to planktonic cells (11). Bacteria in a biofilm are commonly heterogenous and express different phenotypes, in response to variations in for example pH, access to nutrients and oxygen in microenvironments within the biofilm (46). This results in a versatile community, well-equipped to survive various stress and environmental factors (7). *E. coli* is a common biofilm forming pathogen, and this virulent behaviour plays a particularly large role in the causation of urinary tract infections, but also in the outcome of bloodstream infections (6, 47).

The development of an *E. coli* biofilm is a complex process, simplified in Figure 1.2. It initiates with primary adhesion to a biotic or abiotic surface (Step 1, Figure 1.2). To overcome the repulsive forces when approaching a surface, flagella-mediated motility has proven to be an important trait (8). Flagella are appendages on the bacterial cell, mediating active bacterial motility, see Figure 1.3 (13). There are different mechanisms of motility, but only the «swimming» and «swarming» type have been correlated to the presence of flagella (48), as it works as a rotating motor. While swimming is the action of a single bacterium, swarming is the term used for a group in motion (48).

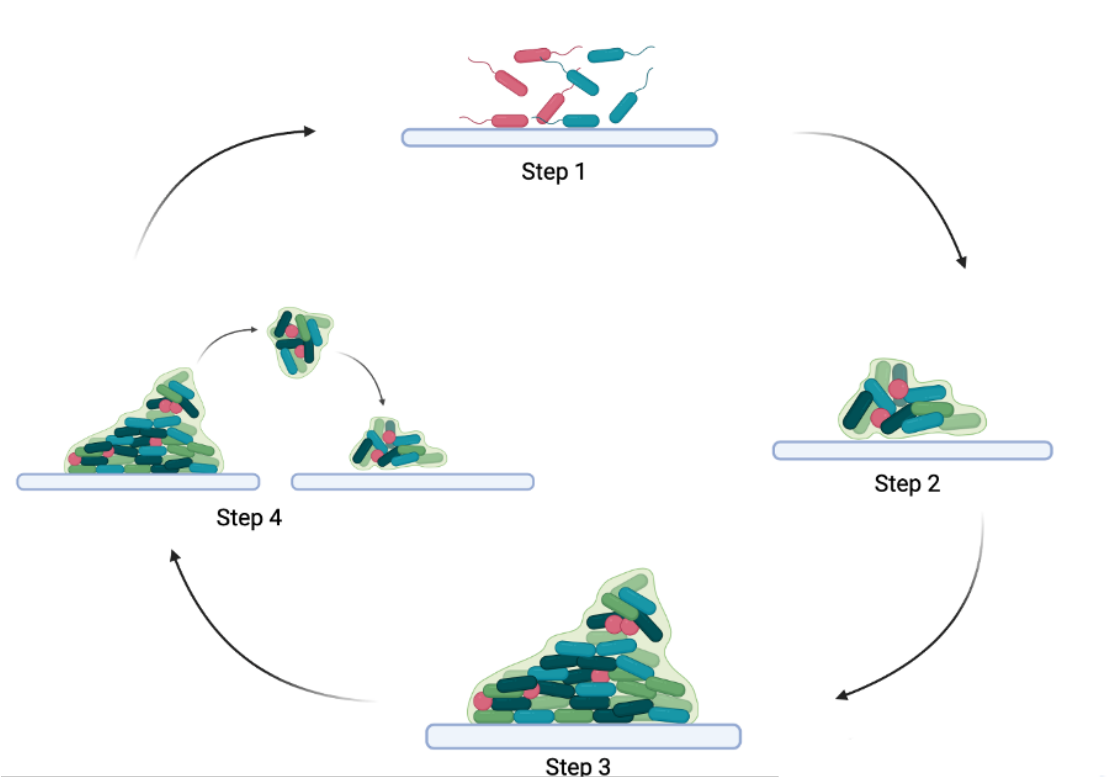

Figure 1.2. A simplified illustration of the main steps involved in biofilm formation. Initial contact with the surface is followed by adherence, biofilm maturation and dissemination. Figure created in BioRender.com

To further adhere irreversibly to the surface, type 1- and type P-fimbriae have proven to be important for host recognition and adhesion (Step 2, Figure 1.2) (8). The type 1- and P-fimbriae are both assembled by the chaperone-usher pathway (15). Type 1-fimbriae are encoded by the *fim* operon which is constitutively expressed, while the type P-fimbriae are encoded by the *pap* operon, a part of a pathogenicity island (13). The type 1-fimbriae are suggested to play an important role in colonization of the bladder, while the type P-fimbriae are associated with pyelonephritis and infection of the kidney (15). The tip of the fimbriae is the actual adhesin protein, encoded by *fimH/papG*, respectively (14), see Figure 1.3.

When attached to the surface, the cells alter their phenotypic expression to produce biofilm matrix components and develop a mature biofilm. They create microcolonies, by recruiting new cells or aggregating with already attached cells, followed by production of extracellular matrix components (7). As the biofilm matures, it results in a three-dimensional structure (Step 3, Figure 1.2) (6, 8). Primarily, the biofilm matrix serves a protective role, by shielding bacteria from host defence recognition and as a barrier to biocides (8). Additionally, it serves a structural role, keeping the adherent cells close to each other and the surface (8). The biofilm matrix

consists of proteins, exopolysaccharides and nucleic acids (49). The secreted polysaccharides cellulose and β-1,6-N-acetyl-D-glucosamine polymer (PGA) have proven to be major constituents of the *E. coli* biofilm matrix (8, 49, 50). The proteinaceous curli fimbriae is a bacterial appendage that is a hallmark for formation of the *E. coli* biofilm matrix (Figure 1.3). Curli is an extracellular amyloid fiber assembled on the surface of many Gram-negative bacteria, including *E. coli* (51). It is composed of a major subunit CsgA and a minor subunit CsgB (8). Curli, cellulose and PGA all contribute to biofilm formation by exhibiting cell to cell contact, cell-surface interactions, and adhesive properties (8, 52). Some strains even exhibit a curli-dependent biofilm formation, making curli a possible target in the development of anti-biofilm agents (51). Curli has also been suggested to induce internalization by eukaryotic cells (53). In *E. coli,* expression of cellulose requires the *bcsABZC* operon, and PGA is encoded by the *pgaABCD* operon, while expression of curli is encoded by the *csgDEFG* and *csgBAC* operons (8, 50).

Bacterial cells can detach from the mature biofilm, reenter the planktonic state and migrate to other surfaces, initiating biofilm production there (Step 4, Figure 1.2) (7). Similar to the surface-attached biofilm, UPEC can adhere to, and invade, bladder epithelial cells and form persistent, biofilm-like reservoirs, named intracellular bacterial communities (IBC) (54). They follow the same stages in development as previously described, beginning with initial adherence mediated by type 1-fimbriae and a FimH-dependent internalization by bladder cells as a mechanism to avoid host defences (54). This is subsequently followed by biofilm matrix production, biofilm maturation, detachment of cells and establishment of new IBCs (54).

Biofilm forming UPEC isolates are responsible for the majority of catheter-related infections and recurrent UTIs (54). The biofilm success of UPEC is attributed to the interplay of various virulence factors expressed, including the ones mentioned and illustrated in Figure 1.3. An example is adherence to the bladder epithelium by type 1- and type P-fimbriae, which is an important property that distinguish UPEC from other types of *E. coli*. By adherence to the bladder epithelium, the bacteria can persist the defence mechanisms of the bladder, like for example the urine flow and immune responses (13, 14).

Biofilm forming capacities and the expression of curli has also been suggested to play a role in the development of blood stream infections. In a study conducted by Zhang *et al.,* biofilm forming *E. coli* from patients with bacteraemia, resulted in a worse clinical outcome than the

non-biofilm formers (47). Bian *et al.* found that more than 50% (24 out of 46) of human *E. coli* sepsis isolates were able to produce curli in vitro at 37 ℃ (55, 56).

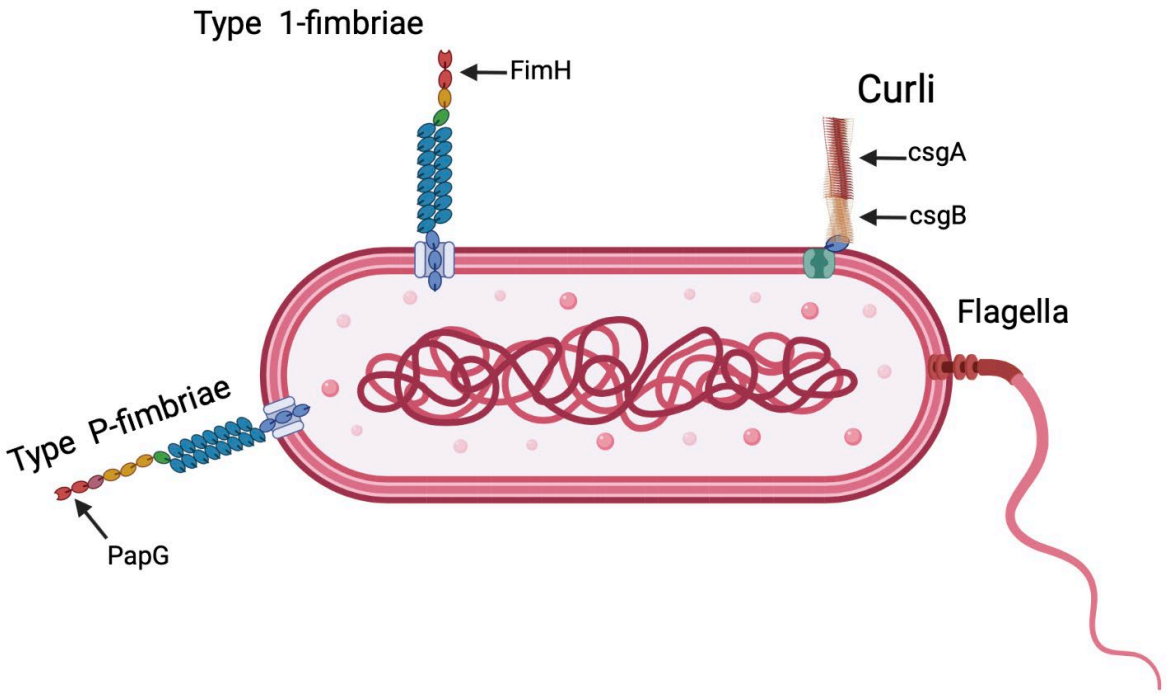

Figure 1.3. Illustration of relevant *E. coli* appendages important for biofilm formation. The figure is based on a figure presented in (13). Figure created in BioRender.com.

1.4.2 Other virulence factors

α-haemolysin

Characteristic for UPEC isolates is the secretion of the haemolytic toxin α-haemolysing, encoded by *hlyA* (15). α-haemolysing can cause lysis of red blood cells, but it also possesses cytotoxic properties against a wide variety of cell types, which can lead to tissue damage (57). α-haemolysing contributes to pore formation in the epithelial cells of the bladder, facilitating uptake of iron and nutrition of the bacteria (15). In addition, α-haemolysing activates exfoliation of the bladder epithelial cells. This is a host defence mechanism to flush the bacteria out in the urine, but it also exposes deeper layers of the bladder tissue, making them available for colonization by the bacteria (13). The expression of α-haemolysing in UPEC isolates has proven to be correlated with the severity of UTI, as α-haemolysing expression is present in up to 78% of the pyelonephritis cases (57).

Capsules

E. coli produce high-molecular weight surface polysaccharides, which constitutes a surrounding capsule, also called K-antigen (58). The capsule is a virulence factor, protecting the cell from opsonization and phagocytosis by the host immune system (58). The 80 different capsular serotypes are classified into 4 distinct group, where group II and III share the same assembly system, and are the ones associated with ExPEC (58). The release of group II capsular polysaccharides has been suggested to weaken adhesion to the surface and also cell-cell interactions and thereby inhibiting biofilm formation (59). This have been suggested as a strategy to prevent biofilm formation on abiotic surfaces, like indwelling catheters (8).

2 Aims and hypothesis

The production of biofilm provides a challenge in treatment of ExPEC infections, like UTIs and blood stream infections. Biofilm offers an increased tolerance to host immune defenses and antibiotic therapy. Hence, increased knowledge about the biofilm phenotype is needed to better combat these infections.

The aim of this study was to contribute to this knowledge gap. By correlating the biofilm production to phenotypic and genotypic traits of clinical ExPEC isolates, we aim to generate novel knowledge in this field. Based on previous studies, we formulated the following hypotheses:

- Expression of major matrix components curli and cellulose are associated with more biofilm formation compared to isolates not expressing curli and cellulose.
- Expression of curli and cellulose at 28℃ is a hallmark for UTI isolates, while expression at 37℃ is more pronounced in isolates from blood stream infections.
- ST648 is a better biofilm former compared to other ExPEC STs.
- Motile strains produce more biofilm than non-motile strains.
- α-haemolytic activity increases biofilm formation
- Expression of β-lactamases inhibit biofilm formation, while expression of tetracycline resistance induces biofilm production.
- Virulence genes with adhesive properties are associated with increased biofilm formation, while presence of capsule-related genes inhibit biofilm formation.

3 Materials and Methods

3.1 Bacterial strains

In this project, 35 clinical isolates of ExPEC are included, which are presented in Table 3.1. Five of the isolates originate from cases of UTIs in Europe (The EcoSens study (40)). In this project, the five ST73 strains are included as they are pansensitive, plasmid-free strains from UTIs. One of the five strains are WGS. The remaining 30 come from blood culture samples from Norwegian hospitals with unknown origin of infection. All the ST131 and ST648 strains in this project are collected from Norwegian hospitals by NORM and WGS for a previous study (29). Nineteen of the strains belong to ST131 and eleven belong to ST648.

Table 3.1. *E. coli* strains included in the project with information about which collection they come from, which year they are isolated and classification. Classifications are based on WGS conducted in (29).

Strain	Year	Collection	FimH allel	O-type	H-type	ST	ST131 Clades
MP23-01	2016	NORM	30	25	4	131	C2
MP23-02	2014	NORM	30	25	4	131	C1
MP23-03	2011	NORM	30	25	4	131	C1
MP23-04	2017	NORM	58	1	42	648	
MP23-05	2016	NORM		153	6	648	
MP23-06	2013	NORM	25	1	6	648	
MP23-07	2014	NORM	41	16	5	131	A
MP23-08	2013	NORM	25	1	6	648	
MP23-09	2013	NORM		1	6	648	
MP23-10	2011	NORM		1	6	648	
MP23-11	2009	NORM		1	6	648	
MP23-12	2009	NORM	30	153	6	648	
MP23-13	2017	NORM	58	4	42	648	
MP23-14	2013	NORM		153	6	648	
MP23-15	2013	NORM		1	6	648	
MP23-47	2017	NORM	30	25	4	131	C2
MP23-48	2011	NORM	30	25	4	131	C2
MP04-02	2000	EcoSens	10	6	1	73	

MP04-17	2000	EcoSens				73	
MP04-23	2000	EcoSens				73	
MP04-24	2000	EcoSens				73	
MP04-25	2000	EcoSens				73	
MP15-01	2013	NORM	41	16	5	131	A
MP15-03	2006	NORM	41	16	5	131	A
MP15-04	2017	NORM	22	25	4	131	B
MP15-05	2017	NORM	22	25	4	131	B
MP15-06	2005	NORM	22	25	4	131	B
MP15-07	2015	NORM	30	25	4	131	C1
MP15-08	2017	NORM	30	25	4	131	C1
MP15-09	2013	NORM	30	25	4	131	C1
MP15-10	2017	NORM	30	25	4	131	C2
MP15-11	2012	NORM	30	25	4	131	C2
MP15-12	2013	NORM	30	25	4	131	C2
MP15-13	2017	NORM	30	25	4	131	C2
MP15-14	2009	NORM	30	25	4	131	C2

3.2 Media and equipment

3.2.1 Liquid media and chemicals

Table 3.2. Liquid media and chemicals used in the project.

Media	Producer	Recipe	Storage
Miller LB liquid medium	LB Broth: BD, Difco LB Broth, Miller (Luria Bertani). LOT 9039644	10 g LB Broth 400 mL deionized H_2O Final concentration 25g/L.	Stored at 4°C until opened, then at room temperature.
80% glycerol	87% glycerol from VWR Chemicals. LOT 18E154003	73.6 mL glycerol 87% 6.4 mL deionized H_2O	Room temperature
Phosphate buffered saline (PBS)	PBS tablets: VWR chemicals. Lot 18K1556345	1 PBS tablet per 100 mL deionized H_2O.	Room temperature
Crystal violet solution 0.1%	Crystal Violet from Sigma Aldrich	Dissolved in H_2O[a]	Room temperature, protected from light

96% absolute ethanol	VWR chemicals. LOT 19G024030		Room temperature
Congo Red stock solution	C6277 Sigma dye content >85%	Transfer Congo red to sterile tubes with ethanol-disinfected spoon. Dissolve in sterile water, final concentration 10mg/ml.	
Brilliant Blue stock solution	B0770 Sigma.	Transfer Brilliant blue to sterile tubes with ethanol-disinfected spoon. Dissolve in sterile water, final concentration 1mg/ml.	
Ampicillin Gradient Strip	Ampicillin susceptibility testing.	LiofilChem. LOT 120518024	
Tetracycline 30 µg disk	Tetracycline susceptibility testing.	OXOID. LOT 1017275.	

[a] Prepared at the University Hospital of Northern Norway (UNN).

3.2.2 Solid media

Table 3.3. Solid media used in the project.

Media	Producer	Recipe	Storage
Miller LB agar plates	BD, Difco LB Broth, Miller (Luria Bertani), LOT 9039644. Select Agar, Sigma Aldrich, LOT MKCK6323	12 g Agar (15 g/L) 20 g LB Broth (25 g/L) 800 mL deionized H_2O.	Stored at 4°C.
Low Salt LB agar plates with Congo Red and Brilliant Blue	Low-salt LB Broth, Sigma 0.05% NaCl. Lot SLCC1206	12 g Agar (15 g/L) 12,4 g low salt LB (15.5 g/L)	Stored at 4°C, protected from light.

	Select Agar, Sigma Aldrich, LOT MKCK6323 Congo Red stock solution 10mg/ml Brilliant blue stock solution 1mg/ml.	16 mL Coomassie Brilliant Blue, final concentration 20 mg/L (60). 3.2 mL Congo Red, final concentration 40 mg/L (60). 800 mL deionized H_2O.	
Low Salt LB agar plates with Calcofluor	Calcofluor White Stain, Sigma Aldrich. LOT BCCD6645. 1g/L Calcofluor M2R. Select Agar, Sigma Aldrich, LOT MKCK6323 Low-salt LB Broth, Sigma 0.05% NaCl. Lot SLCC1206	12 g Agar (15g/L) 12,4 g low salt LB (15.5 g/L) 160 mL sterile filtrated calcofluor staining (200 mg/L) 640 mL deionized H_2O.	Stored at 4°C, protected from light.
Mueller-Hinton (MH) agar plates	BD Mueller Hinton Broth[a]		Stored at 4°C.
Mannitol tubes		Contains mannitol, a source of nitrate and phenol red in a semisolid 0.2% agar[a]	Stored at 4°C.
Blood agar plates	Oxoid Blood-agar base Cm271, 5 M NaOH, with horseblood: ThermoFisher, dH_2O[a]		Stored at 4°C.

[a] Prepared at the University Hospital of Northern Norway (UNN).

3.2.3 Equipment

Table 3.4. Equipment used in the project.

Equipment	Function	Supplier
Autoclave	Autoclave media and equipment.	SHP Steriltechnik AG Laboklav ECO
Incubator with shaker	Shaking overnight cultures	Infors HT Multitron Standard
96-well microtiter plate	Used in biofilm cultivation for both biofilm procedures.	Thermo Scientific (Nunclon Delta Surface) of polystyrene.
3D printed reusable pegs with lid	Used in biofilm cultivation for CFU quantification.	3D printed from Uppsala University
Lid with pegs	Used in biofilm cultivation for biomass staining.	Thermo Scientific Nunc Immuno TSP lids of polystyrene
ELISA reader	Read the optical density (OD) in 96-well microtiter plates for biomass quantification.	VersaMax PLUS, serial number BNR06652. SoftMax Pro Software version 5.4.6.005, serial number SMP500-17426-FDSA.
UV Transilluminator	Detect binding of calcofluor	UVP High Performance Transilluminator Model TFM-20 95-0286-02

3.3 Standard procedures for bacterial cultivation

Some of the techniques involving bacterial cultivation and preparation of media recured in several procedures and will therefore be described in this sub-chapter.

3.3.1 Preparation of media

Liquid media

The liquid media was prepared according to the recipes given in Table 3.2. The media was subsequently autoclaved at 121°C.

Solid growth media

The agar plates were prepared according to the recipes presented in Table 3.3. The media was autoclaved at 121°C and cooled to approximately 40-50°C on a magnetic stirrer. If dyes were

added, the equivalent amount of water was withdrawn before autoclaving, to maintain the acquired agar/dye concentration. The media was poured at petri-plates on the bench, besides a bunsen burner, and solidified at room temperature overnight before storage at 4°C. Agar plates with dye were protected from light during cooling and storage.

3.3.2 Isolation of single colonies

Working with single colonies will ensure that you have numerous of identical bacteria originating from the same single bacterium and thereby minimizing the risk of genetic variations. This was desirable for all our experiments and procedures.

To obtain single colonies, a 1 µL sterile loop was used to pick bacteria from the freezing stock and transferred to the agar plate. The same loop was used to drag the bacteria in a zigzag pattern across one third of the plate. A new loop would drag the bacteria in another direction in a zigzag pattern, only going through the first streak once. The same procedure was repeated a third time with a new sterile loop. This process is illustrated in Figure 3.1. By diluting the bacterial density like described, one increases the chance of achieving single colonies. The plate was incubated at 37°C for approximately 18 hours.

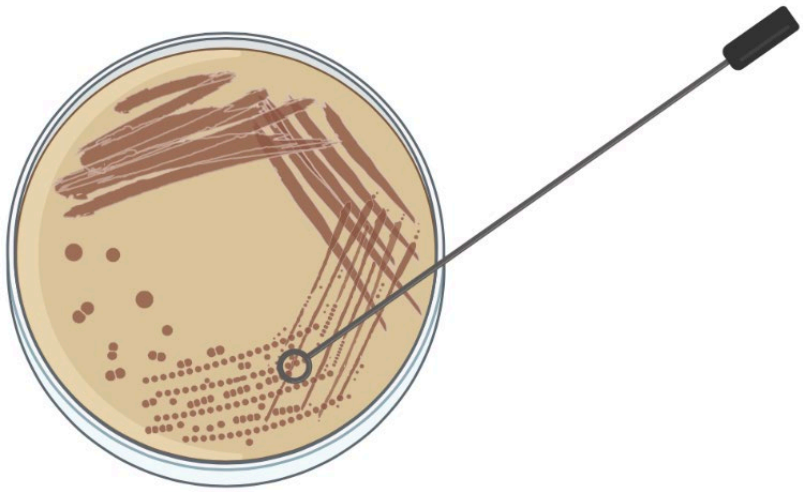

Figure 3.1. Demonstration of the streaking technique to obtain single colonies. Figure created in BioRender.com

3.3.3 Overnight cultures

An isolated colony of the strain of interest (3.3.2) was picked with an inoculation loop and suspended in 5 mL LB Media (Table 3.2) in a 15 mL sterile culture tube. The cultures were incubated for approximately 18 hours at 37°C and shaking at 225 rpm.

3.3.4 Long-Term Storage of Bacterial Strains

The strains used in the project were stored for long-term use in a -80°C freezer. To do so, 2 mL sterile cryovials were filled with 750 µL of the overnight culture and 250 µL of 80% glycerol, for a final concentration of 20% glycerol. The solution was mixed by pipetting. Glycerol works as a cryoprotectant and helps stabilize the bacteria in the cold environment (Appendix A).

3.4 Biofilm cultivation and quantification

The aim of the following procedures was to cultivate biofilm to investigate the amount of biofilm produced by each strain. The procedures are described in detail below. The initial cultivation of the biofilm was similar for the procedures. The final step and interpretation differed. Biofilm amount was quantified either by determining the number of colony-forming units (CFU) in the biofilm on pegs, or total biomass (bacteria + biofilm matrix), both on pegs and in the wells of microtiter plates. Biomass was measured by staining with crystal violet (CV) before the OD_{590nm} was measured. Due to biological variation, 3-5 biological replicates were included, with three technical replicates each. The procedure of biofilm cultivation is described below and illustrated in Figure 3.2.

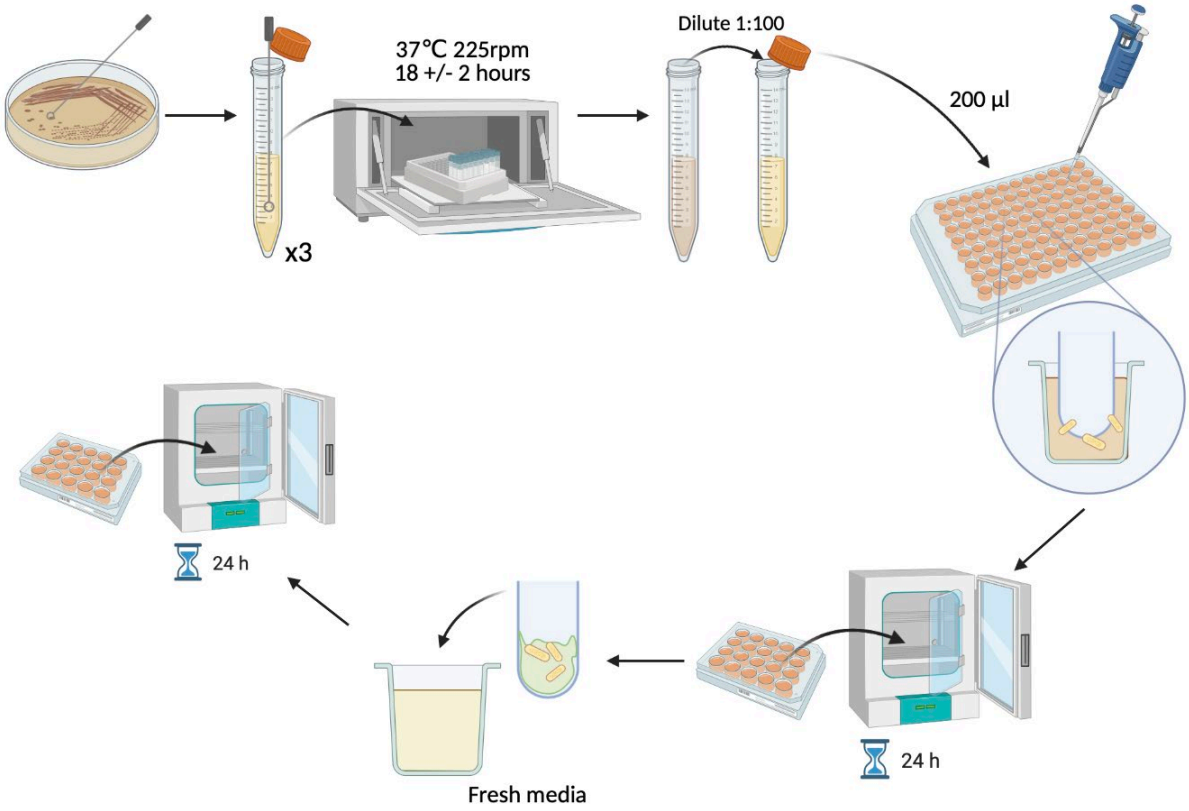

Figure 3.2. Illustration of the steps involved in the biofilm cultivation, the first four days of the two procedures. The enlargement of one well illustrates the well with the peg inside. The pegs were placed in every well with bacteria. Figure created in BioRender.com.

