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Antimicrobial resistance, virulence genes and biofilm forming abilities of *Klebsiella* spp. isolated from healthy domestic animals in Norway

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Preface

This master thesis was compiled at the Norwegian Veterinary Institute in Oslo. The finance was contributed by the KLEB-GAP project (Klebsiella pneumoniae – a key driver in the global spread of antimicrobial resistance and a target for new approaches in diagnostics, surveillance and alternative therapeutics) led by Arnfinn Sundsfjord and Iren Høyland LÖhr, the NoResist project (combating antimicrobial resistance in the Norwegian food production chain) led by Live L. Nesse, and the section for food safety and emerging health threats at the Norwegian Veterinary Institute,.

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There are so many kind, loving and talented people I have had the pleasure to be around this past year at NVI, that I unfortunately can´t thank them all separately (that would take up to much space). But from the bottom of my hart thank you to everyone at the microbiology lab at NVI and University hospital in Stavanger, for always lending me a helping hand and making me feel welcome and included. Also, thank you to the contributors behind NoResist, and KLEB-GAP for making this master thesis possible. Last, but not least to my family, specially my fiancé, Terje Alming, thank you for all the love, and support along this wiggly way to happiness.

Always remember, curiosity is key (*curiositatis agatur clavem*)

Fiona Franklin

Abstract

Klebsiella can be identified in several different environmental habitats, animals and humans. There are many different species and subspecies of *Klebsiella*, with *Klebsiella pneumoniae* being the most medically important species within this genus and is an important cause of hospital-acquired infection (nosocomial infection). *Klebsiella* is part of the "ESKAPE pathogens", which is an acronym used for the six most common nosocomial pathogens; ***E****nterococcus faecium,* ***S****taphylococcus aureus,* ***K****lebsiella pneumoniae,* ***A****cinetobacter baumannii,* ***P****seudomonas aeruginosa* and ***E****nterobacter* species. Most of these bacteria are also opportunistic and could pose a severe threat to patients, especially if they were to become increasingly resistant to antimicrobial agents.

A total of 1290 faecal samples from turkey, swine, broiler, dog and cattle (supplied by NORM-VET) were screened to identify *Klebsiella* spp. From these samples 380 (29.46%) were identified positive for *Klebsiella* spp. Turkey had en overall detection rate of 69.57%, swine 51.79%, broiler 23.51%, dog 15.87% and 4.56% in cattle. Three different isolation methods for Klebsiella spp. were tested on a subset of the samples (n= 343 originating from swine and cattle) with the results indicating that the *Klebsiella* spp. detection rate was higher in samples enriched in LB broth with 10mg/l amoxicillin (45.52% [95% CI: 38-55]), compared to direct out plating (19.53% [95% CI: 15-24%]) and peptone water enrichment (25.56% [95% CI: 21-30%]).

All the 380 *Klebsiella* isolates found were screened for antimicrobial resistance, by being subjected to susceptibility testing that resulted in 7.38% expressing resistance against one or more antimicrobial agent. The prevalence of resistant strains originating from turkey was 60.71%, from broiler 21.43% and from both dog and swine 7.14%. While no resistance was indicated in isolates originating from cattle.

The whole genome sequencing data of the 203 poultry isolates, revealed that 22.66% of the isolates encoded for one or more virulence genes. Yersiniabactin (*ybt*) was identified in 21.18% of the isolates, aerobactin (*iuc5*) in 7.88%, and salmochelin (*iro5*) in 7.39% isolates. All the isolates encoding for salmochelin also encoded for aerobaction, with the detection of IcFII plasmid carrying virulence genes.

There were 64 different sequence types identified among the 203 *K. pneumonia* isolates from poultry. ST35 was identified in 14.29%, followed by ST290-1LV with 7.39%. These sequence types have also been detected in humans.
The average biofilm production for *K. pneumoniae* was OD_{595} 0.66 (STDEV: 0.52-0.80), indicating that they are good producers.

In conclusion, the overall antimicrobial resistance occurrence among *Klebsiella* spp. from Norwegian domestic animals was low. Among the resistant isolates resistance against tetracycline and sulfamethoxazole was most commonly observed. Virulence genes encoding, yersiniabactin, salmochelin and aerobactin, were detected in some of the isolates, with salmochelin and aerobactin encoding genes located on incF plasmids. Further analysis would be recommended in regards to ST290 and ST290-1LV and the detected virulence genes.

Norwegian abstract

Klebsiella kan identifiseres i flere ulike miljøer, dyr og mennesker. Det er mange arter og under arter av *Klebsiella*, og deriblant er *Klebsiella pneumoniae* ansett å være den medisinsk viktigste arten, da den ofte forårsaker sykehuservervede infeksjoner. *Klebsiella* er en del av gruppen ESKAPE patogener, som omslutter de seks mest vanlige sykehuservervede patogene; ***E****nterococcus faecium,* ***S****taphylococcus aureus,* ***K****lebsiella pneumoniae,* ***A****cinetobacter baumannii,* ***P****seudomonas aeruginosa* and ***E****nterobacter* species. Mesteparten av disse bakteriene er opportunistiske og kan bli en trussel mot sykehus pasienter, særlig om de utvikler bred antimikrobiell resistens.

Totalt ble 1290 avføringsprøver fra kalkun, svin, slaktekylling, hund og storfe (innhentet via NORM-VET) undersøkt for tilstedeværelse av *Klebsiella* spp. Fra disse prøvene var 380 (29.46%) positive for *Klebsiella* spp. Deteksjonsraten i prøvene fra kalkun var 69.57%, svin 51.79%, slaktekylling 23.51%, hund 15.87% og 4.56% i storfe. Tre ulike isolasjons metoder ble utprøvd på en andel (n= 343, fra svin og storfe) av avføringsprøvene som viste at deteksjons raten var høyere i LB buljong med 10mg/l amoxicillin(45.52% [95% CI: 38-55]) sammenlignet med direkte utsåing fra avføringen (19.53% [95% CI: 15-24%]) og oppformert i pepton vann (25.56% [95% CI: 21- 30%]).

Alle 380 *Klebsiella* spp. isolatene ble utsatt for antimikrobiell sensitivitets tesing, som resulterte i at 7.38% av *Klebsiella* spp. isolatene indikerte resistens mot ett eller flere antimikrobielle midler. Prevalensen av kalkun isolater var 60.71% de resistente isolatene, 21.43% fra slakte kylling, 7.14% fra både svin og hund, mens ingen av isolatene fra storfe var resistente.

Helgenom sekvensering av fjørfe isolatene viste at av 203 isolater kodet 22.66% for en eller flere virulens gener. Yersiniabactin (*ybt*) ble identifisert i 21.18% av isolatene, aerobactin (iuc5) i 7.88% og salmochelin (iro5) i 7.39% av fjørfe

isolatene. Alle isolatene som kodet for salmochelin kodet også for aerobactin, og blant disse isolatene ble incFII plasmid med virulens genene identifisert.

Totalt ble det identifisert 64 forskjellige sekvenstyper (ST) blant de 203 *K. pneumoniae* isolatene fra fjørfe. ST35 ble identifisert i 14.29%, etterfulgt av ST290-1LV med 7.39%. Overnevnte sekvenstypene har også blitt detektert hos mennesker. Majoriteten av *K. pneumoniae* isolatene ble klassifisert som gode biofilm produsenter, med gjennomsnittlig OD_{595} på 0.66 (STDEV: 0.52-0.80).

Konkluderende viser denne oppgaven at forekomsten av antimikrobiell resistens i *Klebsiella* spp. blant friske dyr (kalkun, svine, slakte kylling, hund og storfe) i Norge er lav. Resistens mot tetrasyklin og sulfamethoxazole hadde størst forekomst blant *Klebsiella* spp. isolatene. Tre viktige virulens gener ble identifisert, og det videre undersøkelser burde utføres ift ST290 og ST290-1LV og plasmider.

1. Introduction

1.1 Bacterial Communities; an Introduction

Bacteria are intricate microorganisms, and vital for a functioning environment and successful symbioses between humans, animals, and plants (Pepper et al., 2014). Bacteria are found anywhere on Earth, from kilometres below the ground, to the top of the highest mountains, from the coldest parts of the Arctic and Antarctic to hot vents in the deep sea, where temperatures can reach in excess of 100°C (Brown et al., 2008; Liu *et al.,* 1997).

In every element there are massive numbers of bacteria at work, either converting nitrogen compounds in the soil so they can be sequestered by plants, fermenting food, producing antibiotics or as a protective layer on the skin or mucosa against pathogenic microbes in humans and animals. But this is just a very narrow spectrum of the diversity of functions bacterium inhabit (Willey et al., 2014). Bacteria, while being single-celled prokaryotic organisms, are among the oldest living organisms on this planet, with fossil records stretching back over 3 billion years (Schopf & Packer, 1987; Schopf, 1993). Because of this, the bacteria have had plenty of time to adapt to all the planet´s environments and many challenges. For instance, the human intestine is home to a large community of bacteria that outnumber the all the human cells in the body by a factor of 10 (Reece *et al*., 2011 p. 617). One way of determining the effect bacterial communities have on eukaryotes is investigating how a complex life form function without them. This has led scientist to create germ-free mice, that are immunological naive and lack any form of microbiota (Lundberg et al., 2016). This allows researchers to investigate the effect of pathogens, drugs and the interactions between certain prokaryotes without the “interference” of other microbes.

Bacterial cells differ from eukaryotic cells in several ways; the presence of a cell wall, flagella used for mobility, a simpler internal structure and a different physical arrangement of their DNA (Reece *et al*., 2011, p. 603-605) are some aspects that separate bacteria from eukaryotic organisms. While cell walls can be found in some eukaryotic organisms (plants and fungi) only prokaryotic cell walls are made from

peptidoglycan, a sugar-based polymer (Reece *et al.,* 2011, p. 603). And though the presence of flagella is not uniquely prokaryotic, they are distinct in their molecular composition and mechanism of propulsion (Willey *et al*., 2014). The internal structure of bacterial cells is without compartments, unlike their eukaryotic counterparts, such as the nucleus or membrane-bound organelles. Lastly, the bacterial DNA is smaller than the eukaryotic and the bacterial genome can be either a circular or linear chromosome. Several species also have smaller rings of independently replicating DNA molecules known as plasmids. DNA replication, transcription and translation are fundamentally similar processes in both eukaryotic and prokaryotic cells. However, small differences in size and RNA content of prokaryotic and eukaryotic ribosomes allow for certain antimicrobial agents, such as tetracycline, to block protein synthesis in bacterial cells only, leaving the eukaryotic host´s cells unaffected (Blackwood *et al*.,1985, p. 318; Connell *et al*., 2003.) Unfortunately, not all bacteria are beneficial for humans and animals and can cause diseases. These are collectively referred to as pathogenic bacteria. Depending on the pathogen, the severity and symptoms of the disease can vary greatly, from fairly harmless nausea to potentially lethal systemic failure (Granum, 2015). Some bacteria, however, are only pathogenic given the right environmental conditions, causing them to be known as opportunistic pathogens. One such bacteria is *Klebsiella*.

1.1.1 *Klebsiella*- classification and taxonomy

In the phylum *Proteobacter*, the class *Gammaproteobacter*, the order *Enterobacteriales,* the highly taxonomically diverse family *Enterobacteriaceae* is where the genus *Klebsiella* spp. is found (biology website, 2019/Willey, Sherwood & Woolverton, 2014, p.509-532). *Klebsiella* spp. can be identified in many different habitats, such as in soil, water, sewage, on plants, and on epithelial and mucosal surfaces in humans and animals (Bagley, 1985). *Klebsiella* spp. is a straight, Gram-negative, rod-shaped bacteria that does not have the ability to produce spores or move (non-motile) (Podshun *et al*., 1998). When identifying *Klebsiella* spp. on blood agar plates, the morphology of the colonies is round, plump and shiny white/grey (Figure 1). Metabolically, *Klebsiella* spp. are facultative anaerobic, and are able to utilize citrate as a carbon source which can be added to a selective agar media to identify *Klebsiella* spp. They can reduce nitrate to nitrite and are both indole and oxidase negative. The latter is due to the lack of cytochrome c, with is also used as an identification tool diagnostically (Wiley, Sherwood & Woolverton, 2014, p. 814).

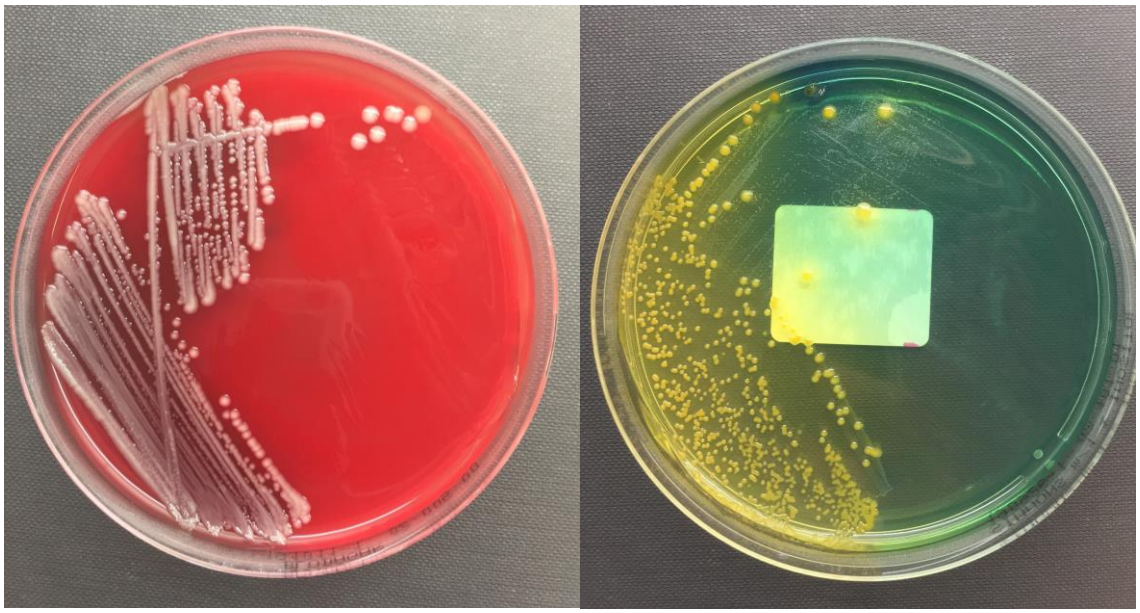

Figure 1. Left: Morphology of *Klebsiella* spp. on blood agar.
Right: Morphology of *Klebsiella* spp. on Simmons citrate agar with 1% inositol.

1.1.2 The *Klebsiella pneumonia* complex

K. pneumoniae is now divided into seven different categories based on their phylogenetic characteristics, but together they form the *Klebsiella pneumoniae* complex (Rodrigues *et al.*, 2019).

Table 1. Different subspecies that form the *Klebsiella pneumoniae* complex, abbreviations for each subspecies and the sources they have been isolated from.

Abbreviation	***Klebsiella* species subsp.**	**Source**
Kp1	*K. pneumoniae*	feces, blood, liver abscess
Kp2	*K. quasipneumoniae subsp. quasipneumoniae*	blood, water, environment
Kp3	*K. variicola subsp. variicola*	blood, banana, maize, fungus garden
Kp4	*K. quasipneumoniae subsp. similipneumoniae*	blood, farmland soil, lakes
Kp5	*K. variicola subsp. tropicalensis*	environment, faecal
Kp6	*K. quasivariicola*	blood, peri-rectal, wound, feces, vagina
Kp7	*K. africanensis*	feces

* modified table from Rodrigues *et al.*, 2019

Whole genome sequencing (WGS) or polymerase chain reaction (PCR) are common methods to identify *Klebsiella*. To distinguish between the *Klebsiella* spp. within the *Klebsiella pneumoniae* complex, gene sequencing of the marker gene *rpoB* is one of the options (Rodrigues *et al.*, 2019). *Klebsiella* spp. in the complex can also be distinguished by molecular typing of beta-lactamase genes. For example, the genome of Kp1 contains the bla_{SHV} gene, while Kp2 contains $bla_{\text{OKP-A}}$, and Kp3 contains bla_{LEN}.

1.1.3 The pathogen *Klebsiella* spp.

Klebsiella spp. is an opportunistic pathogen that can be present in humans and animals but is usually asymptomatic. In humans it is however associated with causing hospital-acquired infection (nosocomial infection) (Selden *et al*., 1971). The individuals who get an infection caused by *Klebsiella* spp. are often immunosuppressed, have an underlying disease such as diabetes mellitus, or have been treated with antimicrobial agents. Such treatments can provide favourable conditions for *Klebsiella* spp. by removing microbes that would normally prohibit further growth for *Klebsiella* spp. There are many different species and subspecies of *Klebsiella*, but *Klebsiella pneumoniae* (*K. pneumoniae*) is the most medically important species within this genus (Holt *et al*., 2015). The lower gastrointestinal tract in colonized patients has long been considered an important reservoir of *K. pneumonia* (Pomakova et al., 2011). *K. pneumonia* is responsible for a significant proportion of urinary tract infections, pneumonia, septicaemia and liver abscess contracted by patients during hospital stays (Lin *et al*., 2006). Some variants of *K. pneumoniae* are associated with high levels of antimicrobial resistance, while others can cause severe infections and are called hypervirulent (Struve *et al*., 2015). A common factor in the latter is an overexpression of the capsule polysaccharides that protects the bacteria from host defence mechanisms such as phagocytosis, along with other mechanisms that help the bacteria evade several components of the immune system (Paczosa *et al*., 2016)

ESKAPE pathogens is an acronym used for the six most common nosocomial pathogens; ***E****nterococcus faecium,* ***S****taphylococcus aureus,* ***K****lebsiella pneumoniae,* ***A****cinetobacter baumannii,* ***P****seudomonas aeruginosa* and ***E****nterobacter* species (Santajit, 2016). Most of these bacteria are also opportunistic and could pose a severe treat to patients, especially if they were to become increasingly resistant to antimicrobial agents (Bialek-Davenet *et al*., 2014).

1.2 Important properties of *Klebsiella* spp.

1.2.1 Cell membrane

Gram-negative bacteria have a complex cell wall divided into three layers (Trondsmo, 2016).

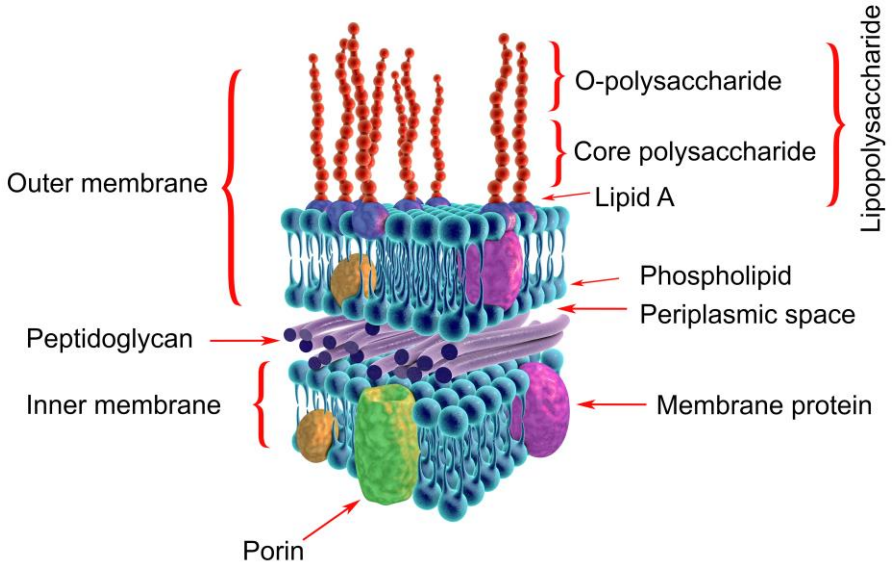

Figure 2. The layering of the gram-negative bacteria cell wall, illustration from Alamy.

Closest to the core of the bacterium is the cytoplasm membrane consisting of a double lipid layer. The cell membrane contains a peptidoglycan network of alternating disaccharides, N-acetylglucosamine (NAG) and N-acetylmuraminc (NAM), bound together with polypeptides in such a way that the structure protects and strengthens the cell, and gives the morphological structure. The periplasm is a gel-like substance containing hydrolytic enzymes, binding proteins, chemoreceptors and transport proteins (Willey, Sherwood & Woolverton, 2014, p. 58). Exposed to the environment is an outer layer of lipoproteins, polysaccharides and phospholipids called the lipopolysaccharide layer. The cell wall has several porins that can transport nutrients and waste across the membranes, but also other compounds like antibiotics (Denyer *et al.*, 2002). If the gene coding for porins gets

mutated, it can alter the porins ability to transport compounds in and out of the cell and thereby prevent certain antibiotics entering the bacteria, making them ineffective (Dé *et al.,* 2001). In the outer layer there are lipopolysaccharides made up by three subunits; lipid A, the core oligosaccharide, and the O-antigenic polysaccharide (Follador *et al*., 2016). Together this forms the O-antigen. Variation in the different compositions of the oligosaccharides makes it possible to identify the different O-antigen types. This gives rise to different modes of targeting in the host such as recognition of invading bacteria and complement- mediated lysis. As of 2016, seven different O-antigens, O1, O2, O3, O4, O5, O8 and O12, had been identified in *K. pneumoniae*, with O1, 02 and O3 being the most commonly found in human isolates (Follador *et al*., 2016; Trautman *et al*., 2004; Trautman *et al.,* 1997).

1.2.2 The polysaccharide capsule

The capsule is an outer layer, in addition to the cell wall, consisting of polysaccharides and protects the bacteria against the host´s immune system response, such as phagocytosis (Schembri *et al.,* 2005). Since there are so many different capsule types, it is difficult to use them to categorize bacteria. However, K1 and K2 are associated with hypervirulence and strains containing these variants show a higher abundance of virulence genes than other strains in *K. pneumoniae* (Struve *et al.*, 2015). The polysaccharide capsule (K-antigen) is encoded on the *cps* locus, and at least 77 different K-antigen capsules are identified in *K. pneumoniae* (Schembri *et al*., 2005). K1, K2, K5, K20, K54, and K57 are all associated with invasive disease in humans, located in the urinary tract or lower respiratory tract (Giske *et al*., 2012). Both O- and K-antigen are important virulence factors for establishing infection.

Pili (or fimbriae) are membrane-bound structures that can be confused with flagella, but pili are not responsible for propulsion and functions as an anchor or a way of attaching the cell to the mucosa or epithelial cell membrane in the urinary-, respiratory- or gastrointestinal tract (Martino *et al*., 2003). This is an important part of infection that gives the bacteria the ability to maintain its position inside the host.

There are several different types of pili, with type 1 and 3 being found in correlation to biofilm formation. After the bacteria has invaded the host, it will start multiplying and, if the conditions are favourable, an infection is imminent.

1.2.3 Molecular typing – Multi locus sequence typing (MLST)

MLST is a technique that identifies variations in the housekeeping genes, and thereby categorises the strains into different sequence types (ST)s.

The ST is based on seven housekeeping genes; *rpoB* (beta-subunit of RNA polymerase), *gapA* (glyceraldehyde 3-phosphate dehydrogenase), *mdh* (malate dehydrogenase), *pgi* (phosphoglucose isomerase), *phoE* (phosphorine E), *infB* (translation initiation factor 2) and *tonB* (periplasmic energy transducer) (Diancourt et al., 2005). Within each of the seven housekeeping genes, there are fragments of 450-500 base pairs that are used for identification. The different sequences present within a sample are assigned as distinct alleles and, the alleles at each of the seven loci define the ST. Most bacterial species have enough variation within the house-keeping genes to provide many alleles per locus, allowing billions of distinct allelic profiles to be distinguished using seven housekeeping loci. The Pasteur institute has developed an online database to compare the query sample to already identified alleles. For example, if all seven loci match the housekeeping genes, the result could be ST290, but if only six loci match, the result would be ST290-1LV. Thus, the only difference between ST290 and ST290-1LV is that one of the genes is either missing or partially deleted in the later. Within *K. pneumoniae* now there are several dominant STs like ST11, ST23 and ST37 identified in humans, and ST35 which is identified in both humans and animals (Jung *et al.*, 2013, Marcade *et al.*, 2013).

There are different clonal lineages assosiated with multi resistance, such as ST258, and increased virulence, ST11. This means that hypervirulent strains are often sensitive to antimicrobial agents (Lee et al., 2017), while lineages that show antimicrobial resistance, rarely have hypervirulent traits. So if an infection were detected early, the correct treatment would prevent a possible fatale outcome.

1.2.4 Mobile genetic elements

Klebsiella spp. are haploid microorganisms, but in addition to their core genome, they can contain up to 30 plasmids, which are passed on to the next generation by vertical gene transfer (Wyres *et al.,* 2018). Plasmids are circular or linear DNA molecules that vary in size and contain between 5-100 genes. While plasmids are not essential for the survival of the bacteria, they contain genetic information that can give the cell a selective advantage in certain environments (Willey, Sherwood & Woolverton, 2014, p. 69). Among these are antibiotic resistance, virulence genes and/or toxin production to name a few. But the plasmid does not contain any housekeeping genes or functions related to reproduction or growth (Tronsmo, 2016). This means that the bacteria do not rely on plasmids for basic metabolic or reproductive functions, the plasmid just adds additional traits that can be beneficial depending on the environment. Plasmids can be non-selective and easily transferred to other bacteria via conjugation. This gives rise to great concern, because plasmids originating from *Escherichia, Yersinia* or any other genus can be taken up by *Klebsiella spp*. There are many variants of plasmids and Inc-plasmid types were identified in *Enterobacteriaceae* with IncF, IncI, IncA/C and IncH being most prevalent (Rozwandowicz *et al*.,2018).