On day 1, the frozen bacteria were streaked out to obtain single colonies like described in section 3.3.2. They were incubated at 37°C for 18 +/- 2 hours. On day 2, one 15 mL sterile culture tube per biological replicate were labelled and filled with 5 mL LB media. For the CFU-quantification (section 3.4.1), three biological replicates per strain were performed. If the CFU varied with a 100-fold difference between biological replicates, another biological replicate was added. Some strains needed in total five biological replicates due to great variation.

For the biomass staining (section 3.4.2), an odd number of biological replicates was desirable, as unusual behaviour is easier to detect with an odd number. It was first performed three biological replicates of each strain, and if the results differed more than two-fold, two more biological replicates were performed. One tube with only LB media was included as a sterile control to make sure the media was not contaminated. One single colony from the agar plates was inoculated to its belonging tube, like described in section 3.3.3. The tubes were incubated at 37°C and shaking at 225 rpm for 18 +/- 2 hours.

On day 3, new sterile culture tubes were filled with 2 mL LB media, one for each biological replicate. The bacteria were diluted 1:100 by inoculating 20 µL from the overnight culture to the 2 mL LB media. This was performed by dipping a 10 µL inoculating loop in the overnight culture and then transferred to the 2 mL LB media, twice. The diluted bacteria were transferred into labelled 96-wells microtiter plates. For the biomass staining (section 3.4.2), all the wells except from the outermost rows were used for biofilm cultivation. The outermost rows were not used due to risk of evaporation. For the CFU determination (section 3.4.1), the bacteria were placed in every other well like illustrated in Figure 3.3. This was done due to the risk of cross-contamination when removing the pegs later.

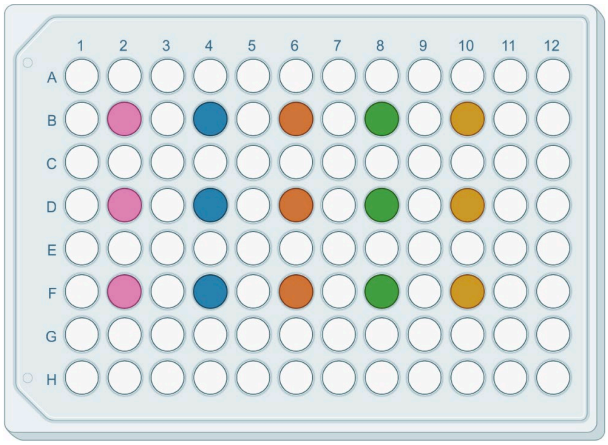

Figure 3.3. Placement of bacteria in microtiter plates. Demonstration of where the bacteria are placed for the CFU quantification. The coloured wells indicate wells where the bacteria are put. Each colour represents one biological replicate of one strain, with three technical replicates. Figure created in BioRender.com.

The diluted bacteria were mixed by pipetting 10-15 times and 200 µL were transferred into the correct wells. A multichannel pipette was used to fill the rest of the wells with pure LB media to create better moisture-retaining conditions and minimize evaporation.

The reusable pegs were autoclaved in advance. These pegs could not be used to investigate the total biomass (section 3.4.2), as CV is used for staining, and it was observed that the pegs adsorbed different amounts of the colorant. See Table 3.4 for equipment.

The pegs were put in the wells with bacteria, whereas the bacteria will stick to the pegs and produce biofilm on the surface. The sterile peg lid was put on top of the microtiter plate. The microtiter plates with pegs were placed in a container with a wet paper towel in the bottom for a moist environment. The plates were incubated at 37°C for 24 hours.

On day 4, all the wells in new microtiter plates were filled with fresh LB media and the peg lids were transferred to the new plates with fresh media. This provided new nutrients for the

bacteria. The plates were placed back in the container with fresh wet towels in the bottom and incubated for another 24 hours at 37°C.

3.4.1 Quantification of colony-forming units (CFU) in biofilm

The procedure continues from the one described in section 3.4 and is illustrated in Figure 3.4 All wells without bacteria (Figure 3.3) are used as negative controls.

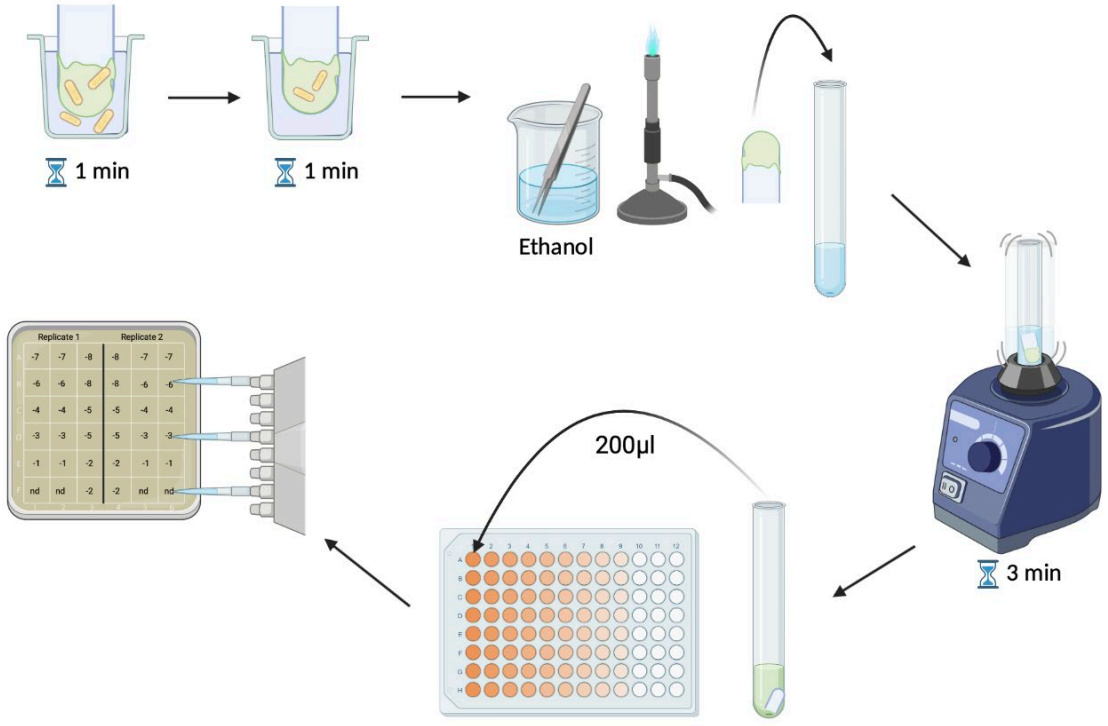

Figure 3.4. Illustration of the stepwise procedure of CFU quantification, following the biofilm cultivation. Single wells with pegs are illustrated, but this goes for the entire microtiter plate. Figure created in BioRender.com

On day 5, two microtiter plates per peg-lid were prepared with 200 µL PBS in the wells where the pegs were placed (Figure 3.3). These were used to wash off planktonic bacteria. The pegs and lid were transferred to the first wash plate for one minute. Next, they were transferred to the second wash plate for another minute.

Each peg was transferred to autoclaved tubes with 2 mL PBS, one tube per peg. A tweezer was used, and the pegs were transferred so that the tip of the peg pointed down in the tube. Ethanol was used to sterilize the tweezer between each peg, and subsequently burned off with a bunsen burner. The tubes with pegs were placed on a vortex for 3 minutes. This removed the biofilm from the peg. Additional two microtiter plates per fifth biological replicate were prepared with 225 µL PBS in every well from column 2 and out. These were used for dilution. Next, 200 µL

of the vortexed solution was transferred to column 1 in the dilution-plates. A multichannel pipette was used to mix all wells in column 1 by pipetting 20 times. Next, 25 µL of the biofilm solution was transferred from column 1 to column 2 on the plate. The pipettes were changed and 25 µL from column 2 was transferred to column 3 and so on. Figure 3.5 demonstrates how the two dilution plates looked like for five biological replicates, each presented with different colours.

Figure 3.5. Illustration of the dilution plates. Each colour represents the same strain and biological replicate, with three different technical replicates. Column 1 is non-diluted, column 2 is diluted to 10^{-1}, column 3 is diluted to 10^{-2} and so on. Figure created in BioRender.com

A multichannel pipette with a pipette tip on every third channel was used to spot the bacterial cultures. With a total of 3 tips at a time, 10 µL of the dilutions on the microtiter plate were spotted on their corresponding place on a square LB agar dish with a grid, like illustrated in Figure 3.6.

The distance between the three pipette tips matched perfectly so that the first time, it was taken 10 µL out of nd, -3 and -6. The second time it was taken from -1, -4 and -7 and the last time it was taken from -2, -5 and -8. The agar dish was split in two, so it was spotted for two pegs on one plate. Two technical replicates were performed for the spotting as well, as you can see two routes for each dilution on each half of the plate. The wells were mixed by pipetting 10-20 times

before 10 µL were taken out and spotted on the agar plate. Pipette tips were changed between each spotting. The drops on the agar dish were dried before the plates were incubated at 37°C for maximum 18 hours.

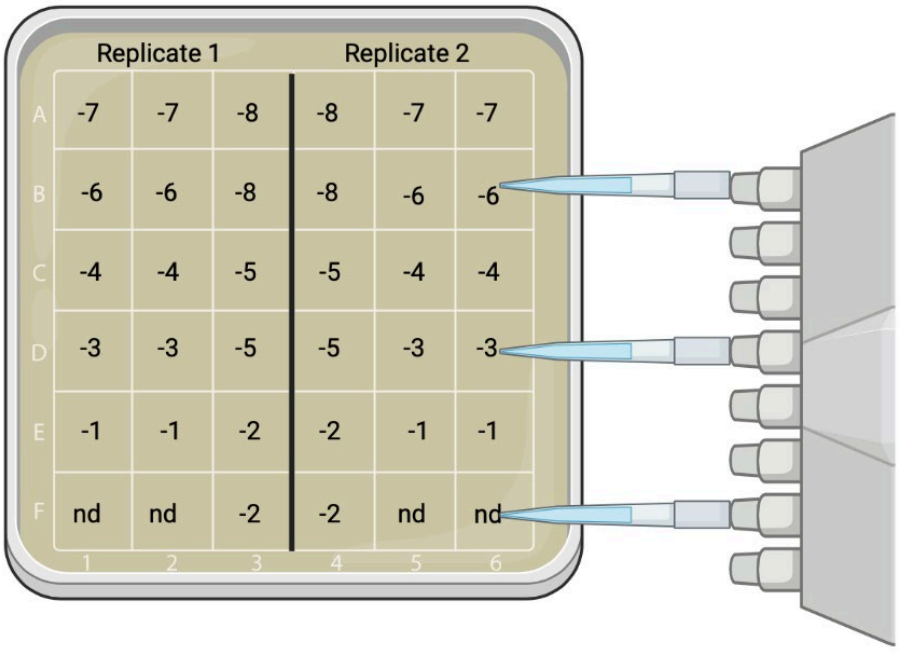

Figure 3.6. Illustration of the different dilutions spotted on a square agar plate. Nd = not diluted. -1 = diluted to 10^{-1} and so on. The thick line illustrates the separation between two technical replicates/two pegs. Figure created in BioRender.com.

On day 6 the number of CFUs were counted on both replicates of the dilution factor that had the most countable colonies. As a rule, it should be between 3 and 30 countable colonies. More than 30 colonies were difficult to count, and if it was fewer than 3 colonies, the previous dilution should in theory be countable. The number of CFU/peg were calculated according to the following formulas:

The number of CFUs from each of the technical replicates were added together:

$$CFU\ in\ 20\mu L = CFU\ in\ 10\ \mu L\ 1 + CFU\ in\ 10\ \mu L\ 2 \quad (1)$$

The number of CFUs were multiplicated by the dilution factor to get expected number of CFUs in the non-diluted spot. E.g. if the countable CFUs were on dilution factor -2, multiplicate with 10^2 or 100:

$$CFU\ in\ nd = CFU\ in\ 20\ \mu L * dilution\ factor \quad (2)$$

CFU/mL were calculated from CFU/20 µL:

$$CFU/mL = \frac{CFU\ in\ nd}{20} * 1000 \quad (3)$$

CFU/peg were calculated from CFU/mL by multiplying with 2, because the biofilm from each peg was suspended in 2 mL of PBS:

$$\frac{CFU}{peg} = \frac{CFU}{mL} * 2\ mL \quad (4)$$

Raw data of the CFU quantification is presented in Appendix F.

3.4.2 Quantification of biofilm mass by crystal violet staining

The procedure continues from the one described in section 3.4.

Staphylococcus epidermidis RP62A was used as positive control for biofilm formation in the wells. The blank outermost rows were used as negative controls. On day 5, the biofilm on the pegs and in the wells were stained with 0.1% CV.

Staining of biomass on the pegs:

The pegs were washed in microtiter plates filled with deionized water for 1 minute, twice. This was to wash away the planktonic cells. The peg lids were incubated at 55°C for an hour with the pegs pointing up, for fixation of the biofilm. Further, the pegs were placed in a new microtiter plate filled with 250 µL 0.1% CV in each well, for 5 minutes. The excess CV was washed off in a container with deionized water before the plates were placed in a new microtiter plate with 250 µL ethanol for 5 minutes. The (OD_{590nm}) in the ethanol wells were read at 590 nm wavelength using an ELISA plate reader (Table 3.4).

Raw data of the quantification of biomass on pegs is presented in Appendix G.

Staining of biomass in the wells:

The wells were emptied on paper towels and washed with deionized water three times to remove excess liquid media and planktonic bacteria. The wells were incubated at 55°C for an hour for fixation of the biofilm. Each well was filled with 250 µL 0.1% CV. After 5 minutes, the CV was emptied on a paper towel. The excess CV was washed off by dipping the plate in a container with deionized water. The water was refreshed, and the plates were washed again until there was no excess CV. All the wells were filled with 250 µL ethanol for the biofilm to resuspend

for 5 minutes. The OD_{590nm} in the ethanol wells were read at 590 nm wavelength using an ELISA plate reader (Table 3.4).

Analysis of the biomass data was also conducted with the average OD of the blank wells withdrawn from the mean OD of each strain. As this did not improve the data, it was decided to just move forward with the crude OD. Raw data of the quantification of biomass in wells is presented in Appendix H.

3.5 Congo Red and Calcofluor staining

To investigate the presence of curli and cellulose in the biofilm matrix, the bacterial cultures were spotted on agar plates with added colorants (Table 3.3) that bind to these biofilm matrix components.

Expression of curli was detected by binding of Congo red when spotted on agar plates with the dye, and expression of cellulose was detected by staining with Calcofluor and observation of fluorescens under UV light (61). The Congo red phenotypes were classified after both colour and surface characteristics. Strains positive for cellulose will bind the calcofluor stain and fluoresce under UV-light at a wavelength between 300-412 nm (62). The fluorescence was observed using a with a wavelength at 302 nm (63). Both the Congo red plates, and the Calcofluor plates were made with low salt LB, like described in Table 3.3, to induce production of these matrix components (51).

After preparation of overnight cultures (section 3.3.3), 3 µL of the overnight culture was spotted on two Congo Red low salt LB agar plates and two Calcofluor low salt LB agar plates. One of each plate were incubated on 28°C and the other two at 37°C, with two replicates per strain per temperature. The results were read after both 24 and 48 hours. If the two replicates did not correspond, a third replicate was performed.

Figure 3.7 illustrates how curli and cellulose production were scored. For curli, the strains were scored 0-4, where 0-1 indicated no expression of curli due to the glossy surface, as curli and cellulose expression is characterized by a dry/rough surface (60). Score 2-4 indicated increasing levels of curli expression. Score 0 was all white, while score 1 had a light colour. Score 2 was characterized by some colour or ring structures, with a matte surface just where the colour is. Score 3 had a dark, even colour accompanied by a matte/dry surface. Score 4 really stood out with a dark colour and a rough, wrinkled colony.

Cellulose expression was scored 0-3, where 0 indicated no cellulose production. Score 1-3 indicated cellulose production, or expression of other polysaccharides similar to cellulose (61).

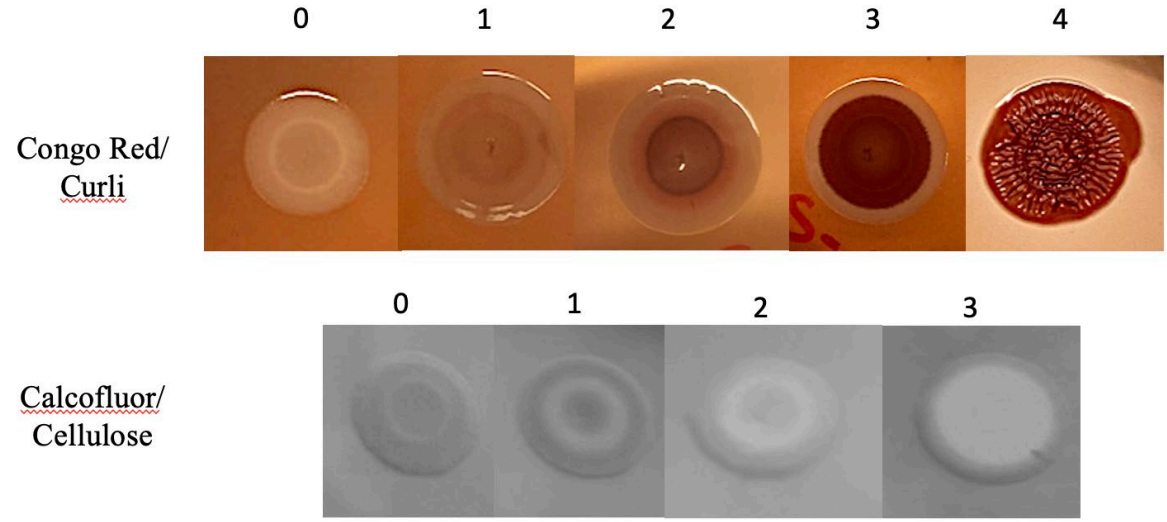

Figure 3.7. Illustration of the scoring system used to classify the expression of curli and cellulose.

Raw data of the Congo red/Calcofluor staining is presented in Appendix E, and the results classified according to the scoring system in Figure 3.7 is presented in Appendix C. The binary result for 28°C and 37°C, used in the statistical analyses, is presented in Appendix I.

3.6 Antibiotic susceptibility tests

To confirm that the genotypic resistance determinants result in resistance, phenotypic antibiotic susceptibility tests were performed for ampicillin and tetracycline. This was investigated by diffusion susceptibility testing with diffusion tests. The antibiotic of interest was applied to an agar surface, previously inoculated with the isolate that was tested. A prerequisite for these tests is that the bacterium is streaked for confluent growth, so that single colonies cannot be detected (64). The antibiotic was applied on the agar as either a disk or a gradient strip, where the antibiotic diffused throughout the agar and the inhibition zone was determined by visual inspection. The concentration of the antibiotic is reduced in a logarithmic scale, as the distance from the disk increases (64). For disk diffusion, the concentration of antibiotic in the disk is based on, among other things, achievable serum concentration (64). The minimum inhibitory concentration (MIC) is based on the diameter of the inhibition zone, whereas a smaller diameter indicates a higher MIC. MIC is the lowest concentration of the antibiotic that will inhibit visible growth of the bacteria in vitro (23). The same concept is used for gradient strip testing, only

they have increasing concentration of the antibiotic along the strip and the MIC value can be read directly.

The ability to diffuse throughout the agar will vary among different antibiotics, dependent on molecular size and hydrophilicity. Cation-adjusted Mueller-Hinton (MH) agar plates are commonly used for susceptibility tests, because of several properties. As an example, they are low on certain inhibitors that interferes with some antibiotics, like thymidine/thymine (inhibits sulphonamides and trimethoprim) and divalent cations (inhibits tetracycline) (65).

For both ampicillin and tetracycline, *E. coli* ATCC25922 were used as quality control. The result for each strain is presented in Appendix I.

3.6.1 Ampicillin susceptibility test

Ampicillin belongs to the group of β-lactam antibiotics. The susceptibility testing was performed as described in method 3.2.1 (Appendix B). Briefly, a bacterial suspension with optical density of 0.5 McFarland was prepared, and the solution was spread on MH agar plates for confluent growth. An ampicillin gradient strip was added to determine the MIC. The MIC value was read by the haze of the inhibition zone, or by any single colonies that grew within the inhibition zone. MIC value ≤ 8 mg/L indicates that the strain is sensitive to ampicillin, while a MIC value >8 mg/L indicates an ampicillin resistant strain (66).

3.6.2 Tetracycline susceptibility test

The tetracycline resistance test was based on Method 3.2.1 (Appendix B). Instead of using a gradient strip, tetracycline 30 µg disks were added for a disk diffusion test. After incubation, the inhibition zone was measured, whereas a diameter <19 mm indicates a resistant strain (66).

3.7 Motility assay

The purpose of the test was to examine if the strains are motile. The tubes used to perform the test were ordered from UNN (Table 3.3) and contains mannitol, a source of nitrate, and phenol red as indicator. This is because the tubes are also used as a part of correctly identifying Enterobacteriaceae. The agar in the tube was semisolid (0.2% agar), which allowed motile bacteria to move in the media. Motile bacteria resulted in a diffused growth throughout the agar

or on the surface, while non-motile bacteria only grew along the inoculating line. With use of <0.3% agar, one will detect the "swimming" type of motility (48, 67).

A single colony (see section 3.3.2) was picked with a 1 µL inoculating loop and the loop was stabbed through the semisolid agar to the bottom of the tube. The tubes were incubated at 37°C for 18 +/- 2 hours.

The motility test was positive if the agar medium were cloudy, or if it was growth along the surface. A negative test was shown by bacterial growth only along the inoculating line. The result for each strain is presented in Appendix I.

3.8 Haemolysis assay

The aim of this assay was to investigate if the strains express haemolysins. Bacteria were picked from the freezing stock with a 1 µL inoculating loop and streaked directly onto agar plates containing horse blood. Three biological replicates per strain were performed. The plates were incubated overnight at 37°C and the results were read the next day. A green/yellow colour around the bacteria indicated production of α-haemolysin, while a complete clearing indicated production of β-heamolysin. No change in the agar indicated a ɣ-haemolytic/non-haemolytic strain.

Enterococcus gallinarum ATCC49608 was used as control strain for α-haemolysis, and *Enterococcus faecalis* ATCC29212 was used as control strain for β-haemolysis. The result for each strain is presented in Appendix I.

3.9 Genotypic characterization

Out of the 35 strains, 31 were whole genome sequenced. Genotypic traits of interest were investigated by uploading the genome of each strain to specific webtools, whose function and settings are presented in Table 3.5.

Table 3.5 Tools and parameters used to investigate genotypic properties of the strains.

Tool version	Function	Settings	References
ResFinder 4.1	Detects resistance genes	Identity: ≥90% Length: ≥60% for both chromosomal point mutations and acquired antibiotic resistance genes.	(68), (69)
VirulenceFinder 2.0	Detects virulence factor genes	Species: *E. coli* Identity: ≥90% Length: ≥60%	(70), (71)
PlasmidFinder 2.0	Detects plasmid replicons	Database: Enterobacteriaceae Identity: ≥95% Length: ≥60%	(72)
SeroTypeFinder 2.0	Detects O:H serotypes	Species: *E. coli* Identity: ≥85% Length: ≥60%	(30)
MLST 2.0	Detects sequence type	Scheme: *E. coli* #1	(73)
CHTyper 1.0	Detects fumC and fimH type	Identity: ≥95%	(74)

All genotypic data collected on the strains are presented in Appendix J.

3.11 Statistical analyses

All statistical analyses were performed in IBM SPSS Statistics, version 27. Guidance to the procedure of each test, its assumptions and interpretations are provided by Laerd Statistics (2017). *Statistical tutorials and software guides*. Retrieved from https://statistics.laerd.com/premium/index.php.

The normality of the data was investigated by Shapiro-Wilk test and by visual inspection of box plots. For non-normally distributed data, means were compared with Mann-Whitney U test (2 groups) or Kruskal Wallis H test (>2 groups). Subsequent pairwise comparison was performed using Dunn's procedure (1964) with Bonferroni adjustment for multiple tests. This is a conservative test, increasing the risk of Type II errors while decreasing the risk of Type I errors. A less conservative test was desirable but was not available in SPSS.

For normally distributed data, an independent samples t-test or a One-way ANOVA with a Games-Howell or Tucey post hoc was conducted. Correlations were investigated with a Spearman-rank correlation test as the data were non-normally distributed. Categorical data were analysed with χ^2 independence test. If the assumptions for a χ^2 was not met, a Fisher's exact test was conducted. All tests were performed with and without outliers. If the results differed, it is commented. A summary of all statistical analyses performed is presented in Appendix D.

4 Experimental results and discussion

E. coli is a biofilm forming pathogen and a leading cause of extraintestinal infections like UTIs and bloodstream infections. Biofilm formation is a major contributor of persistent infections, as it offers the bacteria increased survival compared to their planktonic form. This has made it an increasing field of interest over the last decades. However, the distinct abilities of clinical isolates to form biofilms are yet to be elucidated. By correlating biofilm forming capacities with genotypic and phenotypic traits of the strains, we aim to generate novel knowledge about the pathogenesis and virulence of clinically relevant ExPEC isolates.

4.1 Biofilm quantification and detection of major matrix components

To investigate the strains' ability to produce biofilm, quantification of both CFUs and biomass (bacteria + biofilm matrix) were conducted. The strains displayed a wide specter of *in vitro* biofilm forming capacities. The results from the quantification of CFUs are presented in Figure 4.1. The total biomass was measured both on pegs lids and in the wells of microtiter plates. The results for both variables are presented in Figure 4.2. Considering that curli and cellulose are two important components of the biofilm matrix (50), it is plausible to think that strains expressing these are also producing more biofilm. Therefore, the strains' abilities to produce curli and cellulose were also investigated.