1.2.5 Horizontal gene transfer

Some bacteria species can exchange DNA, RNA or plasmids by horizontal gene transfer (HGT) without it being considered a sexual transfer of genetic information (Tronsmo, 2016). The “new” DNA or RNA can replace existing genes or introduce new genes into the genome. There are three different methods of HGT (figure 3): A; Transformation, where free DNA from the environment is taken up by the bacteria, B; transduction, where the transfer is performed by a bacteriophage that injects its genome into a bacterium and C; conjugation, where mobile elements such as plasmids and transposons are transported between bacteria by utilizing the pili as a connection between the two cells and transfer the plasmid (Willey, Sherwood & Woolverton, 2014, p. 388).

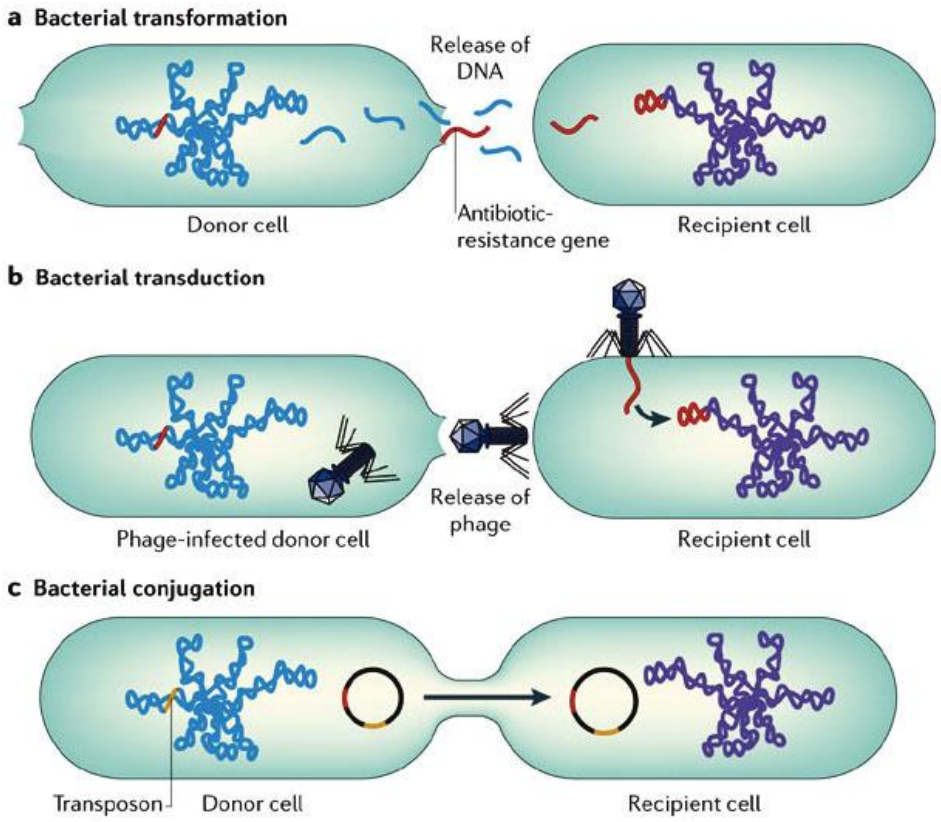

Figure 3. Horizontal Gene Transfer. Transformation, Transduction and Conjugation. (Source: Nature Reviews-Microbiology, 2006)

1.2.6 Virulence factors of importance in *Klebsiella pneumonia*

Virulence genes of special importance in *K. pneumonia* encode for aerobactin, yersiniabactin and salmochelin (Holt *et al*., 2015). Aerobactin and yersiniabactin are siderophores that help the bacteria scavenge iron within a host, since iron is an essential contributor to metabolic pathways such as respiration and DNA replication and is usually a limited resource (Wandersman *et al*., 1999). Aerobactin is a hydroxamate siderophore, it uses hydroxamic acid to bind the iron (Brock *et al*., 1991). Aerobactin has been linked to enhanced virulence in *K. pneumonia* strains in mice by allowing these strains to sequester more iron from their environment than strains that only produced the siderophore enterochelin (Nassif *et al*., 1986). Yersiniabactin is a phenolate siderophore that shows a higher affinity for free Fe^{3+} ions than aerobactin (Perry, *et al*., 1999). As the name suggests, it was first

described for *Yersinia* species but has since been discovered in other species such as *Escherichia* and *Klebsiella*. It allows *K. pneumonia* to scavenge iron and evade the innate immune protein lipocalin 2 (Len2) which binds with certain siderophores, preventing the bacteria from sequestering the necessary iron.
Salmochelin is a catecholate siderophore that also allows bacteria to scavenge iron ions from the host´s transferrin and lactoferrin (Hantke *et al*., 2002). The name stems from *Salmonella* strains, but, like yersiniabactin, it has also been described in virulent *Klebsiella* spp. and is considered a prominent virulence factor (Struve *et al*., 2015).

1.3 Determination of biofilm forming abilities in *Klebsiella* spp.

Biofilm is the production of a matrix of slime, which consists of a network of potentially many different bacteria that can be produced when the environmental conditions become unfavourable and they need to pull their resources together to survive (Costerton *et al*, 1995). The matrix of the biofilm consists of polysaccharides, proteins and extracellular DNA that together form a protective layer for the bacteria against low O_2 levels, unfavourable pH levels, high CO_2 levels and low water availability. Changes in these chemical and physical factors are some of the helping mechanisms the antibiotics utilise to weaken the bacteria. Thus, biofilm will also protect against some antimicrobial agents.
In the biofilm formation there are three steps; first is adhesion to the surface. Second is growth and maturation of the matrix and the third step is detachment which can be either passive or active (Tronsmo, 2016). Bacteria initiate the active detachment when the environment is suitable, while passive detachment is caused by a physical force such as scraping, or the use of chemical components.
Bacteria are able to communicate with each other by a mechanism called quorum sensing (Miller *et al*, 2001). They can communicate about symbiosis, virulence, competence, conjugation, antibiotic production, motility, sporulation and formation of biofilm. Quorum sensing doesn't just happen between cells of the same bacterial species, it can also happen between different species.

Given the fact that biofilm can occur on most surfaces and the bacteria within the biofilm have the ability to exchange DNA and alter their phenotypes in ways that could lead to antimicrobial resistance, it would be of great interest to further characterize the *Klebsiella* spp. abilities to produce biofilm.

1.4 Antimicrobial resistance in general

The term antimicrobial resistance (AMR) is a wider and currently more used term than antibiotic resistance. This is because antimicrobial resistance covers all the different treatment options against bacteria, virus, fungi and parasites that no longer have the inhibiting or lethal effect on the intended microbe because of changes in the target microorganisms (WHO, 2014). Antibiotic resistance, on the other hand, only relates to resistance in bacteria. AMR has been around for millions of years as a defence mechanism in the battle between different microorganisms for resources or for survival (Tronsmo, 2016). Unfortunately, in the clinical aspect, both animal and human, as well as the food industry has had a history of wrongfully administering antibiotics. This includes using antibiotics for viral infections, poor patient compliance by not taking the medication as prescribed and giving antibiotics to livestock as a precaution or as a growth factor by suppressing other important microorganisms (Levy, 1998; Dibner *et al.*, 2005). These are just a few of the many areas and aspects were antibiotics are not administered for its indented purpose. Should a microbe encounter the antimicrobial agent and survive, this can lead to the development of different immunity mechanisms against the same agent, and this trait will be vertically passed on to the next generation.

In the long run, AMR can become a major threat against diseases that are currently considered as harmless, such as urinary tract infection, mastitis in livestock or pneumoniae in both animals and humans (Neu, 1992). AMR could threaten modern medicine in many, making it difficult to perform organ transplants and cancer therapy (WHO, 2018). All because the pathogenic microorganism now is able to continue colonising, using animals or humans as a resource for nutrition and host to continue spreading in the environment. Given the fact that humans and animals

(both pets and livestock) are increasingly moving between different countries and continents, AMR could be distributed on a global scale (WHO, 2018). AMR puts an increased pressure on the healthcare system since it can increase the duration of hospital stays and the amount of medication needed, which in turn will increase the financial burden on private households and/or the government healthcare provisions (ECDC, 2019). According to the European Centre for Disease Prevention and Control (ECDC) in 2018 there has been an increase in resistance against 3rd generation cephalosporin in Norway over the past decade, and an increase in antimicrobial resistance in general. However, the total usage is still less than for the other countries in Europe (ECDC, 2019).

NORM-VET is part of a nationwide surveillance system overlooking the usage of antimicrobial agents and antimicrobial resistance in animals, feed and food since the year 2000, together with NORM that has focused on the human aspect since 1999 (NORM NORM-VET, 2018). The Norwegian livestock industry has a goal of reducing the consumption of antimicrobial agent in humans by 30%, 20% in pets and 10% in production animals by year 2020 (Landbruks- og matdepartementet, 2018). This makes the NORM NORM-VET screening program an essential surveillance tool. Antimicrobial resistance is screened for in meat, vegetables, production animals and pets. In humans, the samples are collected from urine, blood, respiratory tract and cerebrospinal fluid.

The samples used for this thesis are collected through NORM-VET. A guideline for both sample collection and geographical distribution of samplings are key factors to obtain the most accurate and representative data.

1.4.1 Classification of antimicrobial agents and their mode of action

The different types of antimicrobial compounds are often categorized by how they target the microorganism (Kohanski *et al*., 2010). β-lactams and glycopeptides interfere with the cell-wall synthesis, tetracyclines inhibits the protein synthesis, floroquinolones interfere with the synthesis of nucleic acids and trimethoprim-sulfamethoxazole inhibit metabolic pathways. In general, antimicrobial agent can

either have a bactericidal effect, in which case it causes the bacteria to die, or bacteriostatic effect which inhibits cell growth (Tronsmo, 2016).
In this thesis, the ATC-vet categorization is used since this is an international system.

1.4.2 β-lactams

Beta (β)-lactam agents are cephalosporin, carbapenem and penicillin which all contain a β-lactam ring that inhibits the synthesis of peptidoglycan, preventing formation of the cell wall. They do this by covalently binding to the active site of penicillin-binding proteins (Kohanski *et al*., 2010). Cephalosporins have several generations, where 1st generation is most effective against gram-positive bacteria, 2nd and 3rd generation are effective against the gram-negative bacteria that are resistant against the 1st generation cephalosporin, while 4th generation are zwitterions that can penetrate the outer membrane of gram-negative bacteria. Carbapenem is a broad-spectrum antimicrobial agent against gram-negative bacteria. Carbapenem is used when severe infections are suspected or when multi-resistant bacteria are found. Carbapenem resistance is notifiable in Norway due to the severe problem such resistance would entail.

1.4.3 Tetracyclines

Tetracycline disrupts the binding of tRNA to 30S so that the incoming amino acid can´t be elongated to the polypeptide chain (Chopra *et al*., 2001). Tigecycline has a broad-spectrum and is considered 20 times more effective than tetracycline. Both are affective against both gram-positive and negative bacteria, mycoplasmas, and protozoans.

1.4.4 Sulfonamides and trimethoprim

Sulfamethoxazole blocks the synthesis of FAH_2. Trimethoprim inhibits microbial reductases, and it has been shown to have a better effect when combined with sulfamethoxazole, giving a broad-spectrum antimicrobial effect (Kohanski *et al*., 2010).

1.4.5 Quinolones

Nalidixic acid, which is a 1st generation agent and ciprofloxacin, a 2nd generation are both quinolones (Kohanski *et al*., 2010). Quinolones enter the cell through the porins and target topoisomerase and DNA gyrase which prevents the DNA from unwinding and being able to replicate. Floroquinolones are considered a broad-spectrum antimicrobial agent and are used as a last resort or for treatment of infections with multi-resistant microbes. Most quinolones are floroquinolones, which contain a fluorine atom which makes the quinolones effective against both gram- positive and negative bacteria.

1.4.6 Amphenicols

Chloramphenicol is a broad-spectrum antimicrobial agent and works by binding to the 50S- subunit to inhibit translation, thereby inhibiting protein synthesis (Kohanski *et al*., 2010). Since chloramphenicol gives severe side effects, this compound is not used for animals, and only as a last resort for humans.

1.4.7 Aminoglycosides

Aminoglycosides change the structure of the 30S subunit so that mRNA is misread, which inhibits protein synthesis (Kohanski *et al*., 2010). Streptomycin is one of the many agents belonging to this group.

1.4.8 Analysing susceptibility testing

When analysing results obtained by susceptibility tests there are two different ways for categorizing resistant and susceptible phenotypes for antimicrobial agents (EUCAST); epidemiological cut-off and clinical breakpoint. Epidemiological cut-off value is based on the distribution results of hundreds of minimal inhibitory concentration (MIC) samples, giving a pattern of sensitivity and resistance for each species. Clinical breakpoint is based on the recommended dosage given to the patient where the concentration is high enough to have a bactericidal or bacteriostatic effect on the microbe, but not so high as to be a harmful/lethal dosage

for the patient. There is usually a gap between the epidemiological and clinical breakpoints for each antimicrobial agent.

In addition, the possibility of multi drug resistance (MDR), extensively drug resistance (XDR) and pan-drug resistance (PDR) must be considered while interpreting the susceptibility results (Magiorakos *et al*., 2012). MDR is defined as being resistant to three or more antimicrobial groups. WDR are resistant to all antimicrobial groups except two or fewer, and PDR is resistant to all antimicrobial groups.

1.4.9 Antimicrobial resistance mechanisms by the bacteria

The development of antimicrobial resistance is an old and well-established trait amongst certain bacteria (Tronsmo, 2016). These mechanisms are divided into different groups based on how the bacteria combats the antimicrobial agent (figure 4).

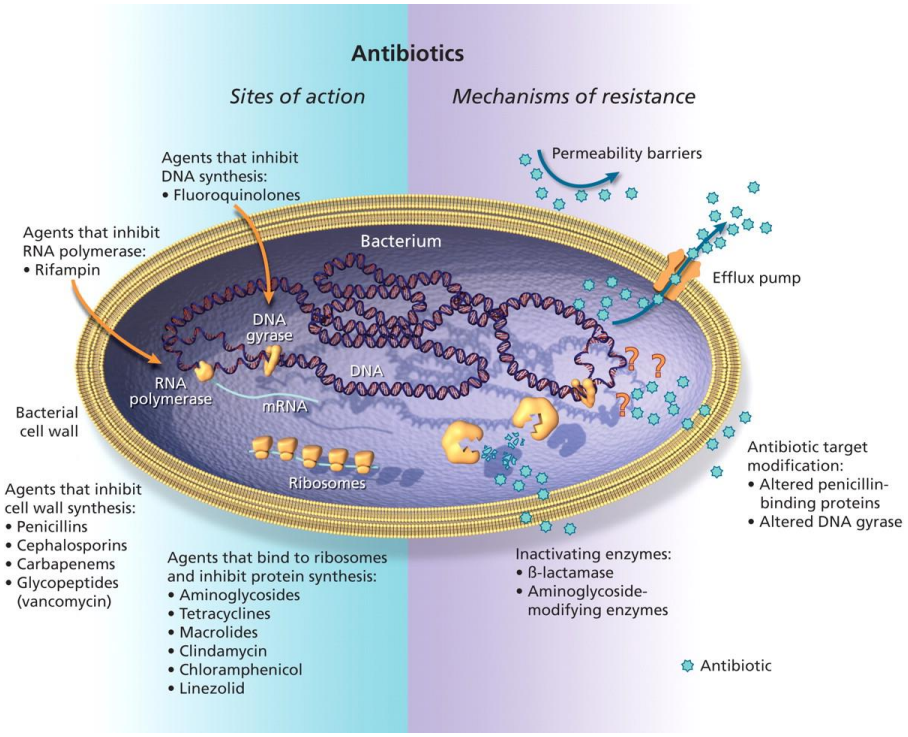

Figure 4. The bacterial mechanisms for antimicrobial resistance and the mode of action by the antimicrobial agents. (Illustration from Alamy).

Group 1: The bacteria can produce enzymes such as penicillinase that changes the antibiotic into an inactive form (Wyres, 2018). *Kp1* has SHV-1 penicillinase in its chromosome, making them resistant against ampicillin. Group 2: Efflux pumps can be used to pump out the antimicrobial agent from the cell, hindering it from reaching high enough concentrations to be lethal or damaging for the bacteria (Tronsmo, 2016). Usually the genes that code for multi-resistant efflux pumps are on the chromosome, but some can be moved to the plasmid. This means that this trait can potentially be transported between bacteria. Group 3: The porins in the cell membrane serve as an access point for antimicrobial agents, so by altering the porin, the bacteria can restrict the agent's entry way. In addition, Gram-negative bacteria are less permeable to antibiotics because of their double membrane. Group 4: The last mechanism for antimicrobial resistance entails changing the metabolic pathway, but without the use of antibiotic blockers.

1.5 The aims of this master thesis

The aim of this thesis was to obtain more knowledge about *Klebsiella* spp. from different animal reservoirs. This was done by studies on *Klebsiella* spp. detection methods, in addition to phenotypic and genotypic AMR profiling, and virulence, biofilm and population structure analysis of obtained *Klebsiella* isolates.

2. MATERIAL & METHODS

2.1 Isolation of *Klebsiella* spp. from domestic animals

2.1.1 Sampling and bacterial cultivation

The *Klebsiella* spp. isolates used for this thesis where collected from January 2018 until August 2019 from the NORM-VET screening program, and as part of a collaborative project (REDUCEAMU) in Thailand. All broiler and turkey (poultry) samples where isolated from January 2018 until December 2018 by staff at NVI in Oslo. From January 2019 until mid-October 2019 *Klebsiella* spp. from swine, dog and cattle was collected by the student of this thesis. The isolation of *Klebsiella* spp. strains in Thailand was done by Thongpan Leangapichart from September to December 2018. The samples collected at NVI from broiler, turkey, swine and cattle were all faecal samples taken from the appendix of healthy animals. From dogs, the samples were collected using rectal swabs. The samples were enriched overnight in peptone water (Difco) and incubated at 37±1.0°C. Then being spread with one blue loop (10µl) onto Simmons citrate agar with 1% inositol (SCAI) (Oxoid), and incubated at 37±1.0°C for 48 hours. The samples from dogs and Thailand were collected as rectal swabs from healthy swine and enriched in peptone water over night before being plated on SCAI agar and incubated at 37±1.0°C for 48 hours. Colonies suspected to be *Klebsiella* spp. were sub-cultured to blood agar (Oxoid) and made into freezing stocks with glycerol, stored at -80°C.

The majority of *Klebsiella* spp. has the ability to ferment inositol that gives yellow colonies on the agar, and Simmons citrate agar only allows bacterium that can utilize citrate as a carbon source to grow (Podshun *et al.*, 1998). Using a combination agar with both inositol and citrate will give selectivity towards *Klebsiella* spp. After incubation, suspected *Klebsiella* colonies were sub-cultured to blood agar and further incubated for 24 hours at 37±1.0°C before the Matrix Assisted Laser Desorption Time of Flight Mass Spectrometry (MALDI-TOF) was used to confirm that the pure culture was *Klebsiella* spp. Pure cultures were made into freezing stocks containing glycerol, stored at -80°C. The isolates collected via NORM-

VET and from Thailand were species identified as *Klebsiella pneumoniae* complex by MALDI-TOF prior to freezing.

2.1.2 Bacterial species identification

The MALDI-TOF method for bacterial species determination is based on a laser that dissolves the matrix and ionizes larger molecules, so that charged particles are formed and registered according to size (Jurinke *et al*., 2004). The molecule mass of the free ions is detected in a vacuum chamber where the mass and charge are measured. There is a correlation between molecule size and speed, where the small ions are registered faster than the large. The ribosomal protein pattern in the sample is compared to a database over bacteria with already established protein patterns. The score values obtained range from 0 (no similarity) up to 1000 (absolute similarity) between the samples. The score value is presented as log(score value) that gives a result ranging from 0-3. If the result is 0.00-1.69 this indicates "no organism identification possible", 1-70-1.99 indicates "low-confidence" and 2.00-3.00 "high-confidence". The ten most accurate results are given, and the two first should both match on species level and be above 2.0 to be sure that the results are correct and reliable. To get the most accurate result, the suspected *Klebsiella* colony was sub-cultured from the SCAI to blood agar to obtain a pure culture and after 24-hour incubation at 37±1.0°C, a small amount from the fresh colony was placed on the MALDI-TOF steel plate and fixated with 1µl matrix solution before being placed in the machine for analysis.

2.1.3 Culturing methods for isolation of *Klebsiella* spp.

In order to obtain an indication of which method would give the highest *Klebsiella* spp. isolation rate, three different methods for isolation were used. The direct method was based on directly streaking out faecal matter on SCAI by using a blue loop (10µl) and incubating for 48 hours at 37±1.0°C.

The two other methods were based on an enrichment stage prior to isolating the *Klebsiella spp*. on SCAI. The first method was regular enrichment in peptone water, where 1g of faeces was placed in 9ml peptone water and incubated at 37±1.0°C for

24 hours, then 10µl was sub-cultured on SCAI agar, followed by incubation for 48 hours at 37±1.0°C. For samples from dogs, the swab were suspended in the peptone water over night at 37±1.0°C for enrichment. Making the dog samples unusable for testing in LB broth containing 10mg/l amoxicillin.

The second enrichment method used LB broth containing 10mg/l amoxicillin (Merck KGaA) and 1g of faeces in 9ml peptone water, and then followed the same incubation and sub-culture procedure as the peptone water enrichment. Amoxicillin (Sigma) was chosen because it belongs to the antibiotic group penicillins and *Klebsiella* spp. has a natural resistance against this substance (Haeggman *et al.*, 2014).

To evaluate if there was a statistically significant difference between the detection methods the 95% confidence interval (CI) was calculated. By using the Hmisc package in R-studio. The command used was: binconf(x,n) were x is the number of positive samples and n is the total sample number in the category tested.

2.1.4 Polymerase chain reaction (PCR) for identification of *Klebsiella* spp.

To see if PCR could be a possible method for detection of *Klebsiella* spp. DNA was extracted from *K. pneumoniae* isolates from swine samples from Thailand. The DNA extraction and PCR were performed in collaboration with Thongpan Leangapichart postdoc project (REDUCEAMU). The high sensitivity DNA extraction kit QlAamp DNA mini kit (Thermo Fisher Scientific) was used for DNA extraction. The start concentration for the first isolates was 39.3 ng/µl and the second isolate had a DNA concentration of 24.3 ng/µl. Both DNA solutions were diluted to 1:10 and 1:100, giving a total of six DNA solutions to test with the PCR. Two pure *K. pneumoniae* cultures (one broiler, one turkey) were tested with the boil lysis method. A bacterial solution of 0.5 McFarland was boiled at 95°C for 15 minutes, then diluted eight times. Also eleven faecal samples from swine were tested with this method. But prior to boiling 1g of faecal matter was enriched in 9ml peptone water over night at 37±1.0°C. After boiling 500µl of the enrichment was centrifuged for five minutes at 5800g. The supernatant was discarded and the pellet re-suspended using 500µl SDV. The wash step was repeated twice before boiling. PCR is a copying method

used, in this case, to amplify a specific gene, hemolysine. By using fluctuating temperatures, it allows the DNA to denaturise, so that the primers can bind to the DNA. This allows the DNA polymerase to attach nucleotides for the elongation of a new DNA strand. The master mix contained 10µl SYBER GREEN, 4µl water, 0.5µl Forward primer and 0.5µl Reverse primer. To each sample 5µl of extracted DNA, giving a total volume of 20µl. Each isolate had a control, were DNA was not added to the wells.

The primers used were:

Forward: 5' CTAAAACCGCCATGTCCGATTTAA 3'

Revers: 5' TTCCGAAAATGAGACACTTCAGA 3'

The following PCR cycle was used:

95°C for 15 min

94°C for 15 sec

60°C for 1 min

72°C for 30 sec.

This cycle beginning at 94°C was repeated 45 times.

2.2 Antimicrobial susceptibility test (AST) by disc diffusion

Before the susceptibility testing began, a pre-test was conducted on five of the broiler isolates to get an approximate size of the inhibition zones and to see if there were any interactions between the antibiotic discs. Based on these results, and the EUCAST inhibition zone guidelines, 17 antibiotics were selected and divided onto three 90 mm Mueller Hinton (MH) agar plates (Thermo Fisher Scientific) for each isolate (figure 5). In addition, the EUCAST guidelines stated that there could be a maximum of six different antimicrobial discs on one agar plate.

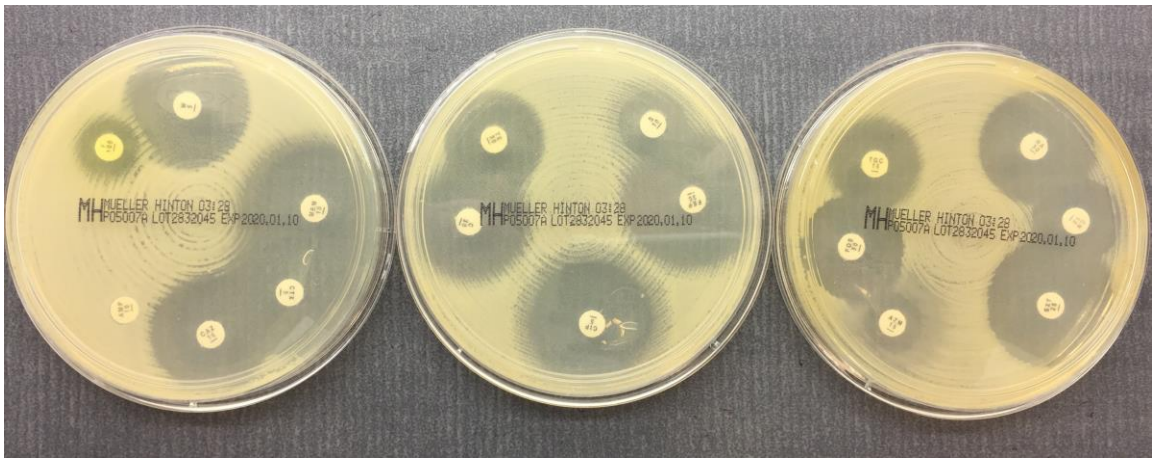

Figure 5. Left plate contained the following discs: nitrofurantoin 100µg, trimethoprim 5µg, meropenem 10µg, cefotaxime 5µg, ceftazidime 10µg and ampicillin 10µg.
Middle plate contained the following discs: streptomycin 10µg, ciprofloxacin 5µg, chloramphenicol 30µg, cefepime 30µg and tetracycline 30µg.
Right plate contained the following discs: azithromycin 15µg, fosfomycin 50µg, tigecycline 15µg, nalidixic acid 30µg, gentamicin 10µg and trimethoprim-sulfamethoxazole 25µg.