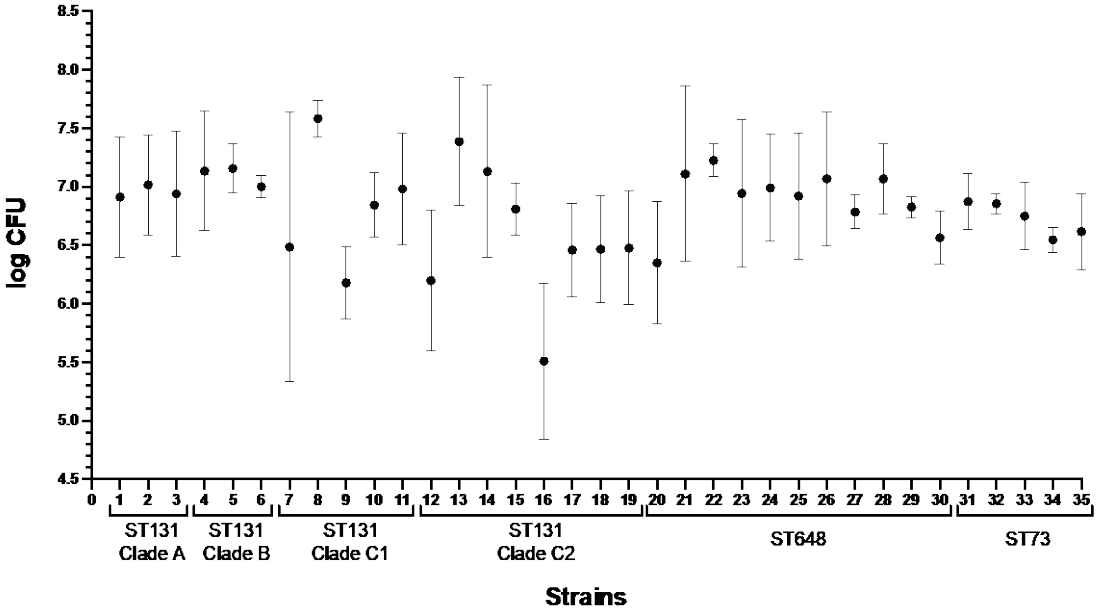

Figure 4.1 Average CFU retrieved from the biofilm of each strain. The 35 strains are presented on the x-axis, sorted by ST and ST131 clades. CFUs are presented on the y-axis, on a base 10 logarithmic scale. Error bars represent the standard deviation (SD). The mean and SD represent the variance between 3-5 biological replicates, with three technical replicates each. The average number of CFUs across strains ranged from 5.5–7.6, with a mean of 6.8.

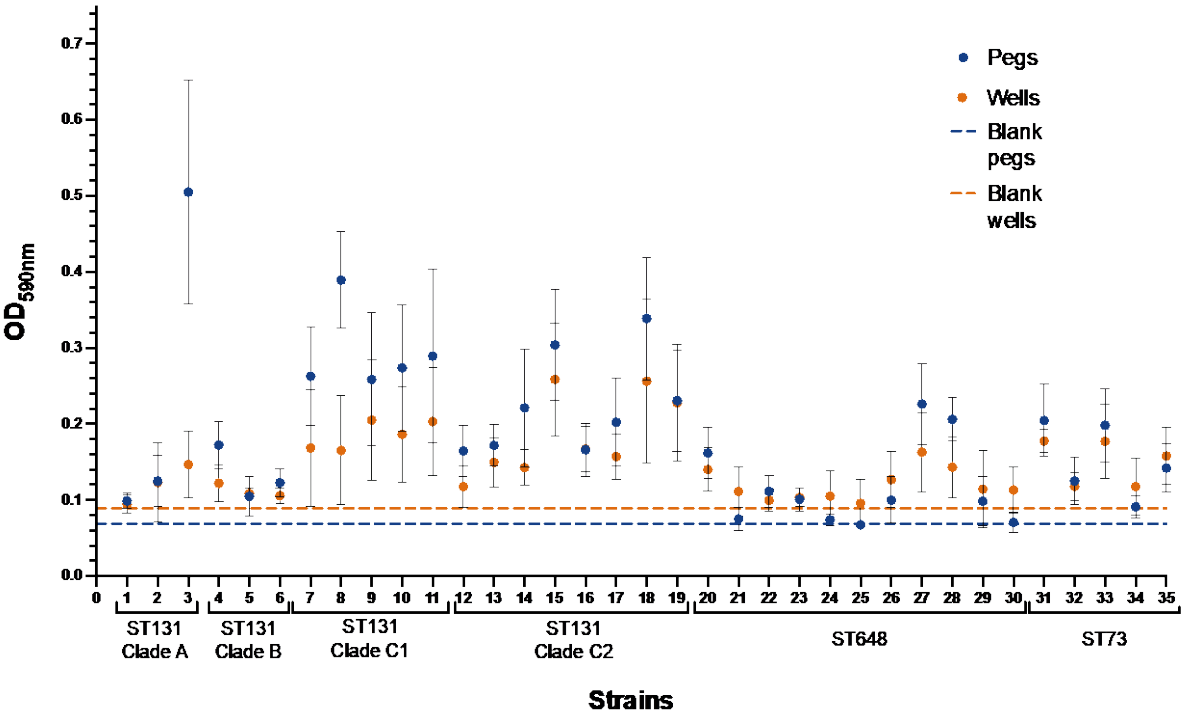

Figure 4.2 Average biomass on pegs and in wells for each strain. The 35 strains are presented on the x-axis, sorted by ST and ST131 clades. The OD_{590nm} values are presented on the y-axis. Error bars represent SD. Mean and SD represent the variance between 3-5 biological replicates, with three technical replicates each. The blanks are negative controls with only LB media. The mean OD_{590nm} for pegs was 0.18, ranging from 0.06 – 0.50, whereas the mean OD_{590nm} for wells was 0.14, ranging from 0.09–0.26.

Correlation with biomass on pegs and in wells, but not with CFU

There was a statistically significant, strong, positive correlation (Spearman-rank correlation test) between biomass on the pegs and biomass in the wells ($p < 0.001$), as illustrated in Figure 4.3

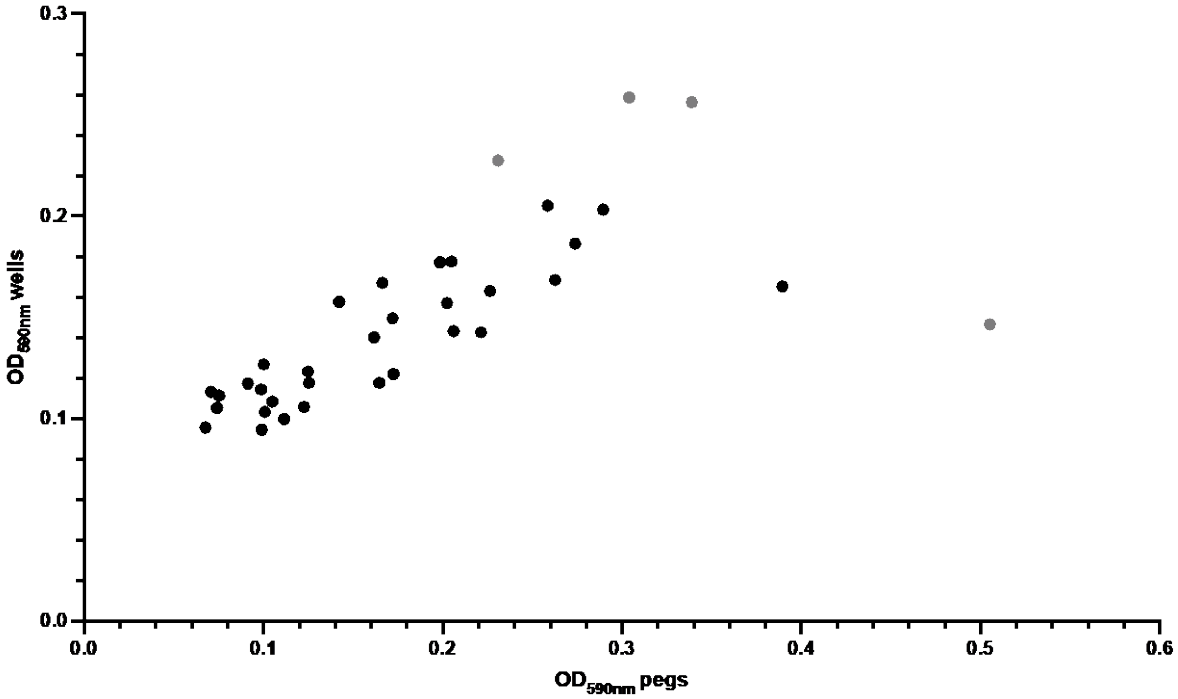

Figure 4.3 Scatterplot illustrating the positive correlation between biomass on pegs and wells (Spearman-rank correlation test, $r_s = 0{,}848$, $p < 0.001$). The plot includes all 35 strains, including outliers (marked in gray). OD_{590nm} is presented for pegs on the x-axis, and for wells on the y-axis.

However, the CFU count did not show any statistically significant correlation (Spearman-rank correlation test) with biomass on pegs ($p = 0.319$), nor biomass in wells ($p = 0.08$). It is important to note that these were not the same pegs, as the 3D printed pegs from Uppsala University used for CFU determination is made of a different material than the peg lids used in the biomass staining. Surface material has been suggested to have an impact on initial adhesion to the surface and subsequent biofilm formation (75). It would be desirable to use the same pegs for both procedures, to make the results more comparable. However, the 3D printed pegs were not possible to use in the CV staining, as we observed different amount of CV adsorption by the pegs themselves, independent of the bacterial strain. It should also be noted that biomass staining also reflects the presence of dead cells (76).

The positive, significant correlation between biomass on pegs and in wells ($p < 0.001$) seems reasonable, because the two variables were mostly measured from the same experiment,

providing equal sources of variance during the biofilm cultivation. However, the biofilm is cultivated on the same pegs for 48 hours in total (two steps), whereas the biomass in the wells is only represented by the last 24 hours (one step), due to the transfer of the peg lid to fresh media after 24 hours of incubation. Naves *et al.* found significant differences between the use of one-step or two-step protocols (46), which explains why this may provide a source of variation between the two variables. The variation may occur in both directions, dependent on strain and media (46). The strong, positive correlation between biomass on pegs and in wells for this collection ($p < 0.001$), suggests that there is no preference of either of the surfaces for these strains. This despite the fact that the risk of aggregation due to sedimentation in the wells is eliminated on the pegs, which is suggested to play a role by Harrison *et al.* (75). The clinical significance of biofilm measurements on pegs or in wells, or which role the number of live cells play in the pathogenesis of clinical biofilms, is not known.

There are also other technical aspects of these procedures which might skew the results. All three procedures are exposed to variation, probably due to the multistep treatment of the biofilms, such as washing steps. This requires practice in consistent handling (76). CFU quantitation is exposed to some additional sources of variation; aggregation of cells during the serial dilution (76), and the manual counting of CFUs as the endpoint compared to automatic OD measurements for the biomass-methods. The number of CFUs for some replicates disagreed with the corresponding dilution factor, e.g. a ten-fold dilution did not always display a ten-fold difference in CFUs, which may imply inconsistent handling during the procedure.

Expression of curli and cellulose wass a widespread phenotype among clinical ExPEC strains

The procedure of curli and cellulose detection was optimized with regards to shaking speed of overnight cultures (225rpm vs static), culture medium (LB or low salt LB), and incubation time (24h-72h). We registered results after 24h and 48h for all isolates, but the results at 48h were used for further analysis as they provided more clearly distinguishable phenotypes. The colonies displayed a wide phenotypic diversity, as illustrated in Figure 3.7, offering a challenge in classification and interpretation. A strong correlation was seen between curli- and cellulose-expression at the two temperatures tested: 28°C and 37°C. Twentynine out of the 35 isolates (83%) showed identical expression of both curli and cellulose at 28°C and 37°C after 48 hours. Curli expression at the two temperatures exhibited a redundant association (χ^2 independence

test, $\phi = 0.715$, $p < 0.001$), the same did cellulose expression (Fishers exact test, $\phi = 0.878$, $p < 0.001$). The association remained the same for blood culture isolates and UTI isolates separately ($p < 0.001$). The strong correlation between expression of curli and cellulose at the two temperatures is not in agreement with what we hypothesized. Based on literature, we expected thermoregulation of the expression of the two components (50, 56). It is previously suggested by Bokranz *et al.*(50) that the expression of the extracellular components at 28°C is typical for UTI isolates, whereas Bian *et al.* suggests that expression of curli at 37°C is typical for sepsis isolates (56). However, Bokranz *et al.* compared the UTI isolates to fecal isolates, and no blood culture isolates were investigated in this study. Bian *et al.* suggested a role for curli and CsgA *in vivo* in human sepsis. This was supported by the detection of anti-CsgA in the bloodstream of sepsis patients, and by the fact that 65% of the isolates were stained by Congo red at 37°C. However, a larger proportion (67%) was stained by Congo red on 28°C, and only 7 of 46 isolates displayed thermoregulation, which more coincides with our results (56). However, these observations have been made with procedures differing from the ones conducted in this project, with regards to incubation time and interpretation schemes. Their findings were supported by additional analyses, like western blot for curli or immunoblotting for CsgA (50, 56).

Further analyses were only performed with the expression of curli and cellulose at 37°C, as this is more clinically relevant and more comparable to the other experiments which are all performed at 37°C. A summary of the number of isolates expressing only cellulose, only curli, both or neither at 37°C is presented in Figure 4.4.

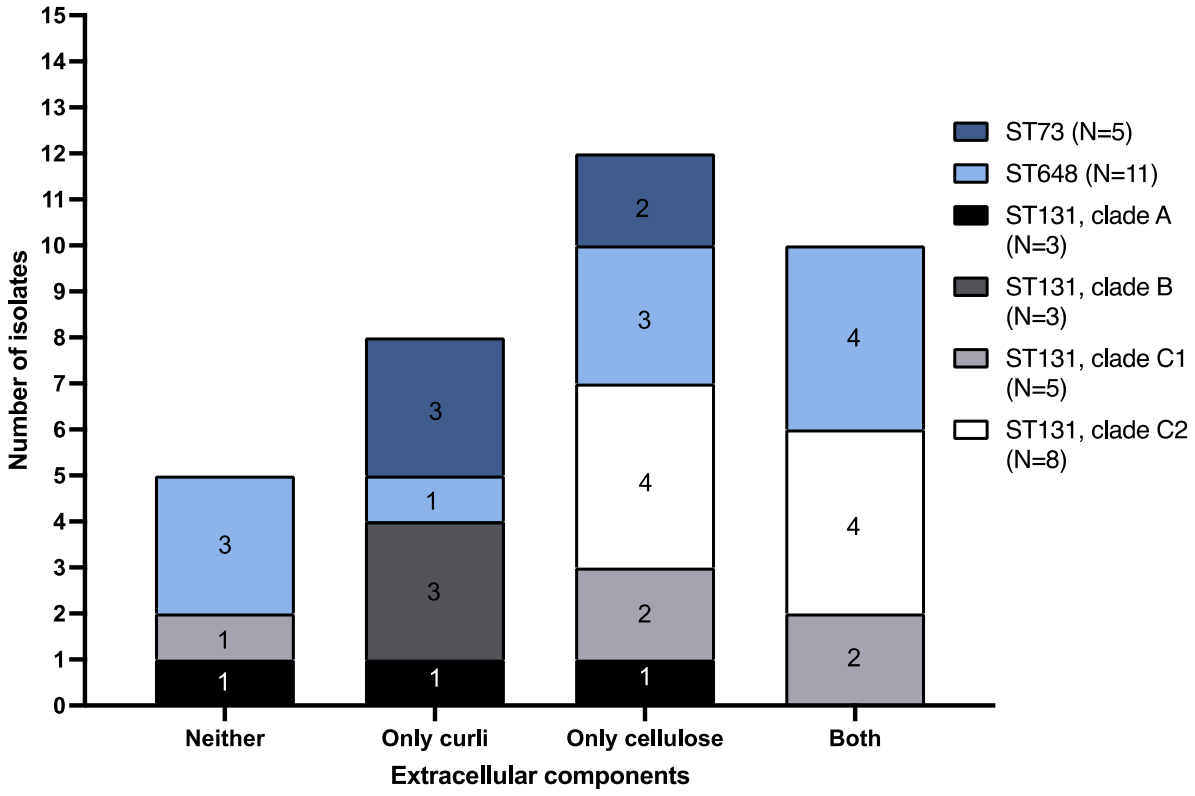

Figure 4.4 Expression of curli and cellulose at 37℃. For each bar, the contribution from each of the STs and ST131 clades are presented with different colours, with number of isolates indicated within each box.

In total, 63% of the strains expressed cellulose and 51% expressed curli. Only five out of 35 strains were not able to produce any of the components at 37℃. The UTI isolates (ST73) in this thesis either produce cellulose or curli, whereas diverse profiles were displayed for ST131 and ST648. ST131 clade B only produced curli. Even though there were no statistically significant differences in expression of curli across STs or clades (Fisher's exact test, STs; p = 1.00, clades; p = 0.434), it could be an interesting trend to investigate with a larger sample size.

No correlation between expression of curli and cellulose

In this project, the expression of curli and cellulose at 37℃ were independent of each other. Literature describes that curli and cellulose are co-regulated by a regulatory cascade involving RpoS, MlrA and CsgD regulators (61, 77). CsgD is required for activation of the *csgBAC* operon and for cellulose synthesis via *adrA* and the synthesis of c-di-GMP (51, 61). However, also a CsgD-independent pathway for cellulose production has been suggested, because deletion of CsgD displayed the same cellulose phenotype on Congo red agar as the wildtype, demonstrated in a commensal *E. coli* strain (61). Alternative regulatory pathways have also

been reported for curli (78). It would be desirable to perform additional analyses on the expression of the two components with a different approach than the one done in this project. Instead of comparing the biofilm phenotype to a binary result of curli and cellulose separately, one could also compare it to the four groups displayed in Figure 4.4. This would be interesting as other have indicated that co-overproduction of curli and cellulose together enhanced bacterial adhesion and could thereby result in increased biofilm formation (79).

Biofilm formation and expression of matrix components across STs and ST131 clades

There was a statistically significant lower biomass on pegs and in wells for ST648 (N = 11, mean rank 10.00 and 10.54, respectively) compared to ST131 (N = 19, mean rank 23.26 and 21.58, respectively) (Kruskal Wallis H test, post hoc: Dunn's procedure with Bonferroni correction. Pegs; $p = 0.002$, wells; $p = 0.014$). There were no differences between ST73 (N = 5, mean rank 20.60) and any of the other STs (Kruskal Wallis H test, Dunn's post hoc, $p > 0.05$.) Based on the findings of Schaufler *et al.* (36) on ST131, ST10 and ST648, we hypothesized that ST648 is superior in biofilm formation compared to other pathogenic STs, but the results obtained in this project imply the contrary. We did not detect a difference in curli and cellulose production between the STs in this study either (Fisher's exact test, curli; $p = 1.000$, cellulose; $p = 0.555$). However, Schaufler *et al.* (36) used solely a qualitative evaluation of biofilm formation, in terms of curli and cellulose production, and no biofilm quantitative assay was performed. They also used a different interpretation scheme for the qualitative evaluation, had a larger sample size and different strain collection with regards to origin (date, geography, host, and infection type) compared to this study.

Even though curli and cellulose are important components of the biofilm matrix, there are discrepancies in the literature concerning the correlation between the two matrix components and the effect on biofilm abundance. In this project, expression of curli did not correlate to any of the biofilm-abundance variables (biomass on pegs, wells and CFU) (CFU; Mann-Whitney U test, $p = 0.503$, pegs; Independent samples t-test, $p = 0.305$, wells; Mann-Whitney U test, $p = 0.708$). The strains that expressed cellulose displayed a higher biomass on pegs (mean ± SD, 0.18 ± 0.08), compared to the strains negative for cellulose (0.13 ± 0.05), a statistically significant difference of 0.057 (95% CI, 0.004 to 0.11) (Independent samples t-test, $p = 0.035$). However, this difference was only seen with the exclusion of outliers, and it was not seen for biomass in the wells, which weakens the soundness of this result. Da Re *et al.* have suggested

that the binding of calcofluor is not strictly correlated to the ability to produce biofilm (61), while Bokranz *et al.* have seen a reduced biofilm formation following deletion of genes required for curli and cellulose production (50). More in correspondence with our study, Da Re *et al.* reported that cellulose, not curli, was required for biofilm formation in a commensal *E. coli* strain (61).

Among the strains belonging to four different ST131 clades in this study, a significant difference with regards to biofilm abundance in the wells were detected (Welch ANOVA, df = 3, p = 0.002). Biomass increased from clade B (mean ± SD = 0.112 ± 0.01) < Clade A (0.121 ± 0.03) < Clade C2 (0.184 ± 0,05) < Clade C1 (0.185 ± 0,02), presented in Figure 4.5. The strains belonging to clade B displayed a significant difference compared to those from clade C1 (Games-Howell post hoc, p = 0.001) and C2 (Games-Howell post hoc, p = 0.029). However, this difference was not seen for biomass on the pegs.

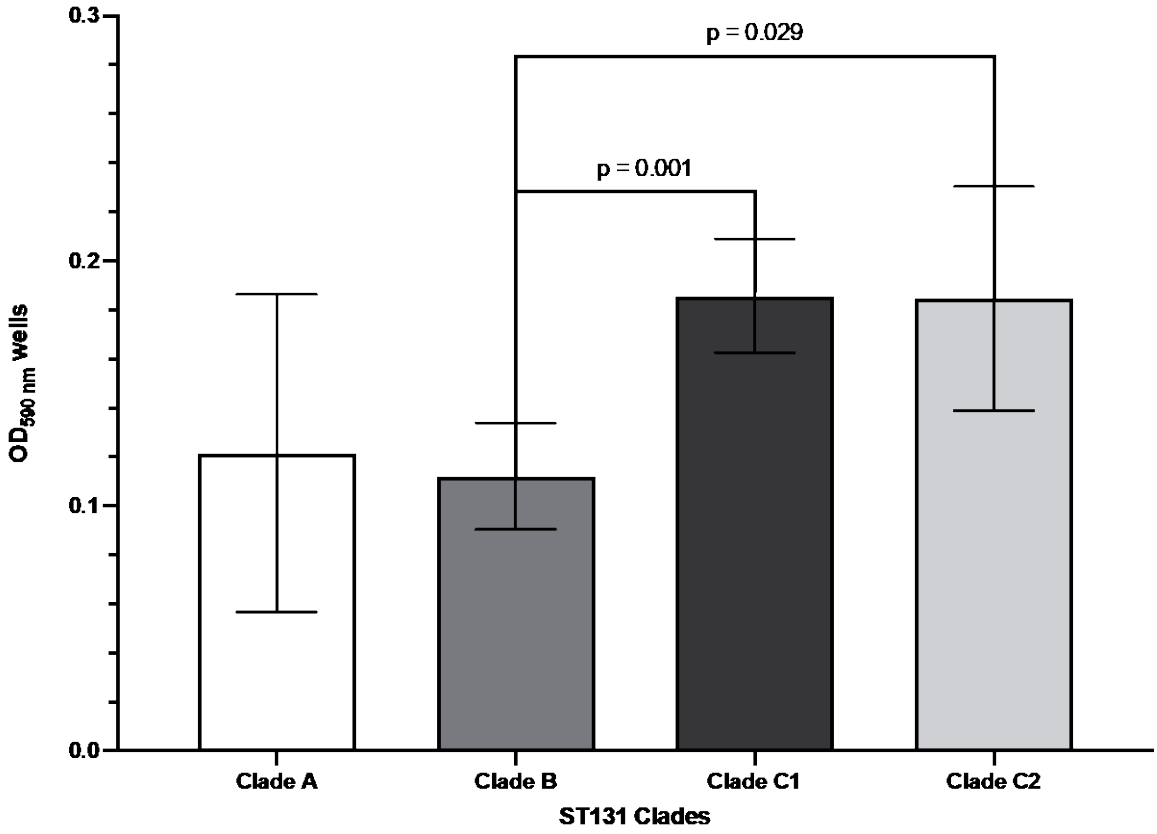

Figure 4.5 Mean biomass in the wells across ST131 clades. Error bars presented as 95% CI. The increase from clade B to clade C1 (0.07, 95% CI (0.03-0.10) p = 0.001), as well as the increase from clade B to clade C2 (0.072, 95% CI (0.008-0.136), p = 0.029) was statistically significant (Welch ANOVA, Games Howell post hoc test).

Little is known about the biofilm forming capacities across ST131 clades, but Duprilot *et al.* described that clade B strains were better at producing early biofilms compared to clade C strains (80). However, it is measured after 2, 3 and 5 hours, thereby reflecting the initial stage of biofilm formation, and not after 48 hours like in this project, which reflects accumulation of biofilm. With regards to biofilm matrix components, we observed no statistically significant difference in curli expression across ST131 clades (Fisher's exact test, $p = 0.434$). However, none of the clade B isolates, with the lowest mean biofilm abundance, expressed cellulose, whereas all isolates in clade C2, with second highest biofilm abundance, did (Fisher's exact test, $p = 0.004$). Albeit small numbers, this, supported by the increased biomass by cellulose producing strains in this material (Independent samples t-test, 0.057, $p = 0.035$) can reinforce the conclusion that cellulose is a significant contributor to the biomass of the isolates in this collection.

Methodological considerations

We included a control strain in the biomass-experiments, *Staphylococcus epidermidis RP62A,* due to its known strong biofilm-forming capacities (81). However, this species grew poorly in LB media and hence little/no biofilm formation was observed. A more appropriate control strain would be desirable. This observation introduced the possible need to correct for growth rate, as different growth between strains was observed by visual inspection of the wells. Others have suggested to extract the values of the blanks and correct for growth rate to normalize the data (46, 76). However, extraction of blanks did not improve our results (data not shown), so the crude OD was further utilized. Low salt LB is frequently used to detect the expression of important components of the biofilm matrix, as such conditions are suggested to induce biofilm formation (78). For that reason, it would be interesting to perform the biofilm assays in low salt conditions as well (60). Nonetheless, pilot experiments previously conducted in the MicroPop lab, indicated no difference between the two media for CFU determination from pegs.