For the antibiotic susceptibility test the standardised method from EUCAST was utilized (EUCAST protocol).
The first step was to sub-culture *Klebsiella* spp. from the frozen stock on to blood agar plates, and incubate for 24 hours at 37±1.0°C. After incubation, one colony was picked to run through the MALDI-TOF to re-confirm that the isolate was a *Klebsiella* spp. before continuing the procedure. Further, when making the bacterial suspension to spread on the MH agar, the concentration had to be 0.5 McFarland (1-2x10^8 CFU/ml) in 0.85% distilled salt-water (Merc KGaA, Darmstadt), and the bacteria was selected from several colonies.
MH agar is used in routine susceptibility testing because of its combination of beef extract, acid hydrolysate of casein, starch, agar and distilled water, and also because most microorganisms can grow on MH agar (Aryal *et al*., 2018). Beef and acid hydrolysate provide nitrogen, vitamins, carbon, amino acids, sulphur and other

essential nutrients. Starch absorbs toxic metabolites produced by the bacteria so they will not interfere with the antimicrobial agent being tested, and hydrolysis of dextrose is the energy source. In addition, MH agar allows for good diffusion of the antibiotics, which gave a good representation of the actual inhibition zone. According to the EUCAST procedure, were MH agar is used, the bacterial suspension had to be spread on to MH agar no more than 15 minutes post-production. Working with gram-negative bacteria, which usually have a high density when collecting from the bacterial suspension with a cotton swab, excess liquid had to be removed before placing the swab on the agar. It is important to get an even layer of bacteria, corresponding to a confluent growth, as both overgrowth and lack of growth can affect the size of the inhibition zones and give false results. Finally, after placing the antibiotic discs, again within 15 minutes of spreading the bacterial suspension on the agar surface, the plates were incubated at 37±1.0°C for 18±2 hours. Incubation must also happen within 15 minutes of placing the antimicrobial discs on the agar. Following incubation, the diameter of the complete clear zone was measured in millimetres. Depending on the combination of bacterial species and antibiotic disc used, EUCAST had different criteria for classification of resistance versus susceptible. The epidemiological cut off values used for this thesis and can be found in the appendix. If the bacteria were susceptible to the exposed antibiotic, that would mean that a therapeutic dose of the antibiotic would likely be sufficient to kill the bacteria. Intermediate needs to increase exposure time and possibly concentration above the therapeutic index to give an inhibitory response (Willey *et al*., 2014). If there was no inhibition zone, the bacteria were resistant to the antibiotic and all doses were ineffective.

2.3 Antimicrobial susceptibility testing by determination of minimal inhibitory concentrations

The minimal inhibitory concentration (MIC) for all the isolates that were resistant against additional antimicrobial agents other than ampicillin were determined by a broth micro dilution method. The relevant isolates were sub-cultured to a new

blood agar plate and incubated at 37±1.0°C for 24 hours, to obtain fresh colonies. Making the bacterial suspension for the MIC test was a two-step process. First, the fresh bacteria were diluted in sterilized distilled water (Merc KGaA, Darmstadt) to a concentration of 0.5 McFarland, then two blue loops (10µl) of the bacterial suspension was inoculated in a 5ml MH broth (by Thermo Fisher Scientific). From the inoculated MH broth, 50µl was dispensed into each well of the preloaded antibiotic Sensititre™ Gram negative MIC plate by using the Sensititre™ automated inoculation delivery system (figure 6). The antimicrobial agents included on the MIC plates were; ampicillin, azithromycin, ceftazidime, cefotaxime, chloramphenicol, ciprofloxacin, colistin, gentamicin, meropenem, nalidixic acid, sulfamethoxazole, tetracycline, tigecycline and trimethoprim.

The MIC plates were sealed with a plastic sheet and placed for incubation for 18 hours at 37±1.0°C before the results were registered.

In addition, as a control to make sure that the inoculated MH broth was free from contaminations, every sample was sub-cultured onto blood agar incubated for 18 hours at 37±1.0°C.

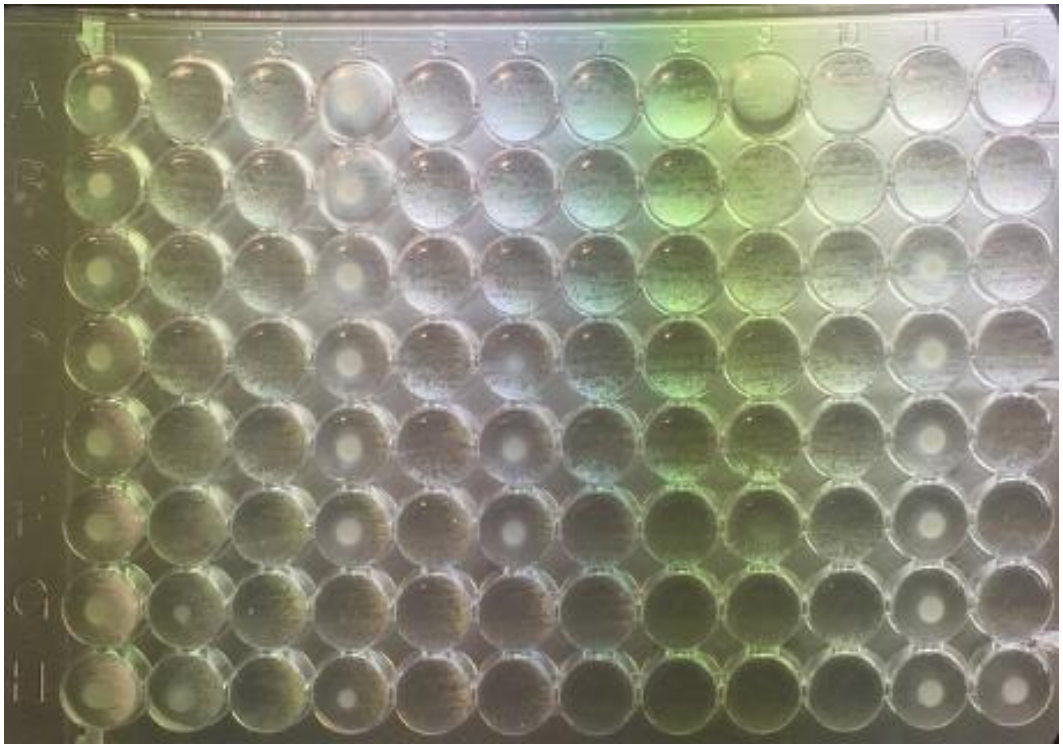

Figure 6. Preloaded antibiotic Sensititre™ Gram negative MIC plate, with bacterial growth expressing resistance against sulfamethoxazole, tetracycline and ampicillin.

As a control ATCC *E. coli* 25922 was used during every run of the disc diffusion and MIC test. In addition, a previously characterized, through WGS, multi resistant *K. pneumoniae* was also used as a control. The use of these controls was to be able to monitor that the procedure had been performed correctly, to control that the antibiotic discs worked properly, and as an indicator that the measured results were credible. Both control isolates followed all the same steps as the test isolates during the whole trial period.

2.4 Biofilm production

Testing for the biofilm forming ability was conducted by following a method described previously (Stepanovic´ *et.al*, 2007). Testing the biofilm forming ability was a five-day process from start to finish. One small loop (1µl) of bacteria, picked from a fresh culture grown on blood agar overnight, was inoculated in 5 ml LB broth (Merc KGaA, Darmstadt) overnight at 37±1.0°C to further develop the bacterial growth to an OD_{595} of approximately 1.0.

The following day, 100µl of LB broth without NaCl and 30µl of the overnight culture was manually added using an automatic multipipette (Thermo Fisher Scientific) to each well in a 96-well Nunc™ Nunclon™ –plate (by Nunc A/S) with lid and flat bottom. The purpose of removing the NaCl from the medium was to stress the bacteria into producing biofilm. The 96-well Nunc™ Nunclon™ -plates were used because they gave the best surface for *Klebsiella* spp. to attached to during biofilm production. The flat bottom was needed so that the OD_{595} could be read correctly by the spectrophotometer. Each isolate was tested as a triplet, meaning that on one 96-well Nunc™ Nunclon™ –plate, 28 isolates could be tested. The entire first row was used as a control that only contained 130µl of LB broth without NaCl. The blank control was used to check for contaminations in the LB broth and to adjust for background noise and any underlying faults from the spectrophotometer. Since biofilm is often produced on surfaces where the temperature is lower than the core temperature of humans, birds or mammals, the isolates were incubated at an average room temperature of 20.0 ±1.0°C for 48 hours.

After the incubation period the bacterial growth was measured using a spectrophotometer at OD_{595} before continuing with cleaning, staining and dissolving the biofilm. Measuring the growth prior to cleaning and staining the potential biofilm was another control point in the procedure. This was to make sure that there were bacteria present in the wells. In case some of the strains did not produce biofilm, the lack of bacterial growth could then be excluded as a reason.

Cleaning of the wells began by emptying the plate by turning it quickly and tapping it firmly onto paper towels until all the excess LB broth was removed. Then, 200µl of tap water was added to each well, followed by emptying the wells again, and this

was repeated twice to make sure only the biofilm was left in the well. To stain the biofilm, 140 µl 1% crystal-violet (Sigma-Aldrich) was added to each well and left at room temperature for 30 minutes in the laminar flow bench. When rinsing off the crystal-violet, the same procedure as for removing the LB broth with 200 µl of tap water was used, only this time it was preformed three times since the colour is harder to remove than the broth. The final step in the procedure was to dissolve the biofilm from the walls of the wells by using 140 µl etanol:aceton (70:30) mixture (Prolab) and after 10 minutes incubation at room temperature, the OD_{595} was measured to gauge biofilm production.

2.5 DNA extraction, purification and sequencing

DNA extraction, purification, library prep and sequencing were performed in collaboration with the microbiology section at the University hospital in Stavanger.

The MagNa Pure 96 machine (Roche) and Hamilton NGS Star (Roche) were used for DNA extraction, purification and library prep prior to whole genome sequencing (WGS) using the Illumina Miseq (Illumina). The method explained below was conducted by the mentioned machines, but the procedure is still explained in detail to get the overall understanding of what happened. The kit used for DNA extraction and purification was Quant-iT™ 1x dsDNA HS assay (Thermo Fisher Scientific).

2.5.1 DNA extraction

Fresh colonies by sub-culturing to a new blood agar plate were obtained by overnight incubation at 37±1.0°C. The following day, one small loop (1 µl) of bacteria was inoculated in 500 µl of saltwater and vortexed to a homolog solution. 200 µl of the bacterial solution were transferred to MagNA Pure 96 Processing Cartridge and placed in the MagNA Pure 96 machine.
The bacterial cells were lysed both enzymatically and mechanically (bead beating) to access the DNA. This process took approximately two hours.

2.5.2 Measurement of DNA concentration with Qbit

The Qbit measured the absorbance at $OD_{260/280}$ in each DNA sample, and if the DNA sample was pure, the OD should be between 1.8-2.0. If the results were higher than 2.0 it could indicate sample contamination.

2.5.3 Library prep by using Hamilton NGS Star

This step fractioned the DNA into many different sizes by using beads with sockets. When all the beads were filled, the remaining DNA that was not attached to a bead got washed away. Since the concentration of beads and DNA were supposed to match, there should be an even distribution or representation of DNA in the pooled end sample.

First, DNA was added to nuclease-free water until the concentration was between 100-500 ng, and then checked to make sure each well had a total volume of 30 µl. Then the tagmentation mix consisting of 10 µl Bead-linked transposomes (BLT), that are used to fragment and tag DNA sequence with adapter sequence, and 10µl Tagmentation buffer 1 (TB1) was added to each sample before placing the plate on a TAG thermal cycle, beginning at 55°C, for 15 minutes. During the thermal cycle the DNA should bind to the BLT. 10 µl of Tagment stop buffer (TSB) was added to the tagmentation solution, then a new thermal cycle at 37°C ran for 15 minutes, before the temperature was reduced to 10°C. The 96-well plate was then placed on a magnetic stand and removed after three minutes, and the supernatant was discarded.

Next was a clean-up step, were the 96-well plate was removed from the magnetic stand and 100 µL of Tagment wash buffer (TWB) was added to each well and the beads were gently resuspended in the solution. That washed the adapter tagged DNA on the BLT prior to PCR. When placing the plate back on the magnetic stand, three minutes were allowed to pass before removing and discarding the supernatant. The wash was then repeated once more. If needed, 100 µl TWB was added to prevent the beads from drying out until further in the procedure.

The next step was PCR, to amplify the DNA fragments of the whole genome prior to sequencing. The Nextera dual index adapter plate contained 96 different indices that attached to each strain so that after pooling the samples and sequencing them, it would be possible to identify which fragments belonged to which isolate.
The master mix for the PCR contained 20 µl Enhanced PCR mix and 20 µl Nuclease free water per DNA sample. Placing the 96-well plate back on the magnetic stand and remove and discard the TWB. After the plate was removed from the magnet and 40µl of the master mix was added to each well to resuspend the beads. Prior to starting the PCR cycle, 10 µl index adapter was added to each well and then the following PCR cycle was run:
68°C for 3 min
98°C for 3 min
98°C for 45 sec -> 62°C for 30 sec -> 68°C for 2 min (the number of cycles depends on DNA input ng)
68°C for 1 min
10°C ∞

The PCR plate was placed on a magnetic stand and 45 µl of the supernatant was transferred to a new plate. 40 µl nuclease free water and 45 µl Sample purification beads (SPB) were added to each well containing the supernatant, then mixed and incubated at room temperature for five minutes. To extract 125 µl of the supernatant, the plate was placed on the magnetic stand and the supernatant was transferred to a new plate with 15 µl SPB and mixed and incubated again at room temperature for five minutes. Placing the plate back on the magnet and removing and discarding the supernatant, the beads were washed with 80% EtOH, without mixing, and incubated for 30 seconds before removing and discarding the supernatant. The wash step was repeated again, and the plate was air dried for five minutes. The plate was removed from the magnet and the beads resuspended by adding 32µl Resuspension buffer (RSB) and incubated for two minutes. The plate was then placed back on the magnet and 30µl of the supernatant was transferred to a new 96 well PCR plate.

Next, the library was pooled by adding five µl of each sample to the same microcentrifuge tube before being vortexed and then centrifuged. To dilute and denaturation the library, the following calculation was used:
(ng /µl x $10^{6)}$ / (660g/mol / average library size (bp)) = Molarity (nM)
RBS is added to get the right concentration, 1.2-1.3 pM.

For the dilution a 5 µl of 0.2 N NaOH and 5 µl 4 nM library were centrifuged and incubated at room temperature for five minutes before adding 990µl of HT1 to further dilute the denaturized DNA to 12pM. PhiX is added as a control.

2.5.4 Sequencing with Illumina MiSeq

The mechanism of Illumina sequencing is based on a process called bridge amplification that occurs on a flow cell that is covered with two types of oligonucleotides (Illumina protocol). The single stranded sequences from the library prep had complementary adapters attached so that the sequence could bind to the flow cell (figure 7). DNA polymerase catalysed the incorporation of deoxyribonucleotide triphosphate (dNTP) during bridge amplification to create clusters of similar sequences. The bridge is created when the opposite end of the sequence binds to another complementary oligo nucleotide attached to the flow cell. After the clustering, the strands were separated, and sequencing could begin. In the added solution were four fluorescent labelled nucleotides (red, yellow, green and blue) and for each cycle, one new dNTP is added to the growing nucleic acid chain, and the fluorescence increases upon binding. After the new dNTP is attached the fluorescence is registered and the fluorescence label is removed to give access to the attachment of the next dNTP.
When the sequencing is done, the reads are ready for quality control and assembly.

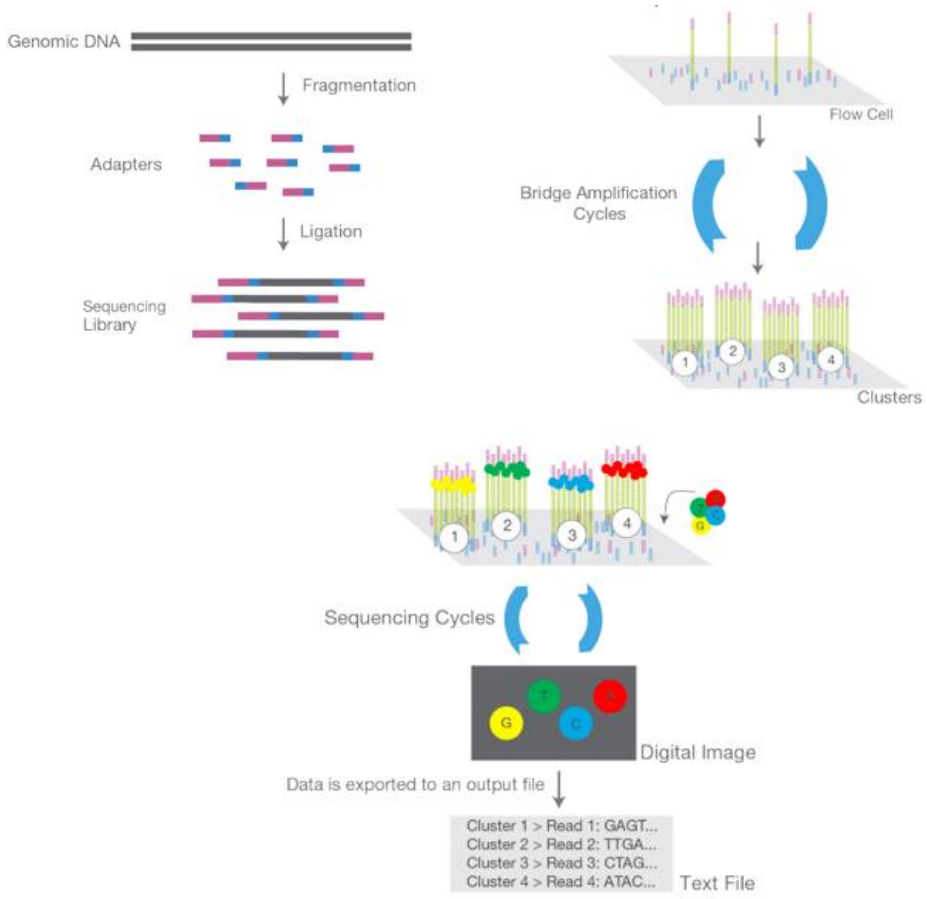

Figure 7. Illustrations are from the Illumina handbook, "An introduction to next-generation sequencing technology", showing the workflow of sequencing.

2.6 Draft genome assembly and analysis

After the WGS was completed, the quality of the reads was controlled, contigs assembled, quality control and Kleborate was performed at the microbiology section at the university hospital in Stavanger by Marit Hetland (figure 8).

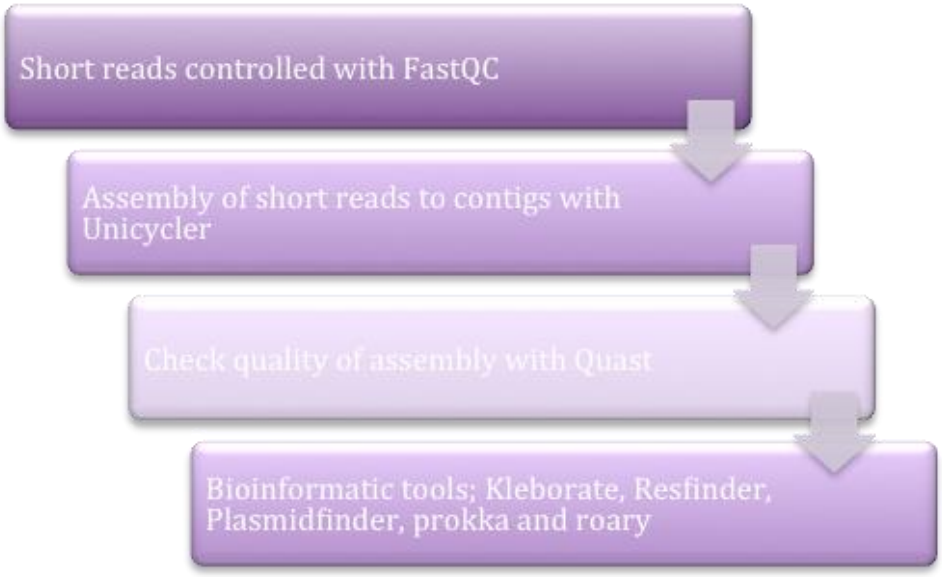

Figure 8. Flowchart of bioinformatic tool used from raw WGS to finished results.

2.6.1FastQC

Fast QC is a tool that performs quality control on the raw sequence data (Andrews, 2018). For example, it displays the quality per base, GC content, per base N content, sequence adaptor and sequence duplication.

2.6.2 Unicycler

Unicycler is an assembly tool that takes short Illumina reads and long nanopore or PacBio reads, and assembles them into larger gene sequences, called contigs (Wick *et al*., 2017). Contigs were created by aligning overlapping reads. Contigs were further assembled into scaffolds. Unicycler software package includes both SPADEs and pilon, which can generate error free assemblies.

2.6.3 Quast

Quast tool is used for comparing genome assemblies. The function is to estimate sequencing coverage of draft assemblies by mapping against reference genome (Gurevich *et al*., 2013).

2.6.4 Kleborate

Kleborate was used to analyse and categorize the *Klebsiella* species, sequence type, identify resistance and virulence genes and capsule types (Katholt, 2019). Kleborate uses the Mash database by NCBI to compare and find the best match among the different *Klebsiella* species. The Kleborate also screens for resistance genes such as *Bla* (beta-lactamases) and *Fcyn* (fosfomycin). But also, for a broad panel of antimicrobial resistance genes such as *AGly* (aminoglycosides), *Bla_broad* (broad spectrum beta-lactamases), *Bla_broad_inhR* (broad spectrum beta-lactamases with resistance to beta-lactamase inhibitors), *Bla_Carb* (carbapenemase), *Bla_ESB*L (extended spectrum beta-lactamases), *Bla_ESBL_inhR* (extended spectrum beta-lactamases with resistance to beta-lactamase inhibitors), *Flq* (fluoroquinolones), *Gly* (glycopeptides), MLS (macrolides), Ntmdz (nitroimidazole), *Phe* (phenicols), *Rif* (rifampin), *Sul* (sulfonamides), *Tet* (tetracyclines) and *Tmt* (trimethoprim). Kleborate utilises curated ARG-ANNOT database to detect possible AMR genes from draft assemblies.

When screening for virulence genes, there were four genes that were screened for; the siderophores yersiniabactin (*ybt*), aerobactin (*iuc*) and salmochelin (*iro*), and the genotoxin colibactin (*clb*).

In addition, genes for hyper mucoviscous phenotype (*rmpA, rmpA2*) that increase/change the capsule structure were also screened for.

Kleborate also contains the Kaptive tool that identifies the different O loci encoded by the polysaccharide capsule and lipopolysaccharide O.

2.6.5 ResFinder

ResFinder is a tool that identifies acquired antimicrobial resistance genes from draft assembly (Zankari *et al*., 2012). The National Centre for Biotechnology Information (NCBI) was used as a reference database to identify the antimicrobial resistance genes. When submitting a sequence to the ResFinder, the genes are compared with the curated ResFinder database by using basic local alignment search tool (BLAST). For ResFinder to acknowledge the presence of a resistance gene, at least 2/5 of the length of the gene must be present in the query sequence. By default, ResFinder

screens for the following antimicrobial resistance genes; aminoglycosides, beta lactam, colistin, fluoroquinoloes, fosfomycin, fusidic acid, glycopeptide, MLS (macrolides, lincosamide and stretogramin B), nitroimidazole, oxazolidinone, phenicol, rifampicin, sulphonamide, tetracycline and trimethoprim. The ID threshold is by default set at 90%, and a minimum of 60% length of the gene.

2.6.6 PlasmidFinder

PlasmidFinder is a web-based tool that is used to identify specific sequences associated with particular plasmid replicons (Carattoli *et al*., 2014). NCBIs BLAST function is used for comparing the query sequence to the nucleotide database. The results are based on similarity given as % ID. The minimum is 60% similarity of the length of the replicon with the NCBI database to identify the correct plasmid. The results also backtrack the origin of the Inc group and possible origins.

2.6.7 MOB-suite

The scripts written to use in MOB-suite, Prokka and Roary were constructed by supervisor Amar A. Telke at NVI. MOB-suite is a software tool used for clustering, reconstruction and typing of plasmids from draft assemblies (Robertson *et al*., 2018).

2.6.8 Prokka

Prokka is an online tool used to annotate genomes from draft assembly after sequencing (Seemann, 2014). The Prokka database uses the NCBI data base to compare with preidentified proteins and their functions, in assistance with Uniprot. When supplying the query genome, a comparison genome is also applied, in this case *K. pneumoniae* from NCBI. The annotation identifies coding sequences, ribosomal RNA, non-coding RNA, etc. and provides the coordinates and labels for the genes.