Congo red staining is frequently used as an indicative of curli production. Most studies differentiate between different colours on the Congo red agar to distinguish between the expression of curli, cellulose, both or neither (50, 60, 61). We found it notoriously difficult to distinguish between different hues of pink and purple, and thereby scored based on colour intensity, where a coloured colony was interpreted as curli positive. This way of classification has also been used in other studies (36, 56). Congo Red can also bind to other extracellular

components, like cellulose, which further complicates the interpretation of the results. Additionally, previous studies have indicated that polysaccharides are responsible for the wrinkled phenotype on agar plates (61). However, this does not necessarily coincide with our results, as some of the wrinkled phenotypes (score 4 for curli, Figure 3.7) showed little/no expression of cellulose as assessed by calcofluor staining. The wrinkled phenotype in this project was thereby interpreted as a high degree of curli expression. The different interpretation schemes used for this kind of assay makes the results challenging to compare across studies. McCrate *et al.* suggests that curli-dependent Congo Red binding must be established in order for the Congo Red staining to be a reliable indicative of curli production. This can be done by deleting curli-specific genes and investigate if binding of Congo Red is abolished (82). Calcofluor binds to cellulose but can also bind to other polysaccharides similar to cellulose (61). This lack of selectivity by the dyes introduces a risk of misclassification and supplementing with other experiments would be desirable to confirm positive results. In short, staining of colonies is considered a fast and easy indication for curli and cellulose expression but should preferably be supplemented with other tests. Genetic analyses to confirm the presence of intact curli- and cellulose related genes or phenotypic analyses of knock-out mutants could complement our results.

4.2 Role of motility and haemolysis in biofilm formation

Flagella-mediated motility was associated with enhanced biofilm formation

As flagella-mediated motility has been suggested to have an impact on the initial contact with a surface and thereby initiating biofilm formation (8), we hypothesized that motile strains would exhibit an increased biofilm abundance compared to non-motile strains. The strains were investigated for their ability to exhibit “swimming” motility, which is correlated to the presence of flagella. Motility behaviour is presented in Table 4.1, and shows that all but four strains are motile. This behaviour was compared to biofilm forming capacities.

Table 4.1 Summary of the isolates' motility, categorized by ST and clade.

	Motile		Non-motile
	Growth only on surface	**Growth throughout the agar**	
ST131 clade A	2		1
ST131 clade B		2	1
ST131 clade C1	1	4	0
ST131 clade C2	2	6	0
ST648	2	8	1
ST73		4	1
Total	**7**	**24**	**4**

Biomass on both pegs and wells was significantly (Mann-Whitney U test, $p = 0.020$ and $p = 0.023$, respectively) higher for motile strains (mean rank 19.42 and 19.39, respectively) compared to non-motile strains (mean rank 7.00 and 7.25, respectively). Some of the strains grew on the surface, and some grew evenly in the entire agar, but this did not exhibit any significant differences in biofilm formation (Mann-Whitney U-test, $p > 0.05$).

These results coincide with our hypothesis and are supported by the results of Pratt and Kolter (83). They showed that flagella-mediated motility was crucial for biofilm formation, by examining the ability to produce biofilm before and after deletion of genes critical for synthesis of flagella. Although our project lacks information on the presence of flagella-associated genes, the swimming motility examined in this assay has previously been correlated to flagella (48).

The inclusion of controls would have improved the quality control of this experiment, but the results were relatively easy to interpret, so we do not consider this a major flaw. A larger sample-size would also have been preferable to avoid skewing the data due to the large difference in numbers between the two groups.

Haemolytic activity was not associated with biofilm production

The phenotypic expression of α-haemolysin (*hlyA*) was investigated. Haemolysin is an important virulence factor and has been suggested as an important trait of UPEC (13). α-haemolysin has previously been suggested to be required for biofilm production in *Staphylococcus aureus* (84). Also among ExPEC causing prostatitis, α-haemolysin was

encoded by a significantly higher proportion of biofilm producing strains than in strains not able to produce biofilms (85). We thereby hypothesized a correlation between biofilm forming ability and haemolysis. Haemolytic activity was determined by visual inspection of the agar around the bacteria after incubation. A complete clearing indicates β-haemolysis, whereas α-haemolysis is recognized by partial lysis, resulting in a green/yellow colour of the agar. The results are shown in Table 4.2 below, showing that approximately half of the strains exhibited β-haemolytic activity, while the remaining isolates were evenly distributed between α- and ɣ-haemolysis.

Table 4.2 Summary of the number of isolates exhibiting haemolytic activity, categorized by ST and ST131 clades.

	α	β	ɣ
ST131 clade A	1	1	1
ST131 clade B	0	3	0
ST131 clade C1	1	3	1
ST131 clade C2	3	2	3
ST648	1	8	2
ST73	1	2	2
Total	7	19	9

We compared the biofilm forming capacities with haemolytic activity of the isolates but found no significant differences (Kruskal Wallis H test) between presence of each of the haemolytic phenotypes, α, β and ɣ, neither with biomass measured from pegs ($p = 0.390$), nor wells ($p = 0.234$). The studies mentioned above, on *S. aureus* and ExPEC from prostatitis, investigated haemolytic activity genotypically, by detection of *hlyA,* encoding the α-haemolysin. Other studies explain and interpret haemolysis tests differently, suggesting that phenotypic distinction between α- and β-haemolysis on blood agar plates, can be challenging (86). None of the genes suggested to result in haemolytic activity on blood agar plates (87) were detected among the strains in this thesis, except for three strains harbouring *hlyF.* Hence, the reason for the haemolytic activity displayed on blood agar plates by 26 of the strains in this project remains unknown.

4.3 Genotypic characterization of the strains

To investigate if certain genotypic characteristics of the strains had an impact on biofilm formation, the strains' genome was uploaded to webtools to detect genes related to virulence, plasmids, and antibiotic resistance. The information on the strains' ST, serotype and *fimH*- and *fumC*-alleles was previously provided from NORM, for a previous study (29). This information was confirmed by the webtools MLST, SeroTypeFinder and CH-Typer. As four of the strains from EcoSens, belonging to ST73, were not WGS, the genotypic traits of these could not be investigated and are not included in the statistical analyses.

4.3.1 Resistance genes and their impact on biofilm formation

As resistance towards tetracycline and the production of β-lactamases has been suggested to impact the ability to produce biofilms (88, 89), phenotypic susceptibility tests towards tetracycline and ampicillin were conducted to confirm the resistance information provided by NORM (29). Tetracycline resistance is suggested to increase biofilm formation (88), while production of β-lactamases are suggested to inhibit biofilm formation (89), which is basis for our hypothesis. One strain showed a phenotypic resistance towards ampicillin, without any β-lactamases detected by ResFinder (68, 69). This was the only strain in our sample previously classified as "intermediate (I)" (29). This suggests that it might be susceptible to higher concentrations of ampicillin, and that the resistance is due to another mechanism than the production of β-lactamases. A summary of the phenotypic resistance are presented in Table 4.3 and a summary of resistance genes detected by ResFinder (68, 69), and the number of isolates harbouring them are presented in Table 4.4

Table 4.3 Number of isolates exhibiting a phenotypic resistance towards tetracycline and ampicillin.

Antibiotic agent	Resistance breaking point	Number of resistant isolates
Tetracycline	< 19 mm inhibition zone	17
Ampicillin	MIC > 8mg/L	13 (+ one intermediate)

Table 4.4. Results from ResFinder. The table shows resistance genes detected in ResFinder (68, 69), and the number of strains in this project harbouring the genes.

Class	Gene	Number of strains
β-lactam	*blaCTX-M-15*	7
	blaOXA-1	5
	blaCTX-M-27	2
	blaCTX-M-14	1
	blaTEM-1B	7
Fluoroquinolones	*gyrA*	22
	parE (mutation)	25
	parC (mutation)	22
Macrolide	*mph(A)*	10
Phenicol	*catB3*	5
	catA1	2
Aminoglycosides	*aac(6')-lb-cr*	5
	aac(3)-lla	5
Folate Pathway Antagonist	*sul1*	10
	sul2	8
	dfrA17	11
	dfrA1	1
	dfrA36	1
Tetracycline	*tet(B)*	9
	tet(A)	8

The most frequent resistance genes are the mutations in *gyrA, parE* and *parC,* providing resistance towards fluoroquinolones. In total 13 strains harboured at least one β-lactamase encoding gene, and 17 strains carried genes providing tetracycline resistance. Considering that multi drug resistance is defined as resistance towards three or more classes of antibiotics (10), 16 of 35 strains in this material were multi drug resistant. The two isolates with the highest number of resistance genes, 13, both belonged to ST648. One ST648 strain and the one ST73 strain that was WGS harboured no resistance genes. The three strains belonging to ST131 clade B harboured only one resistance gene, *tetB.*

β-lactamases seem to be associated with decreased biofilm formation

Biomass in wells was higher in non-β-lactamase producers (0.158 ± 0.05) than the ones producing β-lactamase (0.130 ± 0.02), a statistically significant difference (Independent samples t-test, p = 0.036) of 0.028 (95% CI, 0.002 to 0.054). This difference was however not seen for biomass on the pegs (Mann-Whitney U test, p = 0.243). This result agrees with what is previously found by others (46, 89), implying that the acquisition of β-lactamases may come at a cost to other protecting phenotypes, like biofilm formation. Gallant *et al.* found that *Pseudomonas aeruginosa* and *E. coli* that harboured plasmids encoding bla_{TEM-1} (class A) showed decreased adherence and biofilm formation (89). They suggest that β-lactamases affect the peptidoglycan remodelling, and thereby disrupt bacterial adhesion essential for initial formation of biofilm (89). They found that the effect is restricted to Class A and class D β-lactamases, possibly due to their structural similarity with low-molecular weight penicillin-binding proteins. The β-lactamase genes harboured by the strains in this project, belong to either class A or class D. As Gallant *et al*. (89) investigated the effect of TEM- and OXA-β-lactamases, further analyses were conducted with distinction between the CTX-M type of β-lactamases and TEM/OXA type of β-lactamases. The strains encoding TEM/OXA β-lactamases displayed a significantly lower biomass in the wells compared to the strains that do not encode them (Independent samples t-test, p = 0.037), like illustrated in Figure 4.6. The difference was however, not seen for biomass on the pegs (Mann-Whitney U test, p = 0.079). Neither the biomass on the pegs nor the wells were significantly different between the strains that harboured CTX-M β-lactamases and those that did not (Mann-Whitney U test, p = 0.760 and p = 0.460, respectively). This implies that the biofilm inhibition seen by β-lactamases, was due to the bla_{TEM-1B} and bla_{OXA-1}, not the CTX-M.

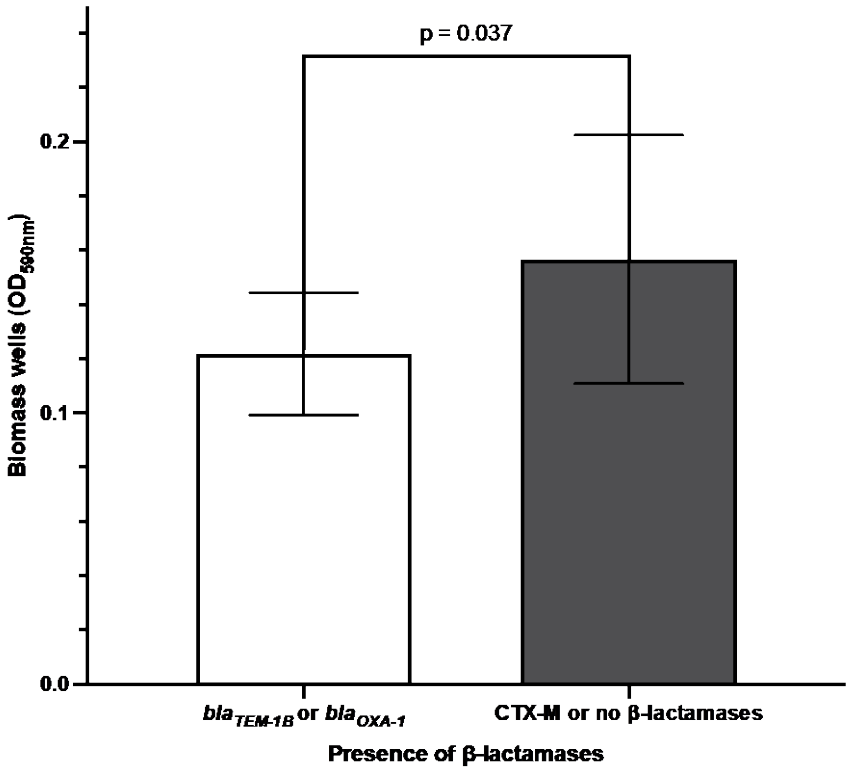

Figure 4.6 Average biomass in wells for two groups: strains harbouring *bla*$_{TEM-1B}$ or *bla*$_{OXA-1}$ and strains that carry either CTX-M type β-lactamase or no β-lactamases. Error bars represents SD. Biomass in wells for the strains carrying *bla*$_{TEM-1B}$ or *bla*$_{OXA-1}$ (N = 9, mean 0.122 ± 0.023) was statistically significantly lower than for the strains that do not harbour these genes (N = 26, mean 0.157 ± 0.046), a significant difference of 0.035 (95% CI 0.002-0.067) (Independent samples t-test, p = 0.037).

Tetracycline resistance and biofilm formation

The presence of any of the two tetracycline resistance genes (*tetA* or *tetB*) did not affect biofilm formation significantly, neither on pegs or in wells (Mann-Whitney U test, p = 0.483 and p = 0.590, respectively). However, when separating the two genes, the strains harbouring *tetA* produced significantly (Kruskal Wallis H test, Dunn's post hoc p = 0.016) more biomass on the pegs (N = 8, mean rank 26.63) compared to the strains harbouring *tetB* (N = 9, mean rank 12.78). Strains not harbouring any tetracycline resistance genes (N = 18, mean rank 16.78) did not differ significantly from any of the groups (Kruskal Wallis H test, Dunn's post hoc, *tetA* p = 0.071, *tetB* p = 1.00). The difference did not apply for biomass in wells (Kruskal Wallis H test, p = 0.079). May *et al.* (88) showed that presence of *tetA*, encoding the tetracycline resistance efflux pump TetA(C), promotes biofilm formation, but only in the presence of sub-inhibitory concentrations of tetracycline. This suggests that the antibiotic exposure induces a stress response (90), resulting in expression of efflux pumps and subsequent biofilm formation. This is supported by Kvist *et al.,* which discovered the abolishment of biofilm formation in the

presence of efflux pump inhibitors (91). It could be interesting to investigate the effect of *tetA* or *tetB* in biofilm formation in the present of sub-inhibitory concentrations of tetracycline, to see if the difference also applies between strains harbouring *tetA/tetB* and strains that harbour no tetracycline resistance genes.

4.3.2 Role of virulence-associated genes in biofilm formation

The presence of virulence-associated genes in each strain was detected with VirulenceFinder (70, 71), to investigate their association with biofilm formation. A lot of genes were detected, and we had to make a cut-off for which genes we wanted to analyse. If we suspected that the genes affect biofilm formation, based on previous literature, and if the gene/group of genes were present/absent in a comparable number of isolates, we considered them suitable for analyses. Genes of interest are presented in Table 4.5. In short, an adhesin-related gene cluster, capsule-related genes and single genes present in >10 strains, with a putative impact on biofilm formation were selected.

Table 4.5 Summary of virulence-factor genes of interest, suitable for statistical analyses. Total number of isolates harbouring them are presented, along with the distribution between the STs. For systems with several genes, the total number of isolates harbouring at least one gene within the system is presented.

			Number of isolates				
Categories	**System**	**Gene**	**Total**	**ST73**	**ST131**	**ST648**	**≥1 gene in the system**
Adhesin	*pap*	*papA_F19*	1	0	1	0	19
		papA_F20	1	0	1	0	
		papA_F43	18	1	15	2	
		papA_F48	1	0	0	1	
		papC	5	0	4	1	
	Single gene	*lpfA*	11	0	0	11	
Capsule	*kps*	*kpsMII*	20	1	15	4	21
		kpsMIII	1	0	0	1	
Toxin		*usp*	20	1	19	0	
Serum resistance		*iss*	19	1	16	2	

The presence of one or more *pap* gene was associated with enhanced biofilm formation

The effect on biofilm formation due to the presence of at least one *pap*-gene, was investigated (Mann- Whitney U test). Biomass both on pegs and in wells, were statistically significantly higher in strains that harboured one or more *pap* genes (N = 19, mean rank 19.11 and 19.79, respectively) compared to the group that did not (N = 12, mean rank 11.08 and 10.00, respectively) (pegs; $p = 0.016$, wells; $p = 0.003$). This finding was expected as it is well-established that the type P fimbriae, encoded by the *pap* operon, plays a role in the initial adhesion required for biofilm formation (8, 13).

The presence of *lpfA* and *usp* was associated with biofilm formation

Long polar fimbriae, encoded by *lpfA,* are colonization factors associated with colonization of the intestines (92), and *usp,* uropathogenic specific protein, is a bacteriocin associated with UPEC (93). The presence of *lpfA* seems to be associated with decreased biofilm formation both on pegs and in wells in this project (Mann-Whitney U test, $p < 0.001$ and $p = 0.003$, respectively), whereas the presence of *usp* seems to be associated with increased biofilm formation both on pegs and in wells (Mann-Whitney U test, $p < 0.001$ and $p = 0.004$, respectively), like illustrated in Figure 4.7. Remarkably, the *lpfA* gene was only carried by the strains belonging to ST648 (all), while the *usp* gene was carried by all strains in this material except for the ST648 strains. The presence/absence of these genes could explain why ST648 produced less biofilm compared to the other STs in this collection (Kruskal Wallis H test, post hoc: Dunn's procedure with Bonferroni correction. Pegs; $p = 0.002$, wells; $p = 0.014$). Considering that Schaufler *et al.* found that ST648 seems to lack virulence factors associated with UTIs (36), and we observe in this study that the ST648 strains are the only one encoding *lpfA*, and the only ones not encoding *usp,* it may imply that biofilm production is more associated with strains from the urinary tract compared to strains from the intestines. However, the presence of these genes is not strictly associated with the origin of the isolates, and ST648, an isolate from the bloodstream, might originate from anywhere (e.g. urinary tract causing urosepsis). Hence, these findings could also be confounded by other traits that distinguish ST648 from ST131 and ST73 as they do not guarantee causality, only associations.

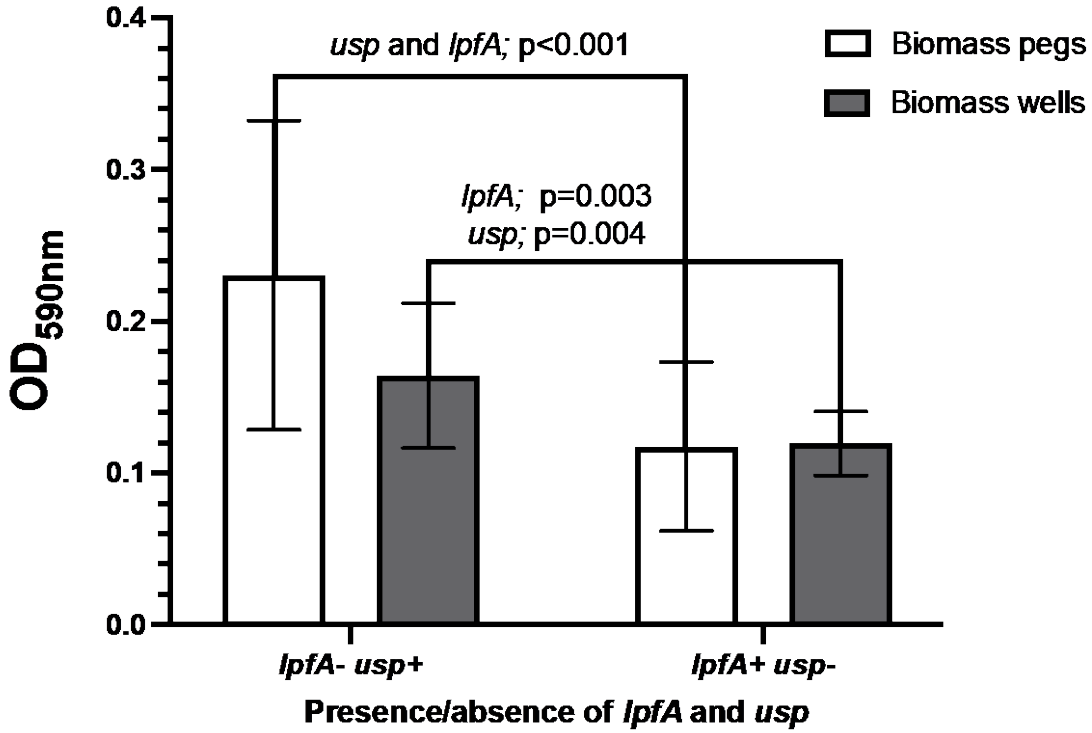

Figure 4.7 Biomass on pegs and in wells, based on the presence/absence of *lpfA* and *usp* genes. The strains harbouring *lpfA* expressed a significantly lower (Mann-Whitney U test, pegs; $p < 0.001$, wells; $p = 0.003$) biomass on the pegs and in the wells (N = 11, mean rank 8.64 and 10.64 respectively), compared to the strains that did not (N = 20, mean rank 20.05 and 21.38 respectively). Biomass on pegs and in wells was significantly higher Mann-Whitney U test, pegs; $p < 0.001$, wells; 0.004) among the strains harbouring the *usp* gene (N = 20, mean rank 20.05 and 19.40 respectively), compared to the strains that did not (N = 11, mean rank 8.69 and 9.82 respectively).

Presence of *iss*, increased serum survival, was associated with enhanced biofilm formation

The strains harbouring *iss* had a significantly higher (Mann-Whitney U test, $p < 0.001$) biomass both on pegs and in wells (N = 19, mean rank 20.26 and 23.11, respectively), compared to the strains that did not (N = 10, mean rank 9.25 and 11.94, respectively). Among the strains included in this project, 54% harboured the *iss* gene. The role of *iss* in human ExPEC and biofilm is unclear and needs to be further investigated. Although not as frequently examined in humans, it has been found to occur in approximately 60% of UPEC (94). The presence of *iss* and its effect on virulence is mostly studied in avian pathogenic *E. coli* (APEC) (94). *iss* in APEC has frequently been associated with complement resistance (95). This complement resistance has been suggested to contribute to the virulence of APEC. It was also found to be present to a higher degree in chickens with colibacillosis, compared to healthy chickens (95). However, the apparent association between biofilm abundance and *iss* could be random, due to them being selected under similar conditions.

Genes encoding capsules was not associated with biofilm formation

The presence of *kpsMII* or *kpsMIII* genes did not show a significant effect on biofilm formation (Mann-Whitney U test, pegs; $p = 0.468$, wells; $p = 0.749$). Another study have found that the release of group II capsular polysaccharides by UPEC inhibits the biofilm formation drastically by reducing the ability to adhere to the surface (59). This effect was not seen in this project. However, their biomass staining was performed in microtiter plates of different material than ours, and it was only one out of seven biofilm assays performed by Valle *et al.* (59).

5 Conclusion and future prospects

The production of biofilm offers a challenge in treatment of ExPEC infections. The aim of this project was to examine the ability of clinical ExPEC isolates to produce biofilms and investigate any correlation to selected phenotypic and genotypic traits of the strains, with the possibility of generating novel knowledge on factors associated with biofilm formation in ExPEC.

We characterized the biofilm formation of the included ExPEC strains with both quantitative and qualitative methods. Based on results from this project, we conclude that total biomass better reflects biofilm abundance, compared to number of bacteria in the biofilm. Expression of major biofilm matrix components, curli and cellulose, appeared to be a widespread phenotype among the ExPEC strains included in this project. The expression of curli and cellulose was independent of temperature and of each other, and our results indicate that cellulose had a greater impact on biofilm abundance than curli.

Higher biofilm abundance was associated with certain phylogenetic groups (STs, clades). ST131, especially clade C1 and C2, was superior in biofilm formation compared to ST648, which is contradictory to what we hypothesized. Detection of *lpfA*, important for colonization of the intestines, in all ST648 strains and of *usp*, encoding uropathogenic specific protein, in all strains but ST648, may suggest an explanation for the inferior biofilm formation in ST648. This might imply that biofilm formation may be a more important trait in the urinary tract compared to the intestines. However, the presence of these genes does not strictly correlate with the origin of the isolate. In this project, the ST648 strains are from the bloodstream, and might originate from both the urinary tract and the intestines. Hence, this finding could also be explained by other properties within the STs.

We confirmed that flagella-mediated motility was associated with increased biofilm formation, whereas haemolysis was not. Our findings also indicated that presence of genes encoding β-lactamases comes at a cost of other protecting phenotypes like biofilm formation, as these strains produced less biofilm. The presence of *tetA* was associated with a higher biofilm biomass compared to *tetB,* but no significant difference was seen with the tetracycline-susceptible strains.

We found that the presence of certain virulence genes is associated with increased biofilm formation. We hypothesized that the presence of adhesin-related genes would contribute to biofilm formation, which was the case for type P-fimbriae (encoded by *pap*). The presence of *iss* (increased serum survival) was associated with enhanced biofilm formation, however, the mechanism for this is unclear. The presence of capsule-related genes has been suggested to be associated with inhibition of biofilm formation, but this was not detected in our results.

This project provides some interesting results, offering preliminary tendencies of the biofilm way of life in clinical isolates of ExPEC. Provision of five different variables as a measurement of biofilm formation, increases the projects robustness. Some of the observations done in this project displays a potential for further investigation, preferably on a bigger sample size. Additional bioinformatic analyses is suggested for future prospects, comparing possible allelic differences between genes associated with biofilm formation, e.g. genes related to curli and cellulose expression, which might shed a light on their expression profiles.

It is also essential to mention that the procedures performed in this project have been conducted under optimal conditions *in vitro* which are not directly transferable to human physiological conditions *in vivo.* Animal models to characterize the biofilms of ExPEC *in vivo* would be essential to make progress in determining the most important aspects of biofilm formation in clinical isolates. We suggest that the findings in this project should be further investigated with larger sample sizes and complemented with other procedures, in order to elucidate what are the central traits of biofilm formation in ExPEC.

References

1. Qadri F, Svennerholm A-M, Faruque ASG, Sack RB. Enterotoxigenic Escherichia coli in Developing Countries: Epidemiology, Microbiology, Clinical Features, Treatment, and Prevention. Clinical Microbiology Reviews. 2005;18(3):465-83.
2. Javaloyas M, Garcia-Somoza D, Gudiol F. Epidemiology and Prognosis of Bacteremia: A 10-y Study in a Community Hospital. Scandinavian Journal of Infectious Diseases. 2002;34(6):436-41.
3. de Lastours V, Laouénan C, Royer G, Carbonnelle E, Lepeule R, Esposito-Farèse M, et al. Mortality in Escherichia coli bloodstream infections: antibiotic resistance still does not make it. Journal of Antimicrobial Chemotherapy. 2020;75(8):2334-43.
4. European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report 2019. Stockholm: ECDC; 2020.