2.6.9 Roary

Roary is a pan genome analysis tool, that uses annotated genomes in GFF3 format (Page., *et al*, 2015). Roary works by converting coding sequences into protein sequences and clustering these protein sequences using several methods. Further, Roary refines the clusters into orthologous genes for each sample and determines if a gene is present or absent. It also produces core gene alignment, using the gene present or absent information to build a tree, using FastTree. Lastly, it produces core gene alignment overall and calculates the number of genes that are shared and that are unique.

2.6.10 Interactive Tree Of Live – ITOL

To visualise the clustering and core gene alignment previously produced in Roary, ITOL is a creative and flexible web-based tool to display the phylogenetic tree (Letunic, *et al*., 2007). For this thesis, the function used was to show clustering of ST, colour code the species origin (turkey or broiler), and mark the isolates encoding AMR genes and virulence genes.

3.RESULTS

3.1 Isolation of Klebsiella spp. from animal samples

3.1.1 Samples from poultry, swine, dog and cattle

A total of 1290 faecal samples from poultry, swine, dog and cattle were screened to identify *Klebsiella* spp. From these samples, 380 (29.46%) were identified positive for *Klebsiella* spp. with 94.21% belonging to the *Klebsiella pneumoniae* complex, 5.0% identified as *K. oxytoca* and 0.79% identified as *K. aerogenes* (Figure 9). These results indicate that *K. pneumoniae* was most frequently detected in the samples.

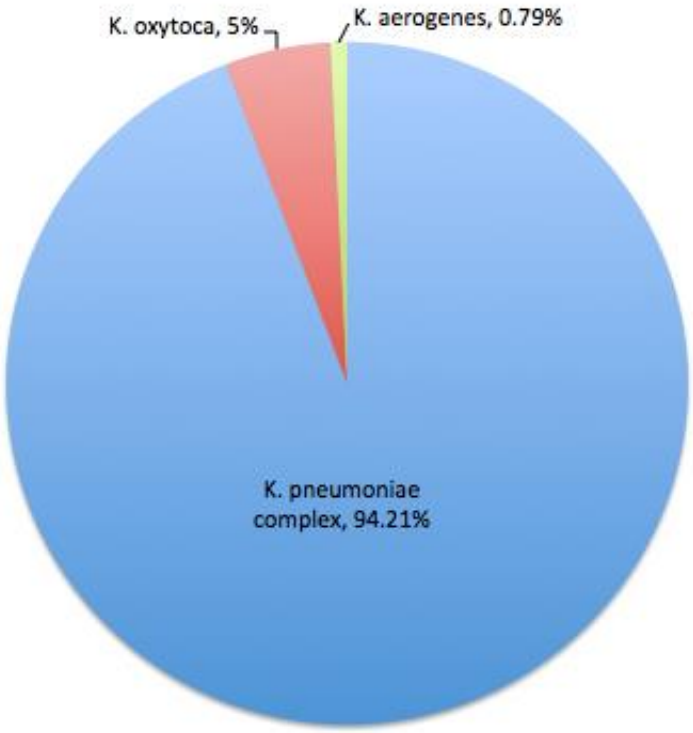

Figure 9. Distribution of *Klebsiella* spp. isolated from Norwegian animal samples in 2018 and 2019.

Further the isolation rate for *Klebsiella* spp. in each animal species was identified. A total of 565 faecal samples from poultry were screened (2018), with the distribution being 404 (71.50%) broiler and 161 turkey (28.50%) (Table 2). Out of the 565 poultry samples, 207 were culture positive for *Klebsiella* spp., which gave an overall detection rate of 36.64%. Broiler had an overall detection rate of 23.51% and turkey 69.57%.

Faecal samples from 725 swine, dogs and cattle were screened (2019) to isolate *Klebsiella* spp. The sample distribution was as follows; 251 (34.62%) swine, 189 (26.07%) dogs and 285 (31.03%) cattle. From swine 130 (51.79%) isolates were *Klebsiella* spp. culture positive, 30 (15.87%) from dogs and 13 (4.56%) from cattle.

Table 2. The sample size and *Klebsiella* spp. detection rate in different animal reservoars

	Turkey	Broiler	Swine	Dog	Cattle
Total samples	161	404	251	189	285
Klebsiella spp.	112	95	130	30	13
Detection rate	(69.57%)	(23.51%)	(51.79%)	(15.87%)	(4.56%)
[95% CI]	[62-77%]	[19-28%]	[45-58%]	[11-22%]	[2-7%]
K.pneumoniae	112	91	106	20	9
K.variicola	0	0	16	2	2
K.oxytoca	0	4	6	7	2
K.aerogenes	0	0	2	1	0

3.1.2 Klebsiella spp. isolated from swine, dog and cattle using three different methods

Over a five-month trial period, 343 faecal samples collected from swine, dogs and cattle were subjected to three different isolation methods; direct out plating on SCAI media, enrichment in peptone water then plating out on SCAI and LB broth with 10 mg/l amoxicillin followed by plating on SCAI media (Figure 10). The results

indicated that the *Klebsiella* spp. detection rate was higher in samples enriched in LB broth with 10mg/l amoxicillin (45.52%) compared to direct out plating (19.53%) and peptone water enrichment (25.56%).

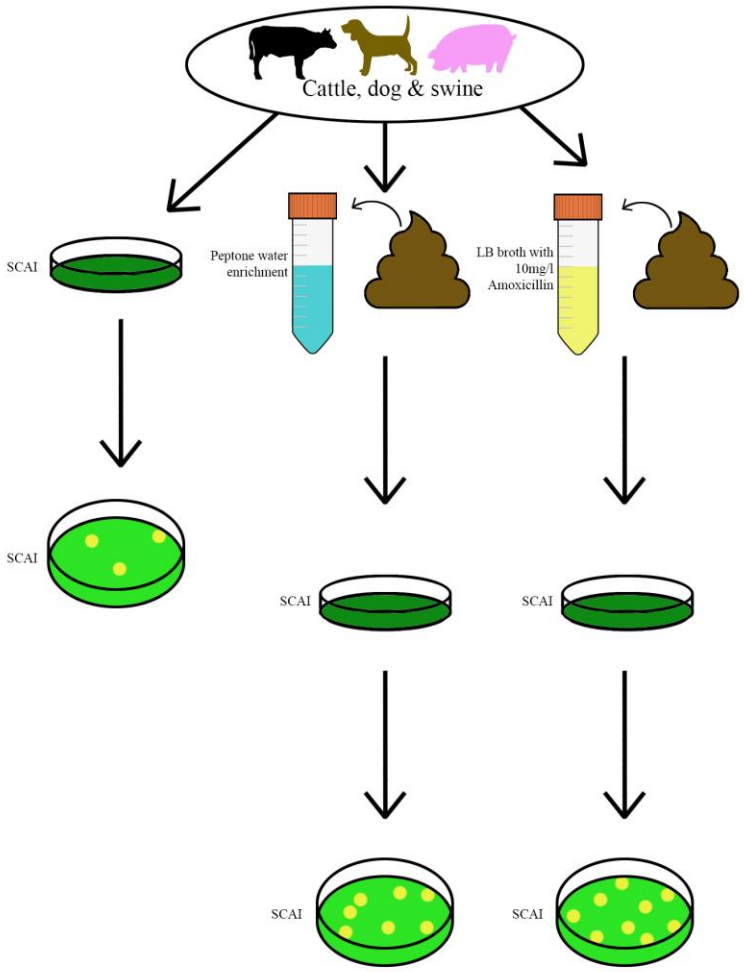

Figure 10. Flowchart of *Klebsiella* spp. isolation methods based on SCAI media culturing with and without enrichments.

When estimating the confidence intervals [CI] for the three isolation methods, there was an overlap in 95% CI between the direct out plating [15-24%] and peptone water enrichment method [21- 30%] (table 3). The LB broth with amoxicillin method however, showed no overlap with the two others (95% CI; [38-55%]).

Table 3. Number of samples, detection rates (%) and 95% CI for the three detections methods for *Klebsiella* spp.

	Direct	Peptone water enrichment	LB broth/ 10mg/l amoxicillin
Total samples	343	343	132
Positive	67 (19.53%)	89(25.95%)	61 (46.21%)
Negative	276 (80.47%)	254 (74.05%)	71 (53.79%)
[95% CI]	[15-24%]	[21-30%]	[38-55%]

From the detection rate for *Klebsiella* spp. in each of the three animal species (swine, dog and cattle, Table 4) it was possible to identify to see if the same pattern was found there as in Table 3.

Table 4. Distribution of *Klebsiella* spp. positive and negative samples for cattle, dog and swine from the three different detection methods.

	Swine	Cattle	Dog
Direct			
Total samples	157	107	79
Positive (%)	56 (35.26%)	6 (5.61%)	4 (5.06%)
Negative (%)	101(64.74%)	101 (94.39%)	75(94.94%)
[95% CI]	[28-43%]	[2-12%]	[1-12%]
Enrichment			
Total samples	157	107	79
Positive (%)	78(49.36%)	8 (7.48%)	3(3.80%)
Negative (%)	79(50.64%)	99 (92.52%)	76(96.20%)
[95% CI]	[41- 57%]	[3- 14%]	[1- 11%]
LB/amoxicillin			
Total samples	82	50	
Positive (%)	53(64.63%)	8 (16%)	
Negative (%)	29 (35.37%)	42 (84%)	
[95% CI]	[53 – 75%]	[7-29%]	

* dog samples were collected with swabs making it unable to test with LB broth with 10mg/l amoxicillin.

3.1.3 Identification of *Klebsiella* spp. by using polymerase chain reaction

Using DNA extracted from two swine samples, confirmed by MALDI-TOF to be *K. pneumoniae,* PCR protocol for *K. pneumoniae* in environmental samples was tested (Figure 11). The CT values ranged from 11.13 to 20.71 depending on the DNA concentration.

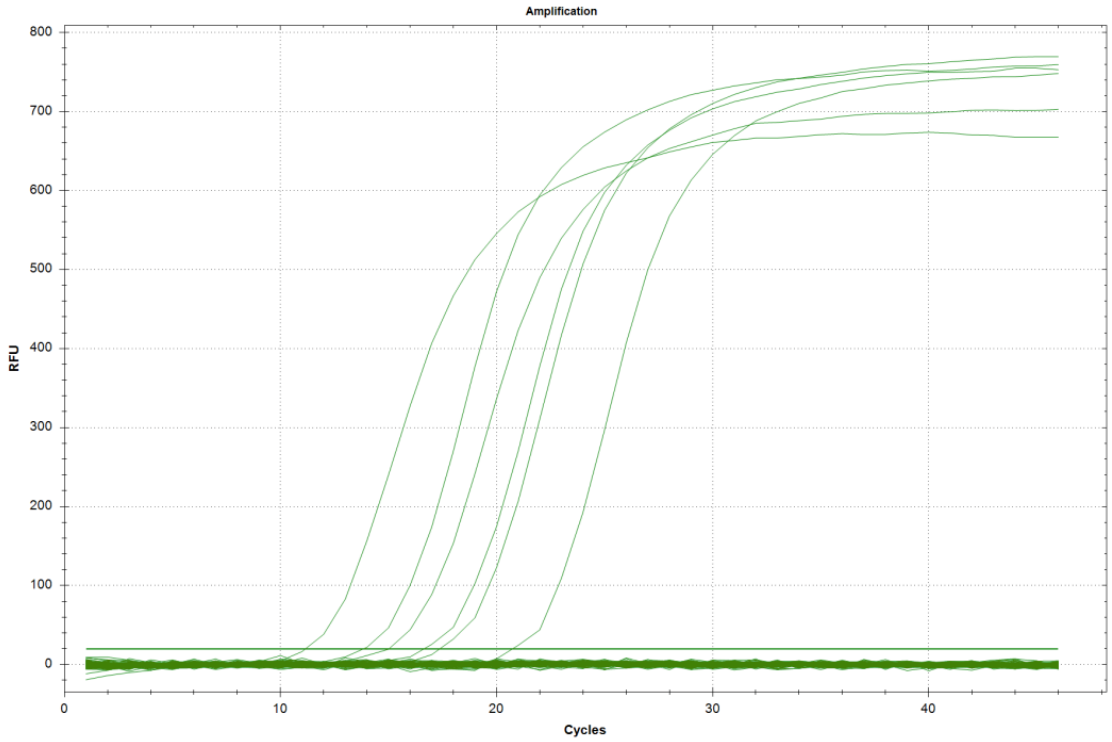

Figure 11. CT values for detection of pure *K. pneumoniae* DNA from isolates from swine.

The DNA extracted from pure *K. pneumoniae* colonies from two poultry isolates by using the boil lysis method was not successful. The lowest CT value was 21.04. In addition, seven pre-confirmed *K. pneumoniae* positive faecal samples from swine were also tested with the boil lysis method. These were also unsuccessful, with the lack of positive results in several of the isolates and high CT values, beginning at 25.35.