Stockholm, November 2020.
5. Goering R. V. DHM, Zuckerman M., Roitt I. M., Chiodini P. L. Chapter 20: Urinary Tract Infections. Mims' Medical Microbiology. Fifth ed: Elsevier Saunders; 2013.
6. Soto SM. Importance of Biofilms in Urinary Tract Infections: New Therapeutic Approaches. Advances in Biology. 2014;2014:543974.
7. Jiang X, Pace JL. Microbial Biofilms. In: Pace JL, Rupp ME, Finch RG, editors. Biofilms, Infection, and Antimicrobial Therapy. United States of America: Taylor and Francis Group; 2006.
8. Beloin C, Roux A, Ghigo J-M, editors. Escherichia coli Biofilms. Department of Microbiology and Immunology, Emory University School of Medicine: Springer; 2008.
9. Cooper GM. The Cell: A Molecular Approach. . Sunderland: Sinauer Associates; 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK9839/.
10. McNally A, Kallonen T, Connor C, Abudahab K, Aanensen DM, Horner C, et al. Diversification of Colonization Factors in a Multidrug-Resistant Escherichia coli Lineage Evolving under Negative Frequency-Dependent Selection. mBio. 2019;10(2).
11. Delcaru C, Alexandru I, Podgoreanu P, Grosu M, Stavropoulos E, Chifiriuc MC, et al. Microbial Biofilms in Urinary Tract Infections and Prostatitis: Etiology, Pathogenicity, and Combating strategies. Pathogens. 2016;5(4).
12. Johnson JR, Russo TA. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) Escherichia coli. International Journal of Medical Microbiology. 2005;295(6):383-404.
13. Terlizzi ME, Gribaudo G, Maffei ME. UroPathogenic Escherichia coli (UPEC) Infections: Virulence Factors, Bladder Responses, Antibiotic, and Non-antibiotic Antimicrobial Strategies. Front Microbiol. 2017;8:1566.
14. Lillington J, Geibel S, Waksman G. Biogenesis and adhesion of type 1 and P pili. Biochim Biophys Acta. 2014;1840(9):2783-93.
15. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015;13(5):269-84.
16. François M, Hanslik T, Dervaux B, Le Strat Y, Souty C, Vaux S, et al. The economic burden of urinary tract infections in women visiting general practices in France: a cross-sectional survey. BMC Health Services Research. 2016;16(1):365.
17. Tumbarello M, Spanu T, Di Bidino R, Marchetti M, Ruggeri M, Trecarichi EM, et al. Costs of bloodstream infections caused by Escherichia coli and influence of extended-

spectrum-beta-lactamase production and inadequate initial antibiotic therapy. Antimicrobial agents and chemotherapy. 2010;54(10):4085-91.
18. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015;40(4):277-83.
19. Organization WH. Antibiotic Resistance Genova: World Health Organization; 2020 [Available from: https://www.who.int/en/news-room/fact-sheets/detail/antibiotic-resistance.
20. European Commission. EU Action on Antimicrobial Resistance. Available from: https://ec.europa.eu/health/antimicrobial-resistance/eu-action-on-antimicrobial-resistance_en.
21. Matsumura Y, Pitout JDD, Peirano G, DeVinney R, Noguchi T, Yamamoto M, et al. Rapid Identification of Different Escherichia coli Sequence Type 131 Clades. Antimicrob Agents Chemother. 2017;61(8).
22. Johnson JR, Tchesnokova V, Johnston B, Clabots C, Roberts PL, Billig M, et al. Abrupt emergence of a single dominant multidrug-resistant strain of Escherichia coli. J Infect Dis. 2013;207(6):919-28.
23. Goering R. V. DHM, Zuckerman M., Roitt I. M., Chiodini P. L. Chapter 33: Attacking the enemy: Antimicrobial Agents and Chemotherapy. Mims' Medical Microbiology. Fifth ed: Elsevier Saunders; 2013.
24. NORM/NORM-VET2019. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø/Oslo; 2020.
25. Anders Bærheim NG. Antibiotikabruk i primærhelsetjenesten: Cystitt: Helsedirektoratet; 2019 [2.5:[Available from: http://www.antibiotikaiallmennpraksis.no/index.php?action=showtopic&topic=vXmA4Spa.
26. Helsedirektoratet(2013). Nasjonal faglig retningslinje for bruk av antibiotika i sykehus [nettdokument]. Oslo: Helsedirektoratet. (Last updated 15.03.2021, read 02.04.2021). Available from https://www.helsedirektoratet.no/retningslinjer/antibiotika-i-sykehus#referere.2013.
27. Georgopapadakou NH. Antibiotic Resistance in Biofilms. In: Pace JL, Rupp ME, Finch RG, editors. Biofilms, Infection, and Antimicrobial Therapy. United States of America: Taylor and Francis Group; 2006.
28. Lewis K, Spoering AL, Kaldalu N, Keren I, Shah D. Persisters: Specialized Cells Responsible for Biofilm Tolerance to Antimicrobial Agents. In: Pace JL, Rupp ME, Finch RG, editors. Biofilms, Infection, and Antimicrobial Therapy. United States of America: Taylor and Francis Group; 2006.
29. Gladstone RAaM, Alan and Pöntinen, Anna K. and Tonkin-Hill, Gerry and Lees, John A. and Skytén, Kusti and Cléon, François and Christensen, Martin O. K. and Haldorsen, Bjørg C. and Bye, Kristina K. and Gammelsrud, Karianne W. and Hjetland, Reidar and Kümmel, Angela and Larsen, Hege E. and Lindemann, Paul Christoffer and Löhr, Iren H. and Marvik, Åshild and Nilsen, Einar and Noer, Marie T. and Simonsen, Gunnar S. and Steinbakk, Martin and Tofteland, Ståle and Vattøy, Marit and Bentley, Stephen D. and Croucher, Nicholas J. and Parkhill, Julian and Johnsen, Pål J. and Samuelsen, Ørjan and Corander, Jukka. Emergence and Dissemination of Antimicrobial Resistance in Escherichia Coli Causing Bloodstream Infections: A Nationwide Longitudinal Microbial Population Genomic Cohort Study in Norway between 2002-2017. 2020. Available from: https://ssrn.com/abstract=3645193 or http://dx.doi.org/10.2139/ssrn.3645193.
30. Joensen KG, Tetzschner AMM, Iguchi A, Aarestrup FM, Scheutz F. Rapid and Easy In Silico Serotyping of Escherichia coli Isolates by Use of Whole-Genome Sequencing Data. Journal of Clinical Microbiology. 2015;53(8):2410-26.
31. Ibarz Pavón AB, Maiden MC. Multilocus sequence typing. Methods Mol Biol. 2009;551:129-40.

32. Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JDD. Global Extraintestinal Pathogenic Escherichia coli (ExPEC) Lineages. Clin Microbiol Rev. 2019;32(3).
33. Johnson JR, Johnston BD, Gordon DM. Rapid and Specific Detection of the Escherichia coli Sequence Type 648 Complex within Phylogroup F. J Clin Microbiol. 2017;55(4):1116-21.
34. Norsk legemiddelhåndbok. Tetrasykliner og glycylsykliner.: Foreningen for utgivelse av Norsk legemiddelhåndbok; 2016 [updated 16.11.2016; cited 2020 16.10.]. Available from: https://www.legemiddelhandboka.no/L1.2.7/Antimikrobielle_midler#Lk-01-infeksjon-1960.
35. Rice LB. Mechanisms of resistance and clinical relevance of resistance to β-lactams, glycopeptides, and fluoroquinolones. Mayo Clin Proc. 2012;87(2):198-208.
36. Schaufler K, Semmler T, Wieler LH, Trott DJ, Pitout J, Peirano G, et al. Genomic and Functional Analysis of Emerging Virulent and Multidrug-Resistant Escherichia coli Lineage Sequence Type 648. Antimicrob Agents Chemother. 2019;63(6).
37. Schaufler K, Semmler T, Pickard DJ, de Toro M, de la Cruz F, Wieler LH, et al. Carriage of Extended-Spectrum Beta-Lactamase-Plasmids Does Not Reduce Fitness but Enhances Virulence in Some Strains of Pandemic E. coli Lineages. Front Microbiol. 2016;7:336.
38. Bogema DR, McKinnon J, Liu M, Hitchick N, Miller N, Venturini C, et al. Whole-genome analysis of extraintestinal Escherichia coli sequence type 73 from a single hospital over a 2 year period identified different circulating clonal groups. Microb Genom. 2020;6(1):e000255.
39. Matsui Y, Hu Y, Rubin J, de Assis RS, Suh J, Riley LW. Multilocus sequence typing of Escherichia coli isolates from urinary tract infection patients and from fecal samples of healthy subjects in a college community. MicrobiologyOpen. 2020;9(6):e1032.
40. Kahlmeter G. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO·SENS Project. Journal of Antimicrobial Chemotherapy. 2003;51(1):69-76.
41. Kahlmeter G, Poulsen HO. Antimicrobial susceptibility of Escherichia coli from community-acquired urinary tract infections in Europe: the ECO·SENS study revisited. International Journal of Antimicrobial Agents. 2012;39(1):45-51.
42. Alhashash F, Wang X, Paszkiewicz K, Diggle M, Zong Z, McNally A. Increase in bacteraemia cases in the East Midlands region of the UK due to MDR Escherichia coli ST73: high levels of genomic and plasmid diversity in causative isolates. Journal of Antimicrobial Chemotherapy. 2015;71(2):339-43.
43. Batt CA. Virulence. Reference Module in Food Science: Elsevier; 2016.
44. Webb SA, Kahler CM. Bench-to-bedside review: Bacterial virulence and subversion of host defences. Crit Care. 2008;12(6):234.
45. Cross AS. What is a virulence factor? Crit Care. 2008;12(6):196.
46. Naves P, Del Prado G, Huelves L, Gracia M, Ruiz V, Blanco J, et al. Measurement of biofilm formation by clinical isolates of Escherichia coli is method-dependent. Journal of Applied Microbiology. 2008;105(2):585-90.
47. Zhang K, Schneider D, Biswas R, de la Espriella MG, Rao J, Baffoe-Bonnie A. 2603. Biofilm Formation as a Predictive Marker of Prognosis for Escherichia coli Sepsis. Open Forum Infectious Diseases. 2019;6(Supplement_2):S904-S5.
48. Harshey RM. Bacterial Motility on a Surface: Many Ways to a Common Goal. Annual Review of Microbiology. 2003;57(1):249-73.

49. Hung C, Zhou Y, Pinkner JS, Dodson KW, Crowley JR, Heuser J, et al. Escherichia coli biofilms have an organized and complex extracellular matrix structure. mBio. 2013;4(5):e00645-13.
50. Bokranz W, Wang X, Tschäpe H, Römling U. Expression of cellulose and curli fimbriae by Escherichia coli isolated from the gastrointestinal tract. J Med Microbiol. 2005;54(Pt 12):1171-82.
51. Zhou Y, Smith DR, Hufnagel DA, Chapman MR. Experimental manipulation of the microbial functional amyloid called curli. Methods Mol Biol. 2013;966:53-75.
52. Reichhardt C, Jacobson AN, Maher MC, Uang J, McCrate OA, Eckart M, et al. Congo Red Interactions with Curli-Producing E. coli and Native Curli Amyloid Fibers. PLoS One. 2015;10(10):e0140388-e.
53. Gophna U, Barlev M, Seijffers R, Oelschlager TA, Hacker J, Ron EZ. Curli fibers mediate internalization of Escherichia coli by eukaryotic cells. Infect Immun. 2001;69(4):2659-65.
54. Hatt JK, Rather PN, editors. Role of Bacterial Biofilms in Urinary Tract Infections. Department of Microbiology and Immunology, Emory University School of Medicine: Springer; 2008.
55. Jubelin G, Dorel C, Lejeune P. Role of Biofilms in Infections Caused by Escherichia coli. In: Pace JL, Rupp ME, Finch RG, editors. Biofilms, Infection, and Antimicrobial Therapy. United States of America: Taylor & Francis; 2006.
56. Bian Z, Brauner A, Li Y, Normark S. Expression of and cytokine activation by Escherichia coli curli fibers in human sepsis. J Infect Dis. 2000;181(2):602-12.
57. Ristow LC, Welch RA. Hemolysin of uropathogenic Escherichia coli: A cloak or a dagger? Biochim Biophys Acta. 2016;1858(3):538-45.
58. Whitfield C. Biosynthesis and Assembly of Capsular Polysaccharides in Escherichia coli. Annual Review of Biochemistry. 2006;75(1):39-68.
59. Valle J, Da Re S, Henry N, Fontaine T, Balestrino D, Latour-Lambert P, et al. Broad-spectrum biofilm inhibition by a secreted bacterial polysaccharide. Proceedings of the National Academy of Sciences of the United States of America. 2006;103(33):12558-63.
60. Lajhar SA, Brownlie J, Barlow R. Characterization of biofilm-forming capacity and resistance to sanitizers of a range of E. coli O26 pathotypes from clinical cases and cattle in Australia. BMC Microbiol. 2018;18(1):41.
61. Da Re S, Ghigo JM. A CsgD-independent pathway for cellulose production and biofilm formation in Escherichia coli. J Bacteriol. 2006;188(8):3073-87.
62. Harrington BJ, Hageage GJ, Jr. Calcofluor White: A Review of its Uses and Applications in Clinical Mycology and Parasitology. Laboratory Medicine. 2003;34(5):361-7.
63. UVP Ultra-violet products. High Performance Ultraviolet Transilluminators: Operating Instructions. . Available from: http://www.company7.com/library/uvp/81016101_trans_hiperf_old.pdf.
64. Jenkins SG, Schuetz AN. Current concepts in laboratory testing to guide antimicrobial therapy. Mayo Clin Proc. 2012;87(3):290-308.
65. Hudzicki J. Kirby-bauer disk diffusion susceptibility test protocol. American Society for Microbiology [Internet]. 2009. Available from: https://asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf.
66. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters 2020 [10.0:[Available from: http://www.eucast.org.

67. Kearns DB. A field guide to bacterial swarming motility. Nat Rev Microbiol. 2010;8(9):634-44.
68. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, et al. ResFinder 4.0 for predictions of phenotypes from genotypes. Journal of Antimicrobial Chemotherapy. 2020;75(12):3491-500.
69. Zankari E, Allesøe R, Joensen KG, Cavaco LM, Lund O, Aarestrup FM. PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. Journal of Antimicrobial Chemotherapy. 2017;72(10):2764-8.
70. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, et al. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic Escherichia coli. J Clin Microbiol. 2014;52(5):1501-10.
71. Malberg Tetzschner AM, Johnson JR, Johnston BD, Lund O, Scheutz F. Genotyping of Escherichia coli Isolates for Extraintestinal Virulence Genes by Use of Whole-Genome Sequencing Data. Journal of Clinical Microbiology. 2020;58(10):e01269-20.
72. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 2014;58(7):3895-903.
73. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, et al. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol. 2012;50(4):1355-61.
74. Roer L, Johannesen TB, Hansen F, Stegger M, Tchesnokova V, Sokurenko E, et al. CHTyper, a Web Tool for Subtyping of Extraintestinal Pathogenic Escherichia coli Based on the fumC and fimH Alleles. Journal of Clinical Microbiology. 2018;56(4):e00063-18.
75. Harrison JJ, Stremick CA, Turner RJ, Allan ND, Olson ME, Ceri H. Microtiter susceptibility testing of microbes growing on peg lids: a miniaturized biofilm model for high-throughput screening. Nature Protocols. 2010;5(7):1236-54.
76. Wilson C, Lukowicz R, Merchant S, Valquier-Flynn H, Caballero J, Sandoval J, et al. Quantitative and Qualitative Assessment Methods for Biofilm Growth: A Mini-review. Res Rev J Eng Technol. 2017;6(4).
77. Mika F, Hengge R. Small RNAs in the control of RpoS, CsgD, and biofilm architecture of Escherichia coli. RNA Biol. 2014;11(5):494-507.
78. Rossi E, Paroni M, Landini P. Biofilm and motility in response to environmental and host-related signals in Gram negative opportunistic pathogens. Journal of Applied Microbiology. 2018;125(6):1587-602.
79. Saldaña Z, Xicohtencatl-Cortes J, Avelino F, Phillips AD, Kaper JB, Puente JL, et al. Synergistic role of curli and cellulose in cell adherence and biofilm formation of attaching and effacing Escherichia coli and identification of Fis as a negative regulator of curli. Environ Microbiol. 2009;11(4):992-1006.
80. Duprilot M, Baron A, Blanquart F, Dion S, Pouget C, Lettéron P, et al. Success of Escherichia coli O25b:H4 Sequence Type 131 Clade C Associated with a Decrease in Virulence. Infect Immun. 2020;88(12):e00576-20.
81. Fredheim EGA, Klingenberg C, Rohde H, Frankenberger S, Gaustad P, Flaegstad T, et al. Biofilm formation by Staphylococcus haemolyticus. Journal of clinical microbiology. 2009;47(4):1172-80.
82. McCrate OA, Zhou X, Cegelski L. Curcumin as an amyloid-indicator dye in E. coli. Chem Commun (Camb). 2013;49(39):4193-5.
83. Pratt LA, Kolter R. Genetic analysis of Escherichia coli biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Mol Microbiol. 1998;30(2):285-93.

84. Caiazza NC, O'Toole GA. Alpha-toxin is required for biofilm formation by Staphylococcus aureus. Journal of bacteriology. 2003;185(10):3214-7.
85. Soto SM, Smithson A, Martinez JA, Horcajada JP, Mensa J, Vila J. Biofilm Formation in Uropathogenic Escherichia coli Strains: Relationship With Prostatitis, Urovirulence Factors and Antimicrobial Resistance. Journal of Urology. 2007;177(1):365-8.
86. Welch RA. Uropathogenic Escherichia coli-Associated Exotoxins. Microbiol Spectr. 2016;4(3):10.1128/microbiolspec.UTI-0011-2012.
87. Kerényi M, Allison HE, Bátai I, Sonnevend A, Emödy L, Plaveczky N, et al. Occurrence of hlyA and sheA genes in extraintestinal Escherichia coli strains. Journal of clinical microbiology. 2005;43(6):2965-8.
88. May T, Ito A, Okabe S. Induction of multidrug resistance mechanism in Escherichia coli biofilms by interplay between tetracycline and ampicillin resistance genes. Antimicrobial agents and chemotherapy. 2009;53(11):4628-39.
89. Gallant CV, Daniels C, Leung JM, Ghosh AS, Young KD, Kotra LP, et al. Common beta-lactamases inhibit bacterial biofilm formation. Molecular microbiology. 2005;58(4):1012-24.
90. Podlesek Z, Žgur Bertok D. The DNA Damage Inducible SOS Response Is a Key Player in the Generation of Bacterial Persister Cells and Population Wide Tolerance. Frontiers in Microbiology. 2020;11(1785).
91. Kvist M, Hancock V, Klemm P. Inactivation of efflux pumps abolishes bacterial biofilm formation. Appl Environ Microbiol. 2008;74(23):7376-82.
92. Torres AG, Blanco M, Valenzuela P, Slater TM, Patel SD, Dahbi G, et al. Genes Related to Long Polar Fimbriae of Pathogenic Escherichia coli Strains as Reliable Markers To Identify Virulent Isolates. Journal of Clinical Microbiology. 2009;47(8):2442-51.
93. Yamamoto S, Nakano M, Terai A, Yuri K, Nakata K, Nair GB, et al. The Presence of the Virulence Island Containing the *usp* gene in UroPathogenic *Escherichia coli is* Associated with Urinary Tract Infection in an Experimental Mouse Model. . Journal of Urology. 2001;165(4):1347-51.
94. Johnson TJ, Wannemuehler YM, Nolan LK. Evolution of the iss Gene in Escherichia coli. Appl Environ Microbiol. 2008;74(8):2360-9.
95. Delicato ER, de Brito BG, Gaziri LCJ, Vidotto MC. Virulence-associated genes in Escherichia coli isolates from poultry with colibacillosis. Veterinary Microbiology. 2003;94(2):97-103.

Appendices

Appendix A: Method 1.2.3: Long-Term Storage of Bacterial Strains

UiT
THE ARCTIC UNIVERSITY OF NORWAY

Faculty of Health Sciences
UiT -The Artic University of Norway

Department of Pharmacy

Microbial Pharmacology and Population Biology (MicroPop)

Method 1.2.3: Long-Term Storage of Bacterial Strains

Version No.	1.0
Author:	Elizabeth G.A. Fredheim
Approved by:	Nicole L. Podnecky
Date:	11 February 2015

1. Purpose/ brief description	To create and maintain pure, frozen stock cultures of bacterial strains for future use.
2. Scope	This protocol can be applied to most aerobic bacteria.
3. Safety precautions	Biosafety precautions based on bacterial strain. Use appropriate protective gear when handling objects in the -75°C freezer.
4. Risk waste	• Standard waste treatment appropriate for the organism. Biological and/or GMO waste in lab should be autoclaved.
5. References	0
6. Attachments	0
7. Notes	

Material and Equipment:

- Liquid growth medium of choice (*e.g.* LB ± antimicrobial for selection)
- Agar plates of choice (*e.g.* LB agar ± antimicrobial for selection)
- Sterile inoculation loops
- Sterile 15 mL culture tubes
- Sterile 2mL cryovials
- Glycerol solution, 80%
- Shaking incubator
- -70 to -80°C freezer

Before you start: Use standard microbiological and aseptic techniques to ensure sterility of the culture. It is recommended to work in a biological safety cabinet to provide an aseptic workspace and protect the sample(s). If the strain is a work in progress, simply making one vial for personal use is fine. Once a strain has been confirmed and will be used for further experiments one vial, a duplicate of the original, must be added to the MicroPop Seed Stock Collection in the IFA-basement, and the original placed in the bacterial biobank, with all information added to the bacterial biobank database found on in the MicroPop server-location.

If necessary, prepare the following reagents.

- Glycerol solution, 80%: For 100 mL - Add 54 mL glycerol (Dens: 1.4746 g/L at 25°C), adjust volume to 100 mL with milliQ water. Autoclave at 121°C for 20 min.

1. **Day 1:** Streak the bacterial strain of interest on appropriate agar to obtain single colonies and incubate at appropriate temperature.
    a. 30-37°C for 18 h +/-2 is the standard incubation time.
2. **Day 2:** Visually inspect plate for any signs of contamination. Select an isolated colony to inoculate 3-5 mL LB media (± antibiotic selection) in a 15 mL sterile culture tube. Incubate the culture for 16-18 hours at appropriate temperature (*e.g.* 37°C) with shaking (≈ 225 rpm).
    a. Always include a negative growth control for the growth media.
3. **Day 3:** Label two sterile 2 mL cryovials per strain with the following information using either an ethanol-proof marker or laminated label (as applicable):
    a. MicroPop Seed Stock Collection number
    b. Genus, species, and strain/isolate name
    c. Date
    d. Your name
    e. Other relevant strain information
        i. Growth Requirements (*e.g.* requires DAP 400 µg/mL; 30°C)
        ii. Resistance traits (*e.g.* AMP 100; CHL 25)
        iii. Plasmid name/number (*e.g.* pDS132; pSV1)
4. Add sufficient 80% Glycerol to 2 mL cryovials (typically 250 µl).
5. Add the culture to the cryovials for a final concentration of 20% glycerol (typically 750 µl culture + 250 µl 80% glycerol), and mix by pipetting.
6. Store the stock vials at -70 to -80°C. One vial in the **MicroPop Bacterial BioBank**, in numbered boxes in freezer on the second floor (for common use)

and one as backup in the **MicroPop Seed Stock Collection** (currently located in freezers in the K1 basement of the IFA building).

7. Enter relevant information about the new strain in the **MicroPop Strain Database** (currently located on the shared network drive at: MicroPop/0 - Laboratory Management /1 - Databases/Strains/MicroPop bacterial biobank MBBB).

8. Restreak the new stocks on appropriate agar and incubate overnight at appropriate temperature to confirm the purity of the samples.

Appendix B: Method 3.2.1: Antimicrobial Gradient Strip Testing

Faculty of Health Sciences
UiT -The Artic University of Norway
Department of Pharmacy
Microbial Pharmacology and Population Biology (MicroPop)

Method 3.2.1: Antimicrobial Gradient Strip Testing

Version No.	1.0
Author:	Elizabeth G.A. Fredheim & Nicole L. Podnecky
Approved by:	Nicole L. Podnecky
Date:	19 February 2018

1. Purpose/ brief description	Determination of the minimal inhibitory concentration (MIC) of *Escherichia coli* isolates to various antimicrobials using gradient strip testing. This is a quick an easy procedure to determine MIC and clinical interpretation using reference cut-off values (*e.g* EUCAST - R, I, S).
2. Scope	This protocol was written specific for *Enterobacteriaceae* according to Liofilchem and BioMerieux guidelines. Check the manufacturer guidelines and EUCAST or CLSI guidelines when working with other bacterial species.
3. Safety precautions	Biosafety precautions based on bacterial strain. Toxic compounds and antimicrobials in the testing strips.
4. Risk waste	Standard waste treatment appropriate for the organism. Autoclave.
5. References	NS-EN ISO 20776-1:2006 http://www.eucast.org/clinical_breakpoints/ CLSI M100-S20
6. Attachments	BioMerieux – Product Insert BioMerieux – MIC reading guide Liofilchem – MIC reading guide
7. Notes	The control strain should be tested regularly and compared to the expected range of MIC values given by EUCAST or CLSI.

Material and Equipment:

- Inoculation loops
- MIC test strips (BioMerieux® or Liofilchem®)
- Sterile cotton swabs
- Sterile forceps
- Sterile toothpicks
- Rotator
- Calibrated densitometer

Enterobacteriaceae specific:

- Mueller Hinton II (cation-adjusted) agar plates (BD – 211438/ 211441/ 212257)
- Sterile saline, 0.85% NaCl
- Incubation at 37°C for **18** +/- 2 hours
- Control strain: *Escherichia coli* ATCC 25922

Procedure:

Before you start: Use standard microbiological and aseptic techniques to ensure sterility of the culture. It is recommended to work in a biological safety cabinet to provide an aseptic workspace, protect the sample(s) and provide ample light for interpretation.

1. Streak for isolation a pure culture of the strain of interest on appropriate agar medium and incubate overnight.
    a. Alternatively an overnight liquid culture may be used.
    b. Subculture and growth to mid-log phase can further reduce inter-assay variability.
2. Allow MIC testing strips to equilibrate to room temperature before testing according to the manufacturer.
    a. Typically 15 minutes for those at 4°C and 1 hour for those at -20°C.
3. Suspend several fresh colonies in 3-5 mL of 0.85% NaCl with a sterile cotton swab to the optical density of a 0.5 McFarland.
    a. 0.5 McFarland(s) should not be prepared more than 15 min in advance of step 4.
    b. 0.5 McFarland has an OD600nm ≈ 0.08 – 0.1 if using a spectrophotometer or can be compared by eye to a reference standard looking through the culture to something dark in color.
4. Place a sterile cotton swab in the inoculum to saturate it. Then press it against the inside of the tube to remove excess liquid.
5. Streak a Mueller Hinton agar plate for confluency.

a. Use a rotator, hold the swab gently to the surface and count to 10 while spiraling the swab to the just past the center. Rotate the swab slightly to use a different surface and then count to 10 spiraling out again.
b. Or streak the plate for confluence 3 times, rotating the plate 60° each time and including the outer edge of the plate and using different sides/surfaces of the swab.

6. Allow the plate to dry for a few minutes (must be completely dry). Maximum 15 mins.
7. Use sterile forceps or strip application device to place gradient strips with scale facing up.
    a. Do not move the strip once it has touched the agar, drug diffusion occurs rapidly.
    b. When using forceps only handle the strip from the top edge - above the highest concentration.
8. Use a sterile toothpick to gently tap the strip and remove any air bubbles from under the strip. Do this in the direction of lowest to highest concentration.
    a. If using forceps for this step, forceps must be re-sterilized before further use.
9. Incubate at 37°C for 18 +/- 2 hours.
    a. Place the plate in the incubator no more than 15 minutes after the strip was applied.
    b. Do not stack more than 5 plates, ideally no more than 3.
10. Analysis: Read the MIC value according to the manufacturer's guidelines.
    a. Typically where the edge of the growth ellipse cuts the strip.
    b. For most bactericidal drugs include any form of growth in the interpretation.
    c. Single colonies within the inhibition zone should be evaluated.
    d. Always increase half-values to the next 2-fold dilution before interpretation of resistance category according to EUCAST or CLSI guidelines.
    e. For bacteriostatic antimicrobials the MIC should be read at ≥ 80% inhibition, the first point of *significant* inhibition as judged by the unaided eye.

Variables that might affect the results:

Low risk:

- The bacteria: colonies should not be older than 24-48h, to ensure a correct relationship between bacterial growth and antimicrobial diffusion. It is ideal to work with pure cultures.
- Growth conditions: Temperature, incubation time, atmosphere (plate stacking)
- MIC strips: diffusion speed, drug solubility, charge, molecular weight, durability, controls, application.

Medium risk:

- Analysis/interpretation: bactericidal antimicrobials should be read at total inhibition, bacteriostatic are read at 80% inhibition, which can be difficult. Single colonies within the inhibition zone have to be evaluated.

- Storage: The strips are very sensitive to moisture, it’s very important to store them dry with a desiccant and bring them to ambient temperature before opening packaging and use.
- Agar: thickness, viscosity, pH, cation concentration, thymine/thymidine content.

Appendix C: Results Congo red and Calcofluor summarized

	Curli		Cellulose	
	28°C	**37°C**	**28°C**	**37°C**
MP23-01	1	0	1	1
MP23-02	4	4	3	3
MP23-03	3	0	0	0
MP23-04	0	0	0	3
MP23-05	0	0	0	0
MP23-06	3	3	3	3
MP23-07	0	1	1	1
MP23-08	3	4	3	3
MP23-09	3	4	0	0
MP23-10	1	1	1	3
MP23-11	0	1	1	3
MP23-12	0	0	0	0
MP23-13	3	4	3	1
MP23-14	0	1	0	0
MP23-15	1	3	3	3
MP23-47	1	2	1	2
MP23-48	0	1	1	2
MP04-02	0	1	1	2
MP04-17	1	1	3	3
MP04-23	3	3	0	0
MP04-24	2	3	0	0
MP04-25	3	3	0	0
MP15-01	2	3	0	0
MP15-03	3	1	1	0
MP15-04	3	2	0	0
MP15-05	3	3	0	0
MP15-06	4	3	0	0

MP15-07	0	1	1	3
MP15-08	0	1	1	3
MP15-09	3	2	3	1
MP15-10	3	1	3	3
MP15-11	0	1	1	1
MP15-12	3	3	3	3
MP15-13	3	3	1	2
MP15-14	3	3	1	2

Appendix D: Summary of statistical analyses

Parameters		Statistical test/ test value	P- value
Correlation CFU and biomass pegs		Spearman rank-correlation / R_s = -0,176	0,319
Correlation CFU and biomass wells		Spearman rank-correlation / R_s = -0,314	0,080
Correlation biomass wells and biomass pegs		Spearman rank-correlation / R_s = 0,848	<0,001
CFU across STs		One-Way ANOVA (Welch)/ 1,355	0,283
Biomass across STs	**Pegs**	Kruskal Wallis / 11,992 Post-hoc: Dunn's (1964) Bonferroni adjustment	0,002 (ST648-ST131)
	Wells	Kruskal Wallis / 8,320 Post hoc: Dunn's (1964) Bonferroni adjustment	0,014 (ST648-ST131)
CFU across ST131 clades		One-Way ANOVA	0,358
Biomass across ST131 clades	**Pegs**	Kruskal Wallis / 6,482	0,090
	Wells	One-Way ANOVA (Welch): 17,866	0,002
		Games-Howell post hoc	0,001 (B-C1) 0,029 (B-C2)
Correlation curli 28°C/ curli 37°C		Chi-Square independence test (Pearson chi-square)	<0,001
		Phi strength of association: 0,715	<0,001

Correlation cellulose 28°C/ cellulose 37°C		Fishers exact test	<0,001
		Phi strength of association: 0,878	<0,001
Correlation curli 37°C/ cellulose 37°C		Chi square independence test (Pearson chi-square)	0,358
Curli37°C across STs		Fishers exact test	1,000
Cellulose37°C across STs		Fishers exact test	0,555
Curli37°C across ST131 clades		Fishers exact test	0,434
Cellulose37°C by ST131 clades		Fishers exact test Post hoc: Multiple z-tests of two proportions with BonFerroni adjustments	0,004 Clade B – Clade C2
CFU across β-lactamase		Mann-Whitney U-test	0,229
Biomass across β-lactamase	**Pegs**	Mann-Whitney U test	0,243
	Wells	Independent samples t-test	0,036
Biomass across OXA/TEM and CTX-M/No β-lactamases	**Pegs**	Mann-Whitney U test	0.079
	Wells	Mann-Whitney U test	0.025
Biomass across CTX-M and non-CTX-M	**Pegs**	Mann-Whitney U test	0.760
	Wells	Mann-Whitney U test	0.460
Curli 37°C across β-lactamases		Chi-square test of independence	0,631
Cellulose37°C across β-lactamases		Fishers exact test	1,000
CFU across tetracycline resistance		Mann-Whitney U test	0,909
Biomass across tetracycline resistance	**Pegs**	Mann-Whitney U test	0,483
	Wells	Mann-Whitney U test	0,590
Biomass across *tetA/tetB*/no tet	**Pegs**	Kruskal-Wallis H test, post hoc: Dunn's (1964) procedure	0.016 *tetA-tetB*

	Wells	Kruskal-Wallis H test	0.079
Curli 37°C across tetracycline resistance		Pearson chi-square	0,127
Cellulose 37°C across tetracycline resistance		Pearson chi-square	0,631
CFU in blood culture/UTI		Mann-Whitney U test	0,299
Biomass in blood culture/UTI	**Pegs**	Mann-Whitney U test	0,598
	Wells	Mann-Whitney U test	0,567
Curli 37°C in blood culture/UTI		Fishers exact test	1,000
Cellulose37°C in blood culture/UTI		Fishers exact test	0,337
CFU across Curli 37°C yes/no		Mann-Whitney U test	0,503
CFU across cellulose 37°C yes/no		Independent samples t-test	0,072
Biomass across curli 37°C yes/no	**Pegs**	Independent samples t-test	0,305
	Wells	Mann-Whitney U test	0,708
Biomass by cellulose37 °C yes/no	**Pegs**	Mann-Whitney U test Without outliers: Independent samples t-test	0,319 0.035
	Wells	Mann-Whitney U test	0,203
CFU across motility		Kruskal-Wallis H test	0.876
Biomass across motility	**Pegs**	Mann-Whitney U test	0.020
	Wells	Mann-Whitney U test	0.023
Motility: Growth on surface / growth in the entire agar	**Pegs**	Kruskal-Wallis H test	0.074
	Wells	Kruskal-Wallis H test	0.789
CFU across hemolysi		Kruskal-Wallis H test	0.412
Biomass across hemolysins	**Pegs**	Kruskal-Wallis H test	0.390
	Wells	Kruskal-Wallis H test	0.234
CFU across ≥ 1 *pap* gene		Mann-Whitney U test	0.217
Biomass across ≥ 1 *pap* gene	**Pegs**	Mann-Whitney U test	0.016

	Wells	Mann-Whitney U test	0.003
CFU across ≥ 1 *afa* gene		Mann-Whitney U test	0.945
Biomass across ≥ 1 *afa* gene	**Pegs**	Mann-Whitney U test	0.631
	Wells	Mann-Whitney U test	0.598
CFU across presence of *lpfA*		Mann-Whitney U test	0.352
Biomass across presence of *lpfA*	**Pegs**	Mann-Whitney U test	<0.001
	Wells	Mann-Whitney U test	0.003
CFU across presence of *usp*		Mann-Whitney U test	0.908
Biomass across presence of *usp*	**Pegs**	Mann-Whitney U test	<0.001
	Wells	Mann-Whitney U test	0.004
CFU across presence of *iss*		Mann-Whitney U test	0.502
Biomass across presence of *iss*	**Pegs**	Mann-Whitney U test	<0.001
	Wells	Mann-Whitney U test	<0.001
CFU across presence of *kpsMII/kpsMIII*		Mann-Whitney U test	0.555
Biomass across presence of *kpsMII/kpsMIII*	**Pegs**	Mann-Whitney U test	0.468
	Wells	Mann-Whitney U test	0.749

Appendix E: Raw data, Congo red (CRA) and Calcofluor (CF) staining

Hours	24 hours															
Degrees	28									37						
	CRA						CF			CRA						
Replicate	1	1	2	2	3	3	1	2	3	1	1	2	2	3	3	1
	Color	Surface	Color	Surface	Color	Surface				Color	Surface	Color	Surface	Color	Surface	
MP23-01	1	0	1	0			0	0		0	0	0	0			0
MP23-02	3	1	3	1			2	2		1	0	1	0			1
MP23-03	1	0	1	0			0	0		0	0	0	0			0
MP23-04	0	0	0	0			0	0		1	0	1	0			1
MP23-05	0	0	0	0			0	0		0	0	0	0			0
MP23-06	3	1	3	1			2	2		3	1	3	1			2
MP23-07	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MP23-08	3	1	3	1			2	2		3	2	3	2			2
MP23-09	3	1	3	1			2	2		3	2	3	2			0
MP23-10	0	0	0	0			0	0		0	0	0	0			1
MP23-11	0	0	0	0			0	0		0	0	0	0			1
MP23-12	0	0	0	0			0	0		0	0	0	0			0
MP23-13	3	1	3	1			2	2		3	1	3	1			0
MP23-14	0	0	0	0			0	0		0	0	1	0			0
MP23-15	0	0	0	0			0	0		3	1	3	1			2
MP23-47	0	0	0	0			0	0		2	1	2	1			1
MP23-48	0	0	0	0			0	0		0	0	0	0			0
MP04-02	0	0	0	0			1	1		0	0	0	0			1
MP04-17	0	0	0	0			1	1		2	1	2	1			1
MP04-23	3	1	3	1			1	1		3	1	3	1			0
MP04-24	0	0	0	0			1	1		0	0	0	0			1
MP04-25	0	0	0	0			1	1		3	1	3	1			0
MP15-01	1	0	1	0			0	0		1	1	1	1			0
MP15-03	1	0	1	0			1	1		1	0	1	1			0
MP15-04	3	1	3	1			0	0		1	1	1	1			0
MP15-05	3	1	3	1			0	0		2	1	2	1			0
MP15-06	3	1	3	1			0	0		3	1	3	1			0
MP15-07	0	0	0	0			1	1		1	0	1	0			2
MP15-08	0	0	0	0			1	1		0	0	0	0			1
MP15-09	1	1	1	1			1	1		1	1	1	1			1
MP15-10	1	0	1	0			1	1		1	1	1	0			1
MP15-11	0	0	0	0			0	0		1	0	1	0			0
MP15-12	1	1	1	1			1	1		2	1	2	1			1
MP15-13	1	0	1	0			1	1		1	1	1	0			1
MP15-14	1	0	1	0			1	1		2	1	2	1			1

Congo red classification:
Colour:
0 = white
1 = rings
2 = weak/light colour
3 = dark, even colour

Surface:
0 = glossy
1 = matte
2 = rough

Calcofluor classification:
0 = negative
1 = weak glow
2 = strong glow

		48 hours													
		28							37						
CF		CRA				CF			CRA				CF		
2	3	1	1	2	2	1	2	3	1	1	2	2	1	2	3
		Color	Surface	Color	Surface				Color	Surface	Color	Surface			
0		1	0	1	0	1	1		0	0	0	0	1	1	
1		3	2	3	2	2	2		3	2	3	2	2	2	
0		3	1	3	1	0	0		0	0	0	0	0	0	
1		0	0	0	0	0	0		0	0	0	0	2	2	
0		0	0	0	0	0	0		0	0	0	0	0	0	
2		3	1	3	1	2	2		3	1	3	1	2	2	
2	1	0	0	0	0	1	0	1	1	0	1	0	0	2	1
2		3	1	3	1	2	2		3	2	3	2	1	1	
0		3	1	3	1	0	0		3	2	3	2	0	0	
1		1	0	1	0	1	1		1	0	1	0	2	2	
1		0	0	0	0	1	1		0	0	1	0	2	2	
1		0	0	0	0	0	0		0	0	0	0	0	0	
0		3	1	3	1	2	2		3	2	3	2	1	1	
0		0	0	0	0	0	0		1	0	1	0	0	0	
2		1	0	1	0	2	2		3	1	3	1	2	2	
1		2	0	2	0	1	1		1	1	1	1	2	2	
0		0	0	0	0	1	1		1	0	1	0	2	2	
1		0	0	0	0	1	2		1	0	1	0	2	2	
1		1	0	1	0	2	2		1	0	1	0	2	2	
0		3	1	3	1	0	0		3	1	3	1	0	0	
1		2	2	2	2	0	0		3	2	3	2	0	0	
0		3	1	3	1	0	0		3	1	3	1	0	0	
0		1	1	1	1	0	0		3	1	3	1	0	0	
0		3	1	3	1	1	1		1	0	1	0	1	0	
0		3	1	3	1	0	0		1	1	1	1	1	1	
0		3	1	3	1	0	0		3	1	3	1	0	0	
0		3	2	3	2	0	0		3	1	3	1	0	0	
2		0	0	0	0	1	1		1	0	1	0	2	2	
1		0	0	0	0	1	1		1	0	1	0	2	2	
1		3	1	3	1	2	2		1	1	1	1	1	1	
1		3	1	3	1	2	2		1	0	1	0	2	2	
0		0	0	0	0	1	1		1	0	1	0	1	1	
1		3	1	3	1	2	2		3	1	3	1	2	2	
1		3	1	3	1	1	1		3	1	3	1	1	2	
1		3	1	3	1	1	1		3	1	3	1	1	2	

Appendix F: Raw data, CFU quantification

Strain	Biological replicte	Technical Replicate	CFU 10 µl 1	CFU 10 µl 2	CFU 20 µl	Dilution Factor	CFU nd	CFU/mL	CFU/peg	CFU/peg biological rep	log	CFU strain (log)	SD
MP23-01	**1**	**1**	15	25	40	100	4000	200000	400000	5.93E+05	5.77	6.20	0.60
		2	5	7	12	1000	12000	600000	1200000				
		3	9	9	18	100	1800	90000	180000				
	2	**1**	23	26	49	100	4900	245000	490000	7.73E+06	6.89		
		2	15	7	22	10000	220000	1E+07	22000000				
		3	4	3	7	1000	7000	350000	700000				
	3	**1**	10	12	22	100	2200	110000	220000	9.83E+05	5.99		
		2	18	15	33	100	3300	165000	330000				
		3	10	14	24	1000	24000	1E+06	2400000				
	4	**1**	4	4	8	1000	8000	400000	800000	6.07E+06	6.78		
		2	5	19	24	1000	24000	1E+06	2400000				
		3	9	6	15	10000	150000	8E+06	15000000				
	5	**1**	28	23	51	100	5100	255000	510000	3.60E+05	5.56		
		2	13	12	25	100	2500	125000	250000				
		3	15	17	32	100	3200	160000	320000				
MP23-02	**1**	**1**	4	6	10	10000	100000	5E+06	10000000	1.97E+07	7.29	6.49	1.15
		2	15	9	24	10000	240000	1E+07	24000000				
		3	13	12	25	10000	250000	1E+07	25000000				
	2	**1**	29	30	59	10	590	29500	59000	1.37E+05	5.14		
		2	30	33	63	10	630	31500	63000				
		3	14	15	29	100	2900	145000	290000				
	3	**1**	26	26	52	10000	520000	3E+07	52000000	3.87E+07	7.59		
		2	13	17	30	10000	300000	2E+07	30000000				
		3	13	21	34	10000	340000	2E+07	34000000				
	4	**1**	7	10	17	1000	17000	850000	1700000	8.40E+05	5.92		
		2	35	32	67	100	6700	335000	670000				
		3	7	8	15	100	1500	75000	150000				
MP23-03	**1**	**1**	15	20	35	10000	350000	2E+07	35000000	3.30E+07	7.52	7.58	0.16
		2	13	18	31	10000	310000	2E+07	31000000				
		3	18	15	33	10000	330000	2E+07	33000000				
	2	**1**	14	10	24	10000	240000	1E+07	24000000	2.93E+07	7.47		
		2	10	13	23	10000	230000	1E+07	23000000				
		3	21	20	41	10000	410000	2E+07	41000000				
	3	**1**	21	18	39	10000	390000	2E+07	39000000	5.83E+07	7.77		
		2	4	5	9	100000	900000	5E+07	90000000				
		3	21	25	46	10000	460000	2E+07	46000000				
MP23-04	**1**	**1**	29	21	50	1000	50000	3E+06	5000000	5.40E+06	6.73	6.35	0.52
		2	29	30	59	1000	59000	3E+06	5900000				
		3	23	30	53	1000	53000	3E+06	5300000				
	2	**1**	17	25	42	1000	42000	2E+06	4200000	4.00E+06	6.60		
		2	12	16	28	1000	28000	1E+06	2800000				
		3	5	0	5	10000	50000	3E+06	5000000				
	3	**1**	11	15	26	1000	26000	1E+06	2600000	3.03E+06	6.48		
		2	17	20	37	1000	37000	2E+06	3700000				
		3	11	17	28	1000	28000	1E+06	2800000				
	4	**1**	9	11	20	100	2000	100000	200000	3.80E+05	5.58		
		2	4	7	11	100	1100	55000	110000				
		3	38	45	83	100	8300	415000	830000				
MP23-05	**1**	**1**	27	29	56	1000	56000	3E+06	5600000	4.35E+07	7.64	7.11	0.75
		2	7	5	12	100000	1E+06	6E+07	120000000				
		3	25	24	49	1000	49000	2E+06	4900000				
	2	**1**	8	6	14	10000	140000	7E+06	14000000	2.10E+07	7.32		
		2	9	15	24	10000	240000	1E+07	24000000				
		3	11	14	25	10000	250000	1E+07	25000000				
	3	**1**	29	26	55	10000	550000	3E+07	55000000	3.00E+07	7.48		
		2	6	9	15	10000	150000	8E+06	15000000				
		3	9	11	20	10000	200000	1E+07	20000000				
	4	**1**	12	9	21	100	2100	105000	210000	1.01E+06	6.00		
		2	42	40	82	100	8200	410000	820000				
		3	11	9	20	1000	20000	1E+06	2000000				
MP23-06	**1**	**1**	20	21	41	1000	41000	2E+06	4100000	1.40E+07	7.15	7.23	0.14
		2	5	11	16	10000	160000	8E+06	16000000				
		3	11	11	22	10000	220000	1E+07	22000000				
	2	**1**	10	16	26	1000	26000	1E+06	2600000	1.39E+07	7.14		
		2	8	8	16	10000	160000	8E+06	16000000				
		3	10	13	23	10000	230000	1E+07	23000000				
	3	**1**	14	4	18	10000	180000	9E+06	18000000	2.43E+07	7.39		
		2	13	11	24	10000	240000	1E+07	24000000				
		3	15	16	31	10000	310000	2E+07	31000000				
MP23-07	**1**	**1**	5	6	11	10000	110000	6E+06	11000000	1.03E+07	7.01	6.91	0.52
		2	12	5	17	10000	170000	9E+06	17000000				
		3	15	14	29	1000	29000	1E+06	2900000				
	2	**1**	17	17	34	1000	34000	2E+06	3400000	1.16E+07	7.06		
		2	17	8	25	10000	250000	1E+07	25000000				
		3	29	35	64	1000	64000	3E+06	6400000				
	3	**1**	6	3	9	100000	900000	5E+07	90000000	4.00E+07	7.60		
		2	16	10	26	10000	260000	1E+07	26000000				
		3	19	22	41	1000	41000	2E+06	4100000				
	4	**1**	14	23	37	1000	37000	2E+06	3700000	4.83E+06	6.68		
		2	21	18	39	1000	39000	2E+06	3900000				
		3	39	30	69	1000	69000	3E+06	6900000				
	5	**1**	11	8	19	1000	19000	950000	1900000	1.60E+06	6.20		
		2	6	9	15	1000	15000	750000	1500000				
		3	8	6	14	1000	14000	700000	1400000				
MP23-08	**1**	**1**	27	26	53	1000	53000	3E+06	5300000	4.00E+06	6.60	6.94	0.63
		2	9	13	22	1000	22000	1E+06	2200000				
		3	25	20	45	1000	45000	2E+06	4500000				
	2	**1**	6	15	21	10000	210000	1E+07	21000000	2.87E+07	7.46		
		2	23	27	50	10000	500000	3E+07	50000000				
		3	8	7	15	10000	150000	8E+06	15000000				
	3	**1**	10	2	12	10000	120000	6E+06	12000000	3.06E+07	7.49		
		2	38	38	76	10000	760000	4E+07	76000000				
		3	10	28	38	1000	38000	2E+06	3800000				
	4	**1**	40	34	74	1	74	3700	7400	1.70E+06	6.23		
		2	25	21	46	1000	46000	2E+06	4600000				
		3	23	26	49	100	4900	245000	490000				
MP23-09	**1**	**1**	30	37	67	1000	67000	3E+06	6700000	9.43E+06	6.97	6.99	0.46
		2	5	11	16	10000	160000	8E+06	16000000				
		3	21	35	56	1000	56000	3E+06	5600000				
	2	**1**	8	5	13	1000	13000	650000	1300000	4.83E+06	6.68		

		2	7	3	10	10000	100000	5E+06	10000000				
		3	17	15	32	1000	32000	2E+06	3200000				
	3	**1**	21	25	46	10000	460000	2E+07	46000000	4.40E+07	7.64		
		2	35	25	60	10000	600000	3E+07	60000000				
		3	10	16	26	10000	260000	1E+07	26000000				
	4	**1**	35	34	69	1000	69000	3E+06	6900000	4.60E+06	6.66		
		2	6	4	10	1000	10000	500000	1000000				
		3	32	27	59	1000	59000	3E+06	5900000				
MP23-10	**1**	**1**	20	29	49	1000	49000	2E+06	4900000	3.02E+06	6.48	6.92	0.54
		2	21	15	36	1000	36000	2E+06	3600000				
		3	30	26	56	100	5600	280000	560000				
	2	**1**	24	27	51	1000	51000	3E+06	5100000	5.80E+06	6.76		
		2	31	31	62	1000	62000	3E+06	6200000				
		3	25	36	61	1000	61000	3E+06	6100000				
	3	**1**	26	19	45	10000	450000	2E+07	45000000	3.33E+07	7.52		
		2	14	13	27	10000	270000	1E+07	27000000				
		3	19	9	28	10000	280000	1E+07	28000000				
MP23-11	**1**	**1**	23	24	47	1000	47000	2E+06	4700000	2.70E+06	6.43	7.07	0.57
		2	7	4	11	1000	11000	550000	1100000				
		3	7	16	23	1000	23000	1E+06	2300000				
	2	**1**	35	30	65	1000	65000	3E+06	6500000	1.68E+07	7.23		
		2	14	11	25	10000	250000	1E+07	25000000				
		3	12	7	19	10000	190000	1E+07	19000000				
	3	**1**	16	10	26	10000	260000	1E+07	26000000	3.53E+07	7.55		
		2	16	14	30	10000	300000	2E+07	30000000				
		3	26	24	50	10000	500000	3E+07	50000000				
MP23-12	**1**	**1**	30	27	57	1000	57000	3E+06	5700000	4.97E+06	6.70	6.78	0.14
		2	25	23	48	1000	48000	2E+06	4800000				
		3	22	22	44	1000	44000	2E+06	4400000				
	2	**1**	8	15	23	1000	23000	1E+06	2300000	8.93E+06	6.95		
		2	28	27	55	1000	55000	3E+06	5500000				
		3	9	10	19	10000	190000	1E+07	19000000				
	3	**1**	4	5	9	10000	90000	5E+06	9000000	5.10E+06	6.71		
		2	23	29	52	1000	52000	3E+06	5200000				
		3	5	6	11	1000	11000	550000	1100000				
MP23-13	**1**	**1**	28	26	54	1000	54000	3E+06	5400000	5.57E+06	6.75	7.07	0.30
		2	5	2	7	10000	70000	4E+06	7000000				
		3	23	20	43	1000	43000	2E+06	4300000				
	2	**1**	23	18	41	10000	410000	2E+07	41000000	2.97E+07	7.47		
		2	7	9	16	10000	160000	8E+06	16000000				
		3	18	14	32	10000	320000	2E+07	32000000				
	3	**1**	20	23	43	1000	43000	2E+06	4300000	1.20E+07	7.08		
		2	12	17	29	10000	290000	1E+07	29000000				
		3	16	10	26	1000	26000	1E+06	2600000				
	4	**1**	8	9	17	10000	170000	9E+06	17000000	9.37E+06	6.97		
		2	33	35	68	1000	68000	3E+06	6800000				
		3	17	26	43	1000	43000	2E+06	4300000				
MP23-14	**1**	**1**	5	13	18	10000	180000	9E+06	18000000	8.60E+06	6.93	6.83	0.09
		2	30	26	56	1000	56000	3E+06	5600000				
		3	12	10	22	1000	22000	1E+06	2200000				
	2	**1**	20	36	56	1000	56000	3E+06	5600000	6.13E+06	6.79		
		2	5	6	11	10000	110000	6E+06	11000000				
		3	7	11	18	1000	18000	900000	1800000				
	3	**1**	19	19	38	1000	38000	2E+06	3800000	5.77E+06	6.76		
		2	37	48	85	1000	85000	4E+06	8500000				
		3	22	28	50	1000	50000	3E+06	5000000				
MP23-15	**1**	**1**	30	36	66	100	6600	330000	660000	2.02E+06	6.31	6.56	0.22
		2	9	16	25	1000	25000	1E+06	2500000				
		3	16	13	29	1000	29000	1E+06	2900000				
	2	**1**	19	14	33	1000	33000	2E+06	3300000	5.13E+06	6.71		
		2	13	8	21	1000	21000	1E+06	2100000				
		3	5	5	10	10000	100000	5E+06	10000000				
	3	**1**	33	35	68	1000	68000	3E+06	6800000	4.76E+06	6.68		
		2	32	38	70	1000	70000	4E+06	7000000				
		3	18	30	48	100	4800	240000	480000				
MP23-47	**1**	**1**	25	30	55	1000	55000	3E+06	5500000	5.66E+06	6.75	7.39	0.55
		2	17	32	49	100	4900	245000	490000				
		3	2	9	11	10000	110000	6E+06	11000000				
	2	**1**	12	9	21	10000	210000	1E+07	21000000	5.30E+07	7.72		
		2	13	15	28	10000	280000	1E+07	28000000				
		3	6	5	11	100000	1E+06	6E+07	110000000				
	3	**1**	28	32	60	10000	600000	3E+07	60000000	4.77E+07	7.68		
		2	19	17	36	10000	360000	2E+07	36000000				
		3	24	23	47	10000	470000	2E+07	47000000				
MP23-48	**1**	**1**	21	31	52	1000	52000	3E+06	5200000	4.70E+06	6.67	7.13	0.74
		2	33	25	58	1000	58000	3E+06	5800000				
		3	17	14	31	1000	31000	2E+06	3100000				
	2	**1**	37	32	69	1000	69000	3E+06	6900000	5.50E+06	6.74		
		2	22	25	47	1000	47000	2E+06	4700000				
		3	21	28	49	1000	49000	2E+06	4900000				
	3	**1**	8	15	23	10000	230000	1E+07	23000000	9.60E+07	7.98		
		2	4	11	15	10000	150000	8E+06	15000000				
		3	10	15	25	100000	3E+06	1E+08	250000000				
MP15-01	**1**	**1**	36	22	58	1000	58000	3E+06	5800000	4.40E+06	6.64	7.02	0.43
		2	11	7	18	1000	18000	900000	1800000				
		3	27	29	56	1000	56000	3E+06	5600000				
	2	**1**	41	38	79	1000	79000	4E+06	7900000	8.40E+06	6.92		
		2	7	6	13	1000	13000	650000	1300000				
		3	7	9	16	10000	160000	8E+06	16000000				
	3	**1**	11	13	24	10000	240000	1E+07	24000000	3.03E+07	7.48		
		2	13	15	28	10000	280000	1E+07	28000000				
		3	18	21	39	10000	390000	2E+07	39000000				
MP15-03	**1**	**1**	15	18	33	1000	33000	2E+06	3300000	2.48E+06	6.40	6.94	0.54
		2	22	23	45	100	4500	225000	450000				
		3	17	20	37	1000	37000	2E+06	3700000				
	2	**1**	20	18	38	1000	38000	2E+06	3800000	9.13E+06	6.96		
		2	28	28	56	1000	56000	3E+06	5600000				
		3	8	10	18	10000	180000	9E+06	18000000				
	3	**1**	17	12	29	10000	290000	1E+07	29000000	2.93E+07	7.47		
		2	8	19	27	10000	270000	1E+07	27000000				
		3	16	16	32	10000	320000	2E+07	32000000				