3.1.4 *Klebsiella* spp. isolated from swine samples in Thailand

When comparing the prevalence of the different species of *Klebsiella* among the swine isolates from Thailand, the same trend as in the Norwegian samples was detected. *K. pneumonia* had the highest prevalence with 46 isolates (88.46*%)*, followed by five *K. variicola* (9.62%) and one *K. aerogenes* (1.92%) (Figure 12).

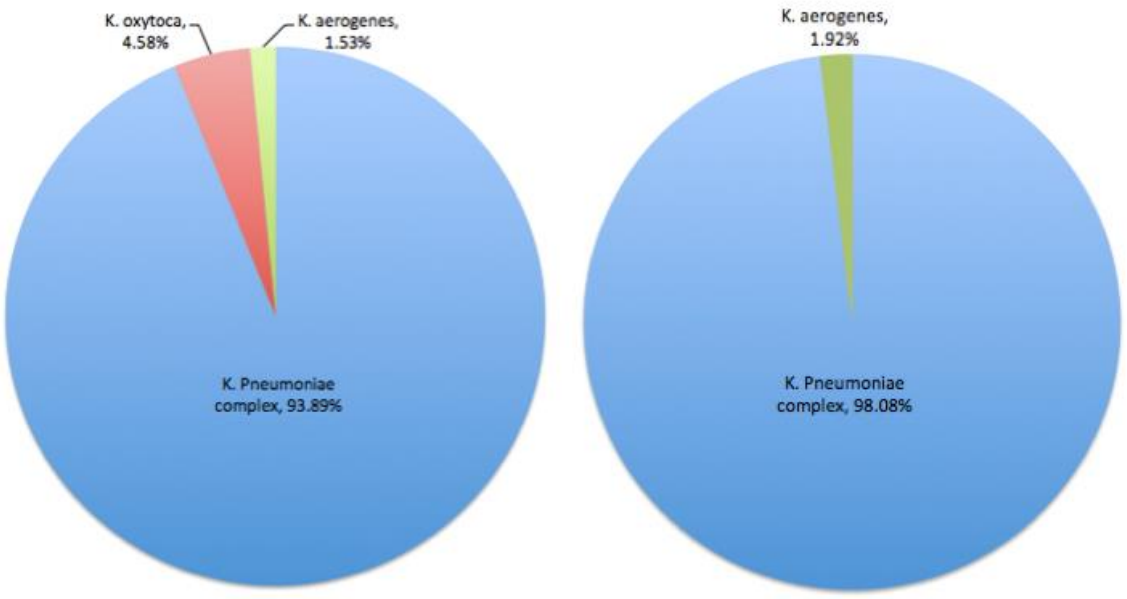

Figure 12. Left: Occurrence and distribution of *Klebsiella* spp. isolated from Norwegian swine. Right: *Klebsiella* spp. distribution from isolates from Thailand.

3.2 Antibiotic Susceptibility Testing & Minimal Inhibitory Concentration

A collection of 380 *Klebsiella* spp. isolates were investigated further. These isolates originated from turkey (n= 112), broiler (n= 95), swine (n= 130), dog (n= 30) and cattle (13). All 380 isolates were subjected to susceptibility testing by disc diffusion, including the following antibiotic groups; amphenicols, aminoglycosides, carbapenems, extended spectrum cephalosporins (3rd and 4th generation), macrolides, penicillin with extended spectrum, sulfonamides, tetracyclines, trimethoprim, quinolones, and other antimicrobial groups. Resistant isolates were further tested to determine minimal inhibitory concentrations.

In the appendix is the complete table of all raw data from both disc diffusion and minimal inhibitory concentration tests. In the raw data, there were three additional antimicrobial agents, fosfomycin, nitrofurantoin and azithromycin, tested that are not represented in these results due to the lack of official breakpoint, or because they were used as an additional control to ampicillin.

3.2.1 Antimicrobial resistance in *Klebsiella pneumoniae* complex

The results showed that 27 (7.99%) of the 338 *K. pneumoniae* isolates were resistant to one or more of the antimicrobial agents within the following five antimicrobial groups; tetracyclines, sulfonamides and trimethoprim, quiolones, amphenicols and aminoglycosides (Table 5).

Of the 27 resistant strains, 17 (62.96%) originated from turkey, six (22.22%) from broiler, two (7.41%) from both dog and swine. Of the 27 resistant isolates, one from broiler showed resistance to four antimicrobial agents; tetracycline, trimethoprim, ciprofloxacin and sulfamethoxazole. Another isolate from broiler was resistant against tetracycline and tigecycline. One isolate from swine showed resistance against tetracycline, trimethoprim, streptomycin and sulfamethoxazole. A single isolate, originating from dog, was resistant to chloramphenicol and sulfamethoxazole. One isolate from turkey was resistant to both sulfamethoxazole and tetracycline. Resistance to tetracycline occurred most frequently, with 16 isolates (59.26%) recorded as resistant. Resistance in sulfamethoxazole was detected in 12 isolates (44.44%). However, this result was not revealed during the disc diffusion test since the disc used was the combination antibiotic trimetoprim-sulfamethoxazole, but by MIC determination plate were the trimethoprim and sulfamethoxazole substances are tested separately. Chloramphenicol and trimetoprim resistance were identified in two (7.41%) isolates. Ciprofloxacin and tigecycline only had resistance in one isolate each (3.70%). From the 121 swine samples, two isolates (1.65%) were resistant to streptomycin, but the MIC for these two isolates were not confirmed further, since the MIC plates used for this thesis did not contain streptomycin. The 13 isolates originating from cattle indicated no resistance other than to Ampicillin.

Table 5. Number of *K. pneumoniae* isolates indicating resistance against each antimicrobial agent from collectively AST and MIC test. (n= 27)

Antimicrobial agent	**Number of isolates**			
	Turkey	**Broiler**	**Swine**	**Dog**
Tetracycline	11	3	1	1
Sulphametoxazole	6	4	1	1
Chloramphenicol	1	-	-	1
Ciprofloxacin	-	1	-	-
Tigecycline	-	1	-	-
Trimethoprim	-	1	1	-
Streptomycin	-	-	2	-
Cefotaxime	-	1	-	-
Trimethoprim-Sulphametoxazole	-	1	-	-

3.2.2 Antimicrobial resistance found in *Klebsiella oxytoca* and *Klebsiella aerogenes*

Of the 19 *K. oxytoca,* only one isolate, originating from broiler, showed resistance against tetracycline when being tested with the disc diffusion. This result was further confirmed by testing the minimal inhibitory concentrations were the isolate had growth in all wells containing tetracycline (> 64mg/l), while showing sensitivity to the remaining antibiotics, except for ampicillin. All the remaining isolates identified as *K. oxytoca* and *K. aerogenes* were susceptible to the antimicrobial agents they were exposed to.

3.2.3 Comparison of antibiotic resistance found in *Klebsiella* spp. and *E. coli*

In 2018, NORM-VET screening program collected and screened for resistance in *E. coli* from the same poultry caecum samples being used in this thesis. The following results are a comparison of the antimicrobial resistance in *Klebsiella* spp. and *E. coli* to investigate if there was a difference in resistance prevalence (Table 6). *E. coli* screening was performed prior to this thesis at the bacteriology lab at NVI, Oslo.

In general, it seems that *E. coli* and *Klebsiella spp.* share a similar AMR pattern, but for tetracycline and sulfamethoxazole *Klebsiella* spp. isolated from broiler has a higher percentage of resistant than *E. coli*. In comparison, the isolates from turkey are very similar regarding resistance to tetracycline. However, *E. coli* isolated from turkey has a twice as high percentage prevalence of resistance against sulfamethoxazole than *Klebsiella* spp. The most striking difference was observed for resistance to ciprofloxacin in isolates from broiler; among E. coli from broiler 10.8% of the isolates were ciprofloxacin resistant compared to 1.05% of *Klebsiella* isolates from broiler.

Table 6. Comparison in prevalence (%) of AMR identified in *Klebsiella* spp. and *E. coli* isolated from the same poultry samples.

	***Klebsiella* spp.**		***E. coli***	
	Broiler	Turkey	Broiler	Turkey
Tetracycline	4.21	9.82	2.2	10.3
Sulfamethoxazole	4.21	5.36	1.4	10.3
Chloramphenicol	0	1.79	0.4	1.3
Ciprofloxacin	1.05	0	10.8	0.6
Tigecycline	1.05	0	1.4	0.6
Trimethoprim	1.05	0	1.1	6.4

3.2.4 Antibiotic resistance found in *Klebsiella* spp. isolates from swine in Thailand

Of the 52 isolates from swine, 46 were classified as *K. pneumoniae,* five for *K. variicola and* one was positive for *K. aerogenes.* Of the 46 *Klebsiella* spp. isolates, 30 were resistant to additional antibiotics other than ampicillin. Resistance to tetracycline (48%) was most commonly observed among swine isolates from Thailand (Figure 13).

Trimethoprim-sulfamethoxazole resistance was indicated in 11 (29%) isolates. Six isolates were resistant to both tetracycline and trimethoprim-sulfamethoxazole, an additional three isolates were also resistant to tetracycline, trimethoprim-sulfamethoxazole, and chloramphenicol. One isolate was resistant against ciprofloxacin, and another was borderline resistant against tigecycline. One isolate was resistant to cefepime, ceftazidime, cefotaxime, tetracycline, trimethoprim-sulfamethoxazole and chloramphenicol. *K. variicola and K.aerogenes* both had one isolate each with resistance against tetracycline.

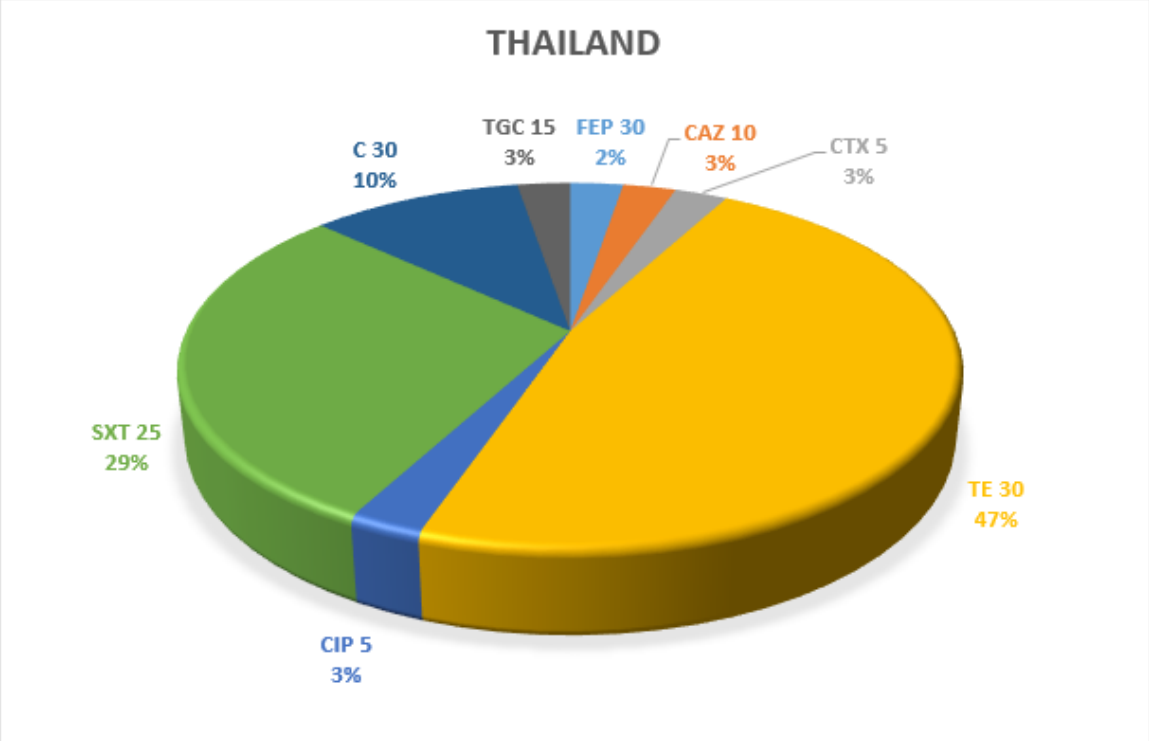

Figure 13. Distribution of antimicrobial resistance found amongst the swine isolates from Thailand (n= 30, resistant isolates).

3.3 Data from whole genome sequencing of *Klebsiella pneumoniae*

3.3.1 Examining the correlation between phenotype and genotype in *Klebsiella pneumoniae*

By using Kleborate, the *Klebsiella* species, ST, AMR genes and virulence genes were identified from the 203 isolates originating from poultry. There were a lot more data included in the Kleborate results, but only the previously mentioned data was relevant for this thesis. The version of Kleborate used identified all the isolates as *K. pneumoniae* with a strong match compared to the *K. pneumoniae* in the database. From the 203 *K. pneumoniae* isolates, antimicrobial resistance genes (characterized by WGS) were identified in 23 isolates, and the genotypic results correlated with the phenotype test results (Table 7). Tetracycline resistance was most prevalent with three different genes: *TetB* (six isolates), *TetD* (five isolates) and *TetA* (three isolates