MP15-04	**1**	**1**	15	12	27	10000	270000	1E+07	27000000	1.36E+07	7.13	7.14	0.51
		2	14	5	19	1000	19000	950000	1900000				
		3	7	5	12	10000	120000	6E+06	12000000				
	2	**1**	32	30	62	1000	62000	3E+06	6200000	4.23E+06	6.63		
		2	23	16	39	1000	39000	2E+06	3900000				
		3	12	14	26	1000	26000	1E+06	2600000				
	3	**1**	9	19	28	10000	280000	1E+07	28000000	4.43E+07	7.65		
		2	21	26	47	10000	470000	2E+07	47000000				
		3	28	30	58	10000	580000	3E+07	58000000				
MP15-05	**1**	**1**	34	33	67	1000	67000	3E+06	6700000	1.89E+07	7.28	7.16	0.21
		2	23	18	41	1000	41000	2E+06	4100000				
		3	22	24	46	10000	460000	2E+07	46000000				
	2	**1**	32	33	65	1000	65000	3E+06	6500000	8.23E+06	6.92		
		2	19	23	42	1000	42000	2E+06	4200000				
		3	6	8	14	10000	140000	7E+06	14000000				
	3	**1**	10	16	26	10000	260000	1E+07	26000000	1.90E+07	7.28		
		2	11	13	24	10000	240000	1E+07	24000000				
		3	30	41	71	1000	71000	4E+06	7100000				
MP15-06	**1**	**1**	14	15	29	1000	29000	1E+06	2900000	1.24E+07	7.09	7.00	0.09
		2	28	34	62	1000	62000	3E+06	6200000				
		3	16	12	28	10000	280000	1E+07	28000000				
	2	**1**	33	43	76	1000	76000	4E+06	7600000	8.00E+06	6.90		
		2	48	34	82	1000	82000	4E+06	8200000				
		3	43	39	82	1000	82000	4E+06	8200000				
	3	**1**	20	19	39	1000	39000	2E+06	3900000	1.02E+07	7.01		
		2	20	26	46	1000	46000	2E+06	4600000				
		3	15	7	22	10000	220000	1E+07	22000000				
MP15-07	**1**	**1**	31	30	61	100	6100	305000	610000	1.84E+06	6.26	6.18	0.31
		2	19	15	34	1000	34000	2E+06	3400000				
		3	10	5	15	1000	15000	750000	1500000				
	2	**1**	2	4	6	1000	6000	300000	600000	1.87E+06	6.27		
		2	14	19	33	1000	33000	2E+06	3300000				
		3	11	6	17	1000	17000	850000	1700000				
	3	**1**	20	16	36	100	3600	180000	360000	2.79E+06	6.45		
		2	50	51	101	100	10100	505000	1010000				
		3	35	35	70	1000	70000	4E+06	7000000				
	4	**1**	25	19	44	100	4400	220000	440000	5.43E+05	5.74		
		2	25	25	50	100	5000	250000	500000				
		3	34	35	69	100	6900	345000	690000				
MP15-08	**1**	**1**	9	11	20	1000	20000	1E+06	2000000	8.77E+06	6.94	6.84	0.28
		2	17	13	30	100	3000	150000	300000				
		3	12	12	24	10000	240000	1E+07	24000000				
	2	**1**	7	15	22	1000	22000	1E+06	2200000	4.86E+06	6.69		
		2	5	7	12	10000	120000	6E+06	12000000				
		3	19	19	38	100	3800	190000	380000				
	3	**1**	35	31	66	1000	66000	3E+06	6600000	1.49E+07	7.17		
		2	13	11	24	10000	240000	1E+07	24000000				
		3	7	7	14	10000	140000	7E+06	14000000				
	4	**1**	4	4	8	100	800	40000	80000	2.87E+06	6.46		
		2	30	33	63	100	6300	315000	630000				
		3	33	46	79	1000	79000	4E+06	7900000				
	5	**1**	10	12	22	10000	220000	1E+07	22000000	8.99E+06	6.95		
		2	18	28	46	1000	46000	2E+06	4600000				
		3	18	19	37	100	3700	185000	370000				
MP15-09	**1**	**1**	2	13	15	10000	150000	8E+06	15000000	7.87E+06	6.90	6.98	0.48
		2	22	19	41	1000	41000	2E+06	4100000				
		3	12	33	45	1000	45000	2E+06	4500000				
	2	**1**	36	24	60	1000	60000	3E+06	6000000	3.57E+06	6.55		
		2	22	20	42	1000	42000	2E+06	4200000				
		3	24	28	52	100	5200	260000	520000				
	3	**1**	8	9	17	10000	170000	9E+06	17000000	3.13E+07	7.50		
		2	16	23	39	10000	390000	2E+07	39000000				
		3	17	21	38	10000	380000	2E+07	38000000				
MP15-10	**1**	**1**	27	35	62	1000	62000	3E+06	6200000	7.10E+06	6.85	6.81	0.22
		2	37	35	72	1000	72000	4E+06	7200000				
		3	42	37	79	1000	79000	4E+06	7900000				
	2	**1**	10	7	17	10000	170000	9E+06	17000000	1.01E+07	7.00		
		2	34	37	71	1000	71000	4E+06	7100000				
		3	33	29	62	1000	62000	3E+06	6200000				
	3	**1**	32	42	74	1000	74000	4E+06	7400000	3.73E+06	6.57		
		2	17	10	27	1000	27000	1E+06	2700000				
		3	6	5	11	1000	11000	550000	1100000				
MP15-11	**1**	**1**	7	5	12	1000	12000	600000	1200000	1.53E+06	6.19	5.51	0.66
		2	5	7	12	1000	12000	600000	1200000				
		3	11	11	22	1000	22000	1E+06	2200000				
	2	**1**	5	8	13	1000	13000	650000	1300000	8.47E+05	5.93		
		2	3	4	7	1000	7000	350000	700000				
		3	20	34	54	100	5400	270000	540000				
	3	**1**	5	4	9	100	900	45000	90000	5.53E+04	4.74		
		2	22	20	42	10	420	21000	42000				
		3	19	15	34	10	340	17000	34000				
	4	**1**	9	11	20	1000	20000	1E+06	2000000	6.92E+05	5.84		
		2	17	10	27	10	270	13500	27000				
		3	29	20	49	10	490	24500	49000				
	5	**1**	9	8	17	100	1700	85000	170000	7.07E+04	4.85		
		2	7	11	18	10	180	9000	18000				
		3	8	16	24	10	240	12000	24000				
MP15-12	**1**	**1**	7	11	18	10000	180000	9E+06	18000000	8.27E+06	6.92	6.46	0.40
		2	28	23	51	1000	51000	3E+06	5100000				
		3	8	9	17	1000	17000	850000	1700000				
	2	**1**	8	9	17	1000	17000	850000	1700000	1.50E+06	6.18		
		2	4	8	12	1000	12000	600000	1200000				
		3	5	11	16	1000	16000	800000	1600000				
	3	**1**	7	9	16	1000	16000	800000	1600000	1.94E+06	6.29		
		2	20	19	39	1000	39000	2E+06	3900000				
		3	16	17	33	100	3300	165000	330000				
MP15-13	**1**	**1**	6	8	14	1000	14000	700000	1400000	1.13E+06	6.05	6.47	0.46
		2	2	6	8	1000	8000	400000	800000				
		3	6	6	12	1000	12000	600000	1200000				
	2	**1**	19	22	41	1000	41000	2E+06	4100000	3.67E+06	6.56		
		2	13	14	27	1000	27000	1E+06	2700000				
		3	18	24	42	1000	42000	2E+06	4200000				

	3	**1**	7	5	12	1000	12000	600000	1200000	1.50E+06	6.18	
		2	4	10	14	1000	14000	700000	1400000			
		3	8	11	19	1000	19000	950000	1900000			
	4	**1**	6	7	13	10000	130000	7E+06	13000000	1.19E+07	7.07	
		2	9	10	19	10000	190000	1E+07	19000000			
		3	19	17	36	1000	36000	2E+06	3600000			
MP15-14	**1**	**1**	6	8	14	1000	14000	700000	1400000	1.87E+06	6.27	6.48	0.48
		2	8	14	22	1000	22000	1E+06	2200000			
		3	9	11	20	1000	20000	1E+06	2000000			
	2	**1**	10	9	19	1000	19000	950000	1900000	1.13E+06	6.05	
		2	3	11	14	1000	14000	700000	1400000			
		3	5	4	9	100	900	45000	90000			
	3	**1**	11	16	27	1000	27000	1E+06	2700000	2.62E+06	6.42	
		2	27	29	56	10	560	28000	56000			
		3	23	28	51	1000	51000	3E+06	5100000			
	4	**1**	10	10	20	10000	200000	1E+07	20000000	1.47E+07	7.17	
		2	6	4	10	10000	100000	5E+06	10000000			
		3	6	8	14	10000	140000	7E+06	14000000			
MP04-02	**1**	**1**	21	16	37	1000	37000	2E+06	3700000	1.32E+07	7.12	6.87	0.24
		2	10	10	20	10000	200000	1E+07	20000000			
		3	10	6	16	10000	160000	8E+06	16000000			
	2	**1**	38	42	80	1000	80000	4E+06	8000000	7.27E+06	6.86	
		2	30	37	67	1000	67000	3E+06	6700000			
		3	40	31	71	1000	71000	4E+06	7100000			
	3	**1**	27	32	59	1000	59000	3E+06	5900000	4.37E+06	6.64	
		2	19	24	43	1000	43000	2E+06	4300000			
		3	15	14	29	1000	29000	1E+06	2900000			
MP04-17	**1**	**1**	16	21	37	1000	37000	2E+06	3700000	8.53E+06	6.93	6.86	0.08
		2	11	9	20	10000	200000	1E+07	20000000			
		3	11	8	19	1000	19000	950000	1900000			
	2	**1**	26	27	53	1000	53000	3E+06	5300000	7.43E+06	6.87	
		2	47	39	86	1000	86000	4E+06	8600000			
		3	42	42	84	1000	84000	4E+06	8400000			
	3	**1**	36	37	73	1000	73000	4E+06	7300000	5.80E+06	6.76	
		2	20	10	30	1000	30000	2E+06	3000000			
		3	36	35	71	1000	71000	4E+06	7100000			
MP04-23	**1**	**1**	33	34	67	1000	67000	3E+06	6700000	1.12E+07	7.05	6.75	0.29
		2	7	9	16	10000	160000	8E+06	16000000			
		3	5	6	11	10000	110000	6E+06	11000000			
	2	**1**	16	27	43	1000	43000	2E+06	4300000	5.37E+06	6.73	
		2	37	43	80	1000	80000	4E+06	8000000			
		3	19	19	38	1000	38000	2E+06	3800000			
	3	**1**	16	16	32	1000	32000	2E+06	3200000	2.97E+06	6.47	
		2	12	13	25	1000	25000	1E+06	2500000			
		3	15	17	32	1000	32000	2E+06	3200000			
MP04-24	**1**	**1**	22	19	41	1000	41000	2E+06	4100000	4.20E+06	6.62	6.55	0.11
		2	10	10	20	1000	20000	1E+06	2000000			
		3	33	32	65	1000	65000	3E+06	6500000			
	2	**1**	19	21	40	1000	40000	2E+06	4000000	2.63E+06	6.42	
		2	6	7	13	1000	13000	650000	1300000			
		3	11	15	26	1000	26000	1E+06	2600000			
	3	**1**	22	17	39	1000	39000	2E+06	3900000	3.97E+06	6.60	
		2	12	11	23	1000	23000	1E+06	2300000			
		3	28	29	57	1000	57000	3E+06	5700000			
MP04-25	**1**	**1**	32	36	68	1000	68000	3E+06	6800000	6.10E+06	6.79	6.62	0.32
		2	32	23	55	1000	55000	3E+06	5500000			
		3	29	31	60	1000	60000	3E+06	6000000			
	2	**1**	8	16	24	1000	24000	1E+06	2400000	3.50E+06	6.54	
		2	27	15	42	1000	42000	2E+06	4200000			
		3	26	13	39	1000	39000	2E+06	3900000			
	3	**1**	7	3	10	1000	10000	500000	1000000	1.57E+06	6.19	
		2	8	13	21	1000	21000	1E+06	2100000			
		3	10	6	16	1000	16000	800000	1600000			
	4	**1**	26	27	53	1000	53000	3E+06	5300000	8.70E+06	6.94	
		2	37	31	68	1000	68000	3E+06	6800000			
		3	8	6	14	10000	140000	7E+06	14000000			

Appendix G: Raw data, biomass on pegs

Strain	Biological Replicate	Technical Replicate	OD	SD	Mean OD biological rep	Mean OD strain
MP23-01	**1**	**1**	0.203	0.034	0.163	0.165
		2	0.132			
		3	0.154			
	2	**1**	0.177		0.148	
		2	0.163			
		3	0.104			
	3	**1**	0.173		0.183	
		2	0.217			
		3	0.158			
MP23-02	**1**	**1**	0.360	0.064	0.344	0.263
		2	0.323			
		3	0.350			
	2	**1**	0.192		0.211	
		2	0.206			
		3	0.234			
	3	**1**	0.257		0.233	
		2	0.223			
		3	0.220			
MP23-03	**1**	**1**	0.441	0.063	0.406	0.389
		2	0.370			
		3	0.406			
	2	**1**	0.313		0.328	
		2	0.351			
		3	0.320			
	3	**1**	0.372		0.435	
		2	0.418			
		3	0.514			
MP23-04	**1**	**1**	0.166	0.034	0.173	0.162
		2	0.171			
		3	0.181			
	2	**1**	0.121		0.124	
		2	0.124			
		3	0.128			
	3	**1**	0.172		0.188	
		2	0.165			
		3	0.227			
MP23-05	**1**	**1**	0.061	0.016	0.056	0.075
		2	0.054			
		3	0.054			
	2	**1**	0.080		0.076	
		2	0.079			
		3	0.070			
	3	**1**	0.068		0.068	
		2	0.069			
		3	0.067			
	4	**1**	0.089		0.083	
		2	0.093			
		3	0.068			
	5	**1**	0.104		0.093	
		2	0.071			
		3	0.102			
MP23-06	**1**	**1**	0.142	0.021	0.134	0.111
		2	0.146			
		3	0.115			
	2	**1**	0.115		0.106	
		2	0.095			
		3	0.108			
	3	**1**	0.088		0.094	

		2	0.100			
		3	0.095			
MP23-07	**1**	**1**	0.087	0.010	0.091	0.099
		2	0.091			
		3	0.094			
	2	**1**	0.113		0.108	
		2	0.111			
		3	0.099			
	3	**1**	0.090		0.098	
		2	0.095			
		3	0.110			
MP23-08	**1**	**1**	0.098	0.015	0.091	0.101
		2	0.082			
		3	0.092			
	2	**1**	0.136		0.116	
		2	0.106			
		3	0.107			
	3	**1**	0.092		0.095	
		2	0.098			
		3	0.095			
MP23-09	**1**	**1**	0.066	0.007	0.066	0.074
		2	0.070			
		3	0.063			
	2	**1**	0.074		0.076	
		2	0.080			
		3	0.075			
	3	**1**	0.075		0.080	
		2	0.086			
		3	0.078			
MP23-10	**1**	**1**	0.062	0.006	0.061	0.068
		2	0.060			
		3	0.060			
	2	**1**	0.072		0.073	
		2	0.078			
		3	0.069			
	3	**1**	0.066		0.069	
		2	0.074			
		3	0.068			
MP23-11	**1**	**1**	0.079	0.030	0.079	0.100
		2	0.089			
		3	0.071			
	2	**1**	0.076		0.114	
		2	0.096			
		3	0.170			
	3	**1**	0.096		0.107	
		2	0.104			
		3	0.120			
MP23-12	**1**	**1**	0.240	0.053	0.213	0.226
		2	0.199			
		3	0.200			
	2	**1**	0.308		0.251	
		2	0.208			
		3	0.238			
	3	**1**	0.277		0.231	
		2	0.201			
		3	0.217			
	4	**1**	0.223		0.270	
		2	0.244			
		3	0.344			

	5	**1**	0.196		0.165	
		2	0.167			
		3	0.133			
MP23-13	**1**	**1**	0.212	0.029	0.209	0.206
		2	0.188			
		3	0.228			
	2	**1**	0.204		0.178	
		2	0.170			
		3	0.159			
	3	**1**	0.246		0.231	
		2	0.232			
		3	0.216			
MP23-14	**1**	**1**	0.073	0.032	0.071	0.099
		2	0.072			
		3	0.067			
	2	**1**	0.069		0.074	
		2	0.078			
		3	0.074			
	3	**1**	0.083		0.086	
		2	0.089			
		3	0.085			
	4	**1**	0.148		0.150	
		2	0.152			
		3	0.150			
	5	**1**	0.083		0.113	
		2	0.125			
		3	0.131			
MP23-15	**1**	**1**	0.054	0.014	0.054	0.071
		2	0.057			
		3	0.052			
	2	**1**	0.072		0.071	
		2	0.070			
		3	0.070			
	3	**1**	0.088		0.086	
		2	0.085			
		3	0.086			
MP23-47	**1**	**1**	0.148	0.027	0.189	0.172
		2	0.193			
		3	0.226			
	2	**1**	0.191		0.165	
		2	0.141			
		3	0.162			
	3	**1**	0.149		0.162	
		2	0.176			
		3	0.163			
MP23-48	**1**	**1**	0.250	0.077	0.304	0.221
		2	0.281			
		3	0.380			
	2	**1**	0.177		0.165	
		2	0.119			
		3	0.200			
	3	**1**	0.180		0.195	
		2	0.163			
		3	0.242			
MP15-01	**1**	**1**	0.157	0.034	0.163	0.125
		2	0.165			
		3	0.167			
	2	**1**	0.128		0.123	
		2	0.116			

		3	0.126			
	3	**1**	0.108		0.088	
		2	0.078			
		3	0.078			
MP15-03	**1**	**1**	0.772	0.147	0.670	0.505
		2	0.653			
		3	0.585			
	2	**1**	0.363		0.362	
		2	0.332			
		3	0.390			
	3	**1**	0.461		0.484	
		2	0.556			
		3	0.434			
MP15-04	**1**	**1**	0.229	0.031	0.196	0.172
		2	0.185			
		3	0.175			
	2	**1**	0.152		0.139	
		2	0.140			
		3	0.124			
	3	**1**	0.173		0.182	
		2	0.179			
		3	0.196			
MP15-05	**1**	**1**	0.154	0.026	0.135	0.105
		2	0.130			
		3	0.121			
	2	**1**	0.091		0.096	
		2	0.098			
		3	0.098			
	3	**1**	0.092		0.084	
		2	0.070			
		3	0.090			
MP15-06	**1**	**1**	0.156	0.019	0.133	0.123
		2	0.123			
		3	0.121			
	2	**1**	0.091		0.106	
		2	0.126			
		3	0.102			
	3	**1**	0.122		0.129	
		2	0.127			
		3	0.137			
MP15-07	**1**	**1**	0.130	0.088	0.130	0.258
		2	0.143			
		3	0.117			
	2	**1**	0.277		0.321	
		2	0.392			
		3	0.293			
	3	**1**	0.279		0.290	
		2	0.323			
		3	0.267			
	4	**1**	0.298		0.335	
		2	0.356			
		3	0.349			
	5	**1**	0.140		0.217	
		2	0.265			
		3	0.247			
MP15-08	**1**	**1**	0.256	0.083	0.226	0.274
		2	0.218			
		3	0.203			
	2	**1**	0.165		0.183	

		2	0.221			
		3	0.162			
	3	**1**	0.307		0.340	
		2	0.410			
		3	0.305			
	4	**1**	0.251		0.238	
		2	0.234			
		3	0.229			
	5	**1**	0.366		0.382	
		2	0.401			
		3	0.380			
MP15-09	**1**	**1**	0.195	0.114	0.186	0.289
		2	0.196			
		3	0.166			
	2	**1**	0.230		0.254	
		2	0.298			
		3	0.232			
	3	**1**	0.378		0.384	
		2	0.426			
		3	0.348			
	4	**1**	0.396		0.445	
		2	0.476			
		3	0.462			
	5	**1**	0.163		0.179	
		2	0.179			
		3	0.194			
MP15-10	**1**	**1**	0.349	0.073	0.309	0.304
		2	0.266			
		3	0.313			
	2	**1**	0.222		0.233	
		2	0.233			
		3	0.245			
	3	**1**	0.324		0.302	
		2	0.285			
		3	0.299			
	4	**1**	0.376		0.420	
		2	0.409			
		3	0.475			
	5	**1**	0.236		0.255	
		2	0.240			
		3	0.289			
MP15-11	**1**	**1**	0.192	0.035	0.182	0.166
		2	0.211			
		3	0.144			
	2	**1**	0.116		0.130	
		2	0.153			
		3	0.121			
	3	**1**	0.168		0.186	
		2	0.195			
		3	0.197			
MP15-12	**1**	**1**	0.155	0.058	0.159	0.202
		2	0.157			
		3	0.166			
	2	**1**	0.160		0.171	
		2	0.185			
		3	0.169			
	3	**1**	0.298		0.276	
		2	0.286			
		3	0.244			

MP15-13	**1**	**1**	0.368	0.081	0.380	0.339
		2	0.335			
		3	0.439			
	2	**1**	0.358		0.308	
		2	0.252			
		3	0.315			
	3	**1**	0.334		0.371	
		2	0.426			
		3	0.352			
	4	**1**	0.386		0.411	
		2	0.374			
		3	0.473			
	5	**1**	0.241		0.224	
		2	0.248			
		3	0.182			
MP15-14	**1**	**1**	0.234	0.067	0.237	0.231
		2	0.231			
		3	0.246			
	2	**1**	0.209		0.206	
		2	0.218			
		3	0.191			
	3	**1**	0.169		0.179	
		2	0.176			
		3	0.192			
	4	**1**	0.278		0.332	
		2	0.276			
		3	0.440			
	5	**1**	0.213		0.200	
		2	0.216			
		3	0.172			
MP04-02	**1**	**1**	0.272	0.048	0.277	0.205
		2	0.257			
		3	0.303			
	2	**1**	0.184		0.203	
		2	0.193			
		3	0.231			
	3	**1**	0.168		0.184	
		2	0.199			
		3	0.186			
	4	**1**	0.237		0.216	
		2	0.202			
		3	0.208			
	5	**1**	0.145		0.144	
		2	0.141			
		3	0.146			
MP04-17	**1**	**1**	0.106	0.031	0.104	0.125
		2	0.100			
		3	0.107			
	2	**1**	0.122		0.108	
		2	0.113			
		3	0.091			
	3	**1**	0.140		0.163	
		2	0.180			
		3	0.170			
MP04-23	**1**	**1**	0.185	0.048	0.203	0.198
		2	0.191			
		3	0.232			
	2	**1**	0.172		0.168	
		2	0.145			

		3	0.189			
	3	**1**	0.236		0.249	
		2	0.251			
		3	0.259			
	4	**1**	0.215		0.226	
		2	0.297			
		3	0.168			
	5	**1**	0.139		0.145	
		2	0.154			
		3	0.143			
MP04-24	**1**	**1**	0.072	0.015	0.074	0.091
		2	0.078			
		3	0.073			
	2	**1**	0.088		0.093	
		2	0.092			
		3	0.100			
	3	**1**	0.111		0.106	
		2	0.106			
		3	0.102			
MP04-25	**1**	**1**	0.141	0.032	0.165	0.142
		2	0.174			
		3	0.180			
	2	**1**	0.126		0.128	
		2	0.141			
		3	0.118			
	3	**1**	0.097		0.095	
		2	0.096			
		3	0.091			
	4	**1**	0.156		0.166	
		2	0.169			
		3	0.174			
	5	**1**	0.141		0.156	
		2	0.141			
		3	0.186			
S. Epi	**1**	**1**	0.087	0.078	0.084	0.165
		2	0.082			
		3	0.084			
	2	**1**	0.275		0.197	
		2	0.223			
		3	0.093			
	3	**1**	0.190		0.214	
		2	0.210			
		3	0.241			

Appendix H: Raw data, biomass in wells

Strain	Biological Replicate	Technical Replicate	OD	SD	Mean OD biological rep	Mean OD strain
MP23-01	**1**	**1**	0.090	0.027	0.086	0.118
		2	0.085			
		3	0.083			
	2	**1**	0.109		0.130	
		2	0.131			
		3	0.149			
	3	**1**	0.138		0.137	
		2	0.151			
		3	0.122			
MP23-02	**1**	**1**	0.096	0.077	0.093	0.169
		2	0.091			
		3	0.092			
	2	**1**	0.143		0.147	
		2	0.142			
		3	0.154			
	3	**1**	0.259		0.266	
		2	0.266			
		3	0.274			
MP23-03	**1**	**1**	0.091	0.072	0.094	0.165
		2	0.087			
		3	0.105			
	2	**1**	0.157		0.147	
		2	0.138			
		3	0.147			
	3	**1**	0.231		0.254	
		2	0.272			
		3	0.259			
MP23-04	**1**	**1**	0.107	0.028	0.117	0.140
		2	0.120			
		3	0.124			
	2	**1**	0.200		0.151	
		2	0.120			
		3	0.134			
	3	**1**	0.144		0.152	
		2	0.162			
		3	0.151			
MP23-05	**1**	**1**	0.117	0.033	0.109	0.111
		2	0.106			
		3	0.103			
	2	**1**	0.168		0.172	
		2	0.173			
		3	0.175			
	3	**1**	0.092		0.090	
		2	0.092			
		3	0.086			
	4	**1**	0.080		0.090	
		2	0.106			
		3	0.083			
	5	**1**	0.090		0.096	
		2	0.095			
		3	0.102			
MP23-06	**1**	**1**	0.084	0.015	0.084	0.100
		2	0.085			
		3	0.083			

	2	**1**	0.097		0.105	
		2	0.097			
		3	0.122			
	3	**1**	0.119		0.110	
		2	0.111			
		3	0.101			
MP23-07	**1**	**1**	0.084	0.012	0.085	0.095
		2	0.093			
		3	0.079			
	2	**1**	0.103		0.093	
		2	0.091			
		3	0.084			
	3	**1**	0.114		0.105	
		2	0.093			
		3	0.109			
MP23-08	**1**	**1**	0.104	0.012	0.103	0.103
		2	0.112			
		3	0.092			
	2	**1**	0.110		0.099	
		2	0.088			
		3	0.099			
	3	**1**	0.127		0.108	
		2	0.102			
		3	0.096			
MP23-09	**1**	**1**	0.159	0.033	0.147	0.105
		2	0.153			
		3	0.130			
	2	**1**	0.085		0.085	
		2	0.087			
		3	0.081			
	3	**1**	0.080		0.084	
		2	0.093			
		3	0.079			
MP23-10	**1**	**1**	0.114	0.031	0.148	0.096
		2	0.184			
		3	0.145			
	2	**1**	0.079		0.078	
		2	0.074			
		3	0.081			
	3	**1**	0.090		0.092	
		2	0.081			
		3	0.105			
	4	**1**	0.079		0.077	
		2	0.075			
		3	0.078			
	5	**1**	0.084		0.083	
		2	0.088			
		3	0.078			
MP23-11	**1**	**1**	0.135	0.037	0.139	0.127
		2	0.136			
		3	0.146			
	2	**1**	0.102		0.134	
		2	0.089			
		3	0.211			
	3	**1**	0.108		0.108	

		2	0.099			
		3	0.116			
MP23-12	**1**	**1**	0.122	0.052	0.107	0.163
		2	0.114			
		3	0.084			
	2	**1**	0.237		0.215	
		2	0.231			
		3	0.176			
	3	**1**	0.211		0.185	
		2	0.169			
		3	0.174			
	4	**1**	0.174		0.200	
		2	0.198			
		3	0.228			
	5	**1**	0.104		0.109	
		2	0.125			
		3	0.098			
MP23-13	**1**	**1**	0.097	0.040	0.105	0.143
		2	0.102			
		3	0.118			
	2	**1**	0.132		0.133	
		2	0.136			
		3	0.131			
	3	**1**	0.210		0.192	
		2	0.198			
		3	0.166			
MP23-14	**1**	**1**	0.203	0.050	0.202	0.114
		2	0.144			
		3	0.258			
	2	**1**	0.084		0.089	
		2	0.091			
		3	0.092			
	3	**1**	0.095		0.095	
		2	0.095			
		3	0.094			
	4	**1**	0.094		0.096	
		2	0.094			
		3	0.100			
	5	**1**	0.096		0.091	
		2	0.089			
		3	0.088			
MP23-15	**1**	**1**	0.130	0.030	0.150	0.113
		2	0.156			
		3	0.162			
	2	**1**	0.083		0.086	
		2	0.083			
		3	0.091			
	3	**1**	0.103		0.104	
		2	0.099			
		3	0.111			
MP23-47	**1**	**1**	0.109	0.032	0.110	0.150
		2	0.111			
		3	0.110			
	2	**1**	0.191		0.174	
		2	0.164			

		3	0.166			
	3	**1**	0.143		0.165	
		2	0.177			
		3	0.175			
MP23-48	**1**	**1**	0.142	0.024	0.163	0.143
		2	0.170			
		3	0.177			
	2	**1**	0.133		0.132	
		2	0.108			
		3	0.154			
	3	**1**	0.127		0.134	
		2	0.117			
		3	0.157			
MP15-01	**1**	**1**	0.198	0.052	0.218	0.123
		2	0.188			
		3	0.267			
	2	**1**	0.096		0.094	
		2	0.089			
		3	0.096			
	3	**1**	0.090		0.092	
		2	0.101			
		3	0.084			
	4	**1**	0.101		0.100	
		2	0.110			
		3	0.091			
	5	**1**	0.117		0.113	
		2	0.121			
		3	0.102			
MP15-03	**1**	**1**	0.108	0.043	0.104	0.147
		2	0.107			
		3	0.097			
	2	**1**	0.143		0.150	
		2	0.115			
		3	0.192			
	3	**1**	0.189		0.186	
		2	0.154			
		3	0.215			
MP15-04	**1**	**1**	0.109	0.024	0.099	0.122
		2	0.095			
		3	0.094			
	2	**1**	0.115		0.116	
		2	0.117			
		3	0.116			
	3	**1**	0.157		0.151	
		2	0.137			
		3	0.159			
MP15-05	**1**	**1**	0.103	0.007	0.105	0.108
		2	0.110			
		3	0.103			
	2	**1**	0.116		0.113	
		2	0.110			
		3	0.113			
	3	**1**	0.111		0.107	
		2	0.094			
		3	0.115			

MP15-06	**1**	**1**	0.098	0.010	0.101	0.106
		2	0.100			
		3	0.106			
	2	**1**	0.098		0.103	
		2	0.106			
		3	0.104			
	3	**1**	0.130		0.114	
		2	0.106			
		3	0.105			
MP15-07	**1**	**1**	0.115	0.079	0.111	0.205
		2	0.102			
		3	0.116			
	2	**1**	0.293		0.309	
		2	0.273			
		3	0.360			
	3	**1**	0.140		0.157	
		2	0.159			
		3	0.171			
	4	**1**	0.210		0.232	
		2	0.275			
		3	0.211			
	5	**1**	0.127		0.218	
		2	0.263			
		3	0.262			
MP15-08	**1**	**1**	0.099	0.063	0.090	0.186
		2	0.080			
		3	0.092			
	2	**1**	0.164		0.184	
		2	0.234			
		3	0.154			
	3	**1**	0.159		0.169	
		2	0.149			
		3	0.199			
	4	**1**	0.236		0.244	
		2	0.252			
		3	0.245			
	5	**1**	0.251		0.245	
		2	0.254			
		3	0.231			
MP15-09	**1**	**1**	0.086	0.071	0.086	0.203
		2	0.082			
		3	0.089			
	2	**1**	0.264		0.263	
		2	0.271			
		3	0.254			
	3	**1**	0.220		0.240	
		2	0.272			
		3	0.228			
	4	**1**	0.251		0.254	
		2	0.274			
		3	0.236			
	5	**1**	0.174		0.173	
		2	0.190			
		3	0.156			
MP15-10	**1**	**1**	0.105	0.074	0.124	0.259

		2	0.162			
		3	0.105			
	2	**1**	0.349		0.312	
		2	0.312			
		3	0.275			
	3	**1**	0.292		0.281	
		2	0.282			
		3	0.270			
	4	**1**	0.270		0.290	
		2	0.300			
		3	0.300			
	5	**1**	0.269		0.286	
		2	0.319			
		3	0.271			
MP15-11	**1**	**1**	0.142	0.030	0.156	0.167
		2	0.176			
		3	0.149			
	2	**1**	0.160		0.155	
		2	0.163			
		3	0.143			
	3	**1**	0.240		0.190	
		2	0.174			
		3	0.157			
MP15-12	**1**	**1**	0.115	0.030	0.119	0.157
		2	0.121			
		3	0.122			
	2	**1**	0.162		0.175	
		2	0.179			
		3	0.184			
	3	**1**	0.179		0.177	
		2	0.191			
		3	0.161			
MP15-13	**1**	**1**	0.093	0.107	0.097	0.256
		2	0.104			
		3	0.095			
	2	**1**	0.314		0.268	
		2	0.290			
		3	0.200			
	3	**1**	0.225		0.286	
		2	0.314			
		3	0.321			
	4	**1**	0.347		0.342	
		2	0.368			
		3	0.310			
	5	**1**	0.332		0.288	
		2	0.407			
		3	0.126			
MP15-14	**1**	**1**	0.094	0.077	0.092	0.228
		2	0.092			
		3	0.092			
	2	**1**	0.260		0.243	
		2	0.241			
		3	0.229			
	3	**1**	0.267		0.243	
		2	0.253			

		3	0.210			
	4	**1**	0.274		0.258	
		2	0.270			
		3	0.229			
	5	**1**	0.260		0.301	
		2	0.296			
		3	0.349			
MP04-02	**1**	**1**	0.186	0.016	0.171	0.178
		2	0.167			
		3	0.160			
	2	**1**	0.195		0.187	
		2	0.166			
		3	0.202			
	3	**1**	0.173		0.169	
		2	0.181			
		3	0.153			
	4	**1**	0.187		0.190	
		2	0.191			
		3	0.192			
	5	**1**	0.160		0.171	
		2	0.160			
		3	0.193			
MP04-17	**1**	**1**	0.101	0.019	0.100	0.118
		2	0.085			
		3	0.112			
	2	**1**	0.121		0.118	
		2	0.123			
		3	0.110			
	3	**1**	0.123		0.136	
		2	0.135			
		3	0.149			
MP04-23	**1**	**1**	0.078	0.049	0.085	0.177
		2	0.081			
		3	0.097			
	2	**1**	0.208		0.203	
		2	0.201			
		3	0.201			
	3	**1**	0.193		0.194	
		2	0.203			
		3	0.185			
	4	**1**	0.210		0.209	
		2	0.232			
		3	0.185			
	5	**1**	0.198		0.195	
		2	0.195			
		3	0.192			
MP04-24	**1**	**1**	0.190	0.038	0.165	0.117
		2	0.151			
		3	0.153			
	2	**1**	0.096		0.092	
		2	0.096			
		3	0.083			
	3	**1**	0.092		0.096	
		2	0.093			
		3	0.103			

MP04-25	**1**	**1**	0.193	0.038	0.220	0.158
		2	0.204			
		3	0.263			
	2	**1**	0.156		0.145	
		2	0.132			
		3	0.147			
	3	**1**	0.129		0.127	
		2	0.127			
		3	0.124			
	4	**1**	0.146		0.138	
		2	0.124			
		3	0.145			
	5	**1**	0.158		0.159	
		2	0.168			
		3	0.152			
S. Epi	**1**	**1**	0.084	0.019	0.084	0.101
		2	0.088			
		3	0.080			
	2	**1**	0.091		0.104	
		2	0.105			
		3	0.116			
	3	**1**	0.093		0.117	
		2	0.133			
		3	0.124			

Appendix I: Summary of experimental results

Strain	year	Selection	FimH allel	O-type	H-type	ST	ST131 Clades	Tetracycline resistance_genotype
MP23-01	2016	NORM	30	25	4	131	C2	none
MP23-02	2014	NORM	30	25	4	131	C1	none
MP23-03	2011	NORM	30	25	4	131	C1	none
MP23-04	2017	NORM	58	1	42	648		none
MP23-05	2016	NORM		153	6	648		tetB
MP23-06	2013	NORM	25	1	6	648		none
MP23-07	2014	NORM	41	16	5	131	A	none
MP23-08	2013	NORM	25	1	6	648		tetA
MP23-09	2013	NORM		1	6	648		none
MP23-10	2011	NORM		1	6	648		none
MP23-11	2009	NORM		1	6	648		none
MP23-12	2009	NORM	30	153	6	648		none
MP23-13	2017	NORM	58	4	42	648		tetA
MP23-14	2013	NORM		153	6	648		tetB
MP23-15	2013	NORM		1	6	648		tetB
MP23-47	2017	NORM	30	25	4	131	C2	none
MP23-48	2011	NORM	30	25	4	131	C2	none
MP04-02	2000	EcoSens	10	6	1	73		none
MP04-17	2000	EcoSens				73		none
MP04-23	2000	EcoSens				73		none
MP04-24	2000	EcoSens				73		none
MP04-25	2000	EcoSens				73		none
MP15-01	2013	NORM	41	16	5	131	A	tetB
MP15-03	2006	NORM	41	16	5	131	A	tetA
MP15-04	2017	NORM	22	25	4	131	B	tetB
MP15-05	2017	NORM	22	25	4	131	B	tetB
MP15-06	2005	NORM	22	25	4	131	B	tetB
MP15-07	2015	NORM	30	25	4	131	C1	none
MP15-08	2017	NORM	30	25	4	131	C1	tetB
MP15-09	2013	NORM	30	25	4	131	C1	tetA
MP15-10	2017	NORM	30	25	4	131	C2	tetA
MP15-11	2012	NORM	30	25	4	131	C2	tetB
MP15-12	2013	NORM	30	25	4	131	C2	tetA
MP15-13	2017	NORM	30	25	4	131	C2	tetA
MP15-14	2009	NORM	30	25	4	131	C2	tetA
LB only								

Tetracycline Resistance phenotype	blaCTX-M-15	blaCTX-M-14	blaCTX-M-27	blaOXA-1 (class D)	blaTEM-1B (class A)	Betalactamase (any kind)
S	+	-	-	+	-	+
S	-	-	+	-	-	+
S	-	+	-	-	-	+
S	-	-	-	-	-	-
R	+	-	-	+	+	+
S	-	-	-	-	-	-
S	-	-	-	-	-	-
R	-	-	+	+	-	+
S	-	-	-	-	+	+
S	-	-	-	-	+	+
S	-	-	-	-	-	-
S	+	-	-	+	+	+
R	-	-	-	-	+	+
R	+	-	-	+	+	+
R	+	-	-	-	-	+
S	+	-	-	-	-	+
S	+	-	-	-	+	+
S	-	-	-	-	-	-
S	-	-	-	-	-	-
S	-	-	-	-	-	-
S	-	-	-	-	-	-
S	-	-	-	-	-	-
R	-	-	-	-	-	-
R	-	-	-	-	-	-
R	-	-	-	-	-	-
R	-	-	-	-	-	-
R	-	-	-	-	-	-
R	-	-	-	-	-	-
R	-	-	-	-	-	-
R	-	-	-	-	-	-
R	-	-	-	-	-	-

Ampicillin resistance phenotype	Hemolysis	Motility	Motility_surface	Average CFU biofilm (log)	SD CFU biofilm
R	alpha	+	surface	6.1987	0.6027
R	none	+	entire agar	6.4858	1.1545
R	alpha	+	entire agar	7.5839	0.1597
S	none	+	entire agar	6.3490	0.5229
R	alpha	+	surface	7.1105	0.7487
S	none	+	surface	7.2251	0.1395
S	beta	-	-	6.9136	0.5155
R	beta	+	entire agar	6.9438	0.6281
R	beta	+	entire agar	6.9913	0.4575
R	beta	+	entire agar	6.9221	0.5392
I	beta	+	entire agar	7.0686	0.5748
R	beta	+	entire agar	6.7849	0.1440
R	beta	+	entire agar	7.0669	0.3037
R	beta	-	-	6.8277	0.0934
R	beta	+	entire agar	6.5645	0.2250
R	none	+	entire agar	7.3852	0.5479
R	beta	+	surface	7.1316	0.7375
S	beta	+	entire agar	6.8744	0.2410
S	beta	+	entire agar	6.8552	0.0850
S	none	+	entire agar	6.7508	0.2897
S	none	-	-	6.5474	0.1106
S	alpha	+	entire agar	6.6160	0.3244
S	alpha	+	surface	7.0166	0.4268
S	none	+	surface	6.9410	0.5364
S	beta	+	entire agar	7.1360	0.5100
S	beta	-	-	7.1574	0.2095
S	beta	+	entire agar	7.0008	0.0947
S	beta	+	surface	6.1789	0.3076
S	beta	+	entire agar	6.8427	0.2754
S	beta	+	entire agar	6.9816	0.4773
S	alpha	+	entire agar	6.8092	0.2192
S	none	+	entire agar	5.5091	0.6642
S	beta	+	entire agar	6.4607	0.3995
S	alpha	+	entire agar	6.4673	0.4594
S	none	+	entire agar	6.4771	0.4833

Biomass staining pegs (OD)	SD OD pegs	Biomass staining wells (OD)	SD OD wells	Curli 28	Curli 37	Cellulose 28	Cellulose 37
0.1646	0.0341	0.1177	0.0270	-	-	+	+
0.2627	0.0644	0.1685	0.0769	+	+	+	+
0.3894	0.0634	0.1653	0.0717	+	-	-	-
0.1617	0.0338	0.1401	0.0284	-	-	-	+
0.0752	0.0158	0.1113	0.0330	-	-	-	-
0.1114	0.0207	0.0998	0.0149	+	+	+	+
0.0990	0.0100	0.0945	0.0120	-	-	+	+
0.1006	0.0153	0.1033	0.0119	+	+	+	+
0.0740	0.0071	0.1053	0.0327	+	+	-	-
0.0676	0.0062	0.0956	0.0311	-	-	+	+
0.1001	0.0304	0.1268	0.0371	-	-	+	+
0.2262	0.0532	0.1630	0.0520	-	-	-	-
0.2061	0.0288	0.1432	0.0402	+	+	+	+
0.0986	0.0323	0.1143	0.0501	-	-	-	-
0.0706	0.0139	0.1132	0.0299	-	+	+	+
0.1719	0.0274	0.1496	0.0324	-	+	+	+
0.2213	0.0775	0.1427	0.0235	-	-	+	+
0.2048	0.0478	0.1776	0.0158	-	-	+	+
0.1252	0.0315	0.1177	0.0187	-	-	+	+
0.1984	0.0481	0.1772	0.0490	+	+	-	-
0.0912	0.0147	0.1174	0.0376	+	+	-	-
0.1420	0.0316	0.1578	0.0375	+	+	-	-
0.1248	0.0338	0.1233	0.0524	+	+	-	-
0.5051	0.1468	0.1466	0.0434	+	-	+	-
0.1724	0.0312	0.1221	0.0240	+	+	-	-
0.1048	0.0257	0.1084	0.0071	+	+	-	-
0.1226	0.0187	0.1058	0.0098	+	+	-	-
0.2584	0.0877	0.2051	0.0795	-	-	+	+
0.2738	0.0829	0.1864	0.0627	-	-	+	+
0.2894	0.1144	0.2032	0.0705	+	+	+	+
0.3040	0.0726	0.2587	0.0742	+	-	+	+
0.1662	0.0346	0.1671	0.0301	-	-	+	+
0.2022	0.0578	0.1572	0.0299	+	+	+	+
0.3388	0.0805	0.2563	0.1072	+	+	+	+
0.2308	0.0670	0.2276	0.0770	+	+	+	+
0.0687		0.0890					

Appendix J: Results from the genotypic characterization

	PlasmidFinder																		
	Col-plasmids						Inc groups							β-lactams					
	ColpVC	Col(MG828)	Col(BS512)	Col8282	Col(MGD2)	Col156	IncFIA	IncFIB	IncFII	IncQ1	IncY	IncX4	p0111	blaCTX-M-15	blaOXA-1	blaCTX-M-27	blaCTX-M-14	blaTEM-1B	
MP23-01	1						2	2	2					1	1				
MP23-02							1	2	1							1			
MP23-03		2					1		1								1		
MP23-04																			
MP23-05							2	2	1					1	1			1	
MP23-06			1				2	2	1				2						
MP23-07							2	2	1	1									
MP23-08			1				2	2	1	2					1	1			
MP23-09			1	2			2	2	2									1	
MP23-10			1	2	1		2	2	2									1	
MP23-11																			
MP23-12											2			1	1			1	
MP23-13	2									1								1	
MP23-14			1				2	2	1					1	1			1	
MP23-15			1				2	2	1		2			1					
MP23-47						2	2	2	1					1					
MP23-48							2	2	2					1				1	
MP04-02																			
MP15-01						2		2	2										
MP15-03						2		2	2										
MP15-04								2	2										
MP15-05								2	2										
MP15-06								2	2										
MP15-07			1				1	2	1			2							
MP15-08							1	2	2										
MP15-09						2	1	2	1										
MP15-10						2	2	2	2										
MP15-11		2					2	2	1										
MP15-12						2	2	2	2										
MP15-13						2	2	2											
MP15-14						2	2	2	1										

1: Match = 100%ID and match length = ref length
2: Match < 100%ID and match length = ref length
3: Match = 100%ID and match length < ref length

ResFinder																		
Fluoroquinolones			Macrolide	Phenicol		Aminoglycosides		Folate Pathway Antagonist					Tetracycline		Genes associated with iron uptake/utilization			
gyrA (mutati	parE (mutation)	parC (mutation)	mph(A)	catB3	catA1	aac(6')-Ib-cr	aac(3)-IIa	sul1	sul2	dfrA17	dfrA1	dfrA36	tet(B)	tet(A)	chuA	fyuA	irp2	iucC
1	1	1	1	3		1	2	1		1					1	1	1	1
1	1	1													1	2	1	1
1	1	1													1	1	1	
															2	1	2	
1	1	1	1	2	2	1		1		1			1		2	1	1	2
1	1	1													2	1	1	1
	1								1						1	2	1	1
1	1	1	1	1		1			1	1				1	2	1	1	1
1	1	1					2								2	1	1	
1	1	1					2								2	1	1	
1	1	1													2	1	2	
1	1	1		3		1	1								2	1	2	
								1	1		1	1		1	2	1	2	
1	1	1	1	2	2	1		1		1			1		2	1	2	1
1	1	1					2						3		2	1	1	
1	1	1	1					1		1					1	1	2	1
1	1	1							1	1					1	1	1	1
															1	1	1	1
	1												1		1	1	1	
	1		1					1	1	1				1	1	1	1	
													1		1	2	2	
													1		1	2	1	
													1		1	2	1	
1	1	1													1	1	1	1
1	1	1											1		1	2	1	1
1	1	1	1					1	1	1				1	1	2	1	1
1	1	1												1	1	1	1	1
1	1	1											1		1	1	1	1
1	1	1	1					1		1				1	1	1	1	1
1	1	1	1					1	1	1				1	1	2	1	
1	1	1	1					1	1	1				1	1	1	1	1

					SPATEs; serum resistance, colonization														
sitA	iutA	ireA	iroN	capU	sat	pic	vat	hra	iss	traT	gad	eilA	ibeA	cnf1	astA	mchB	mchC	mchF	mcmA
1					1			1	1	1	1			1					
1	1				1				1	1	1								
1									1	1	2								
1		2							1		2	2							
1	1				1			2		1	2	2							
1	1									1	2	2							
2	1				1					1	1	2							
1	1			1						1	2	2							
1										1	2	2							
1										1	2	2							
											2	2							
											2	2							
1								2	2		2	2			1	2	1	2	1
1	1				1			1		1	2	2							
2										1	2	2							
1	1				1			1	1	1	1			1					
1	1				1				1	1	2								
1	1		1		1	1	2	2	1		1			1		1	1	1	1
2										1	1								
2										1	1								
2		2	1					2	1	1	1		1					2	
2		2	1					2	1	1	1		1					2	
2		2	1					2	1	1	1		1					2	
1	1				1				1	1	1								
1	1				1				1	1	1								
1	1				1				1	1	2								
1	1				1				1	1	2								
1	1				1				1	1	1								
1	1				1				1	1	1								
	1				1			1	1	1	1								
1								1	1		2								
1	1				1			1	1	1	1								

VirulenceFinder

Toxins								Adhesins											
senB	cea	pic	vat	cma	cvaC	hlyF	usp	iha	papA_F19	papA_F20	papA_F43	papA_F48	papC	yfcV	lpfA	afaA	afaB	afaC	afaD
							2	1	2	2	1		1	1					
							2	1			2			1					
							2							1					
														1	2				
								2			2			1	2				
														2	2				
							2	1			2			1		1		2	1
														2	2				
														1	2				
														1	2				
														2	2				
														1	2				
												2	2	1	2				1
											2			1	2				
														1	2	1	2	2	1
1							2	1			1		2	1					
							2	2			2			1					
	1	1	2				1	1			2			1					
1							2	1			2					1		2	1
1							2	1			2			1					
	1			1	1	1	2							1					
				1	1	1	2							1					
				1	1	1	2							1					
							2	1			2			1					
							2	1			2			1					
1							2	1			2			1					
							2	1			2			1					
1							2	1			2			1					
1							2	1			1		1	1					
							2	1			2			1					
1							2	1			1		1	1					
1							2				2			1		1	2	2	1
1							2	1			2			1					

							Capsule related genes				Increased serum survival			Miscellaneous
afaE	afaE8	nfaE	focC	focG	sfaE	sfaD	kpsMII	kpsMIII	kpsE	neuC	ompT	terC	tcpC	air
											1	2		
							1		1		1	2		
											1	2		
							1		1		1	2		2
							1		2			2		2
									1		1	2		2
		2					1		2		1	2		
									1		1	2		2
									1		1	2		2
									1		1	2		2
									1		1	2		2
								1	2		1	2		2
	2										1	2		2
							1		1		1	2		2
2							1		1		1	2		2
							1		1		1	2		
							1		1		1	2		
			2	1	2	1	1		1		1	2	1	
		2					1		1		1	2		
							1		1		1	2		
							2		2	1	1	2		
							2		2	1	1	2		
							2		2	1	1	2		
									2		1	2		
							1		1		1	2		
							1		1		1	2		
							1		1		1	2		
							1		1		1	2		
							1		1		1	2		
2											1	2		
							1		1		1	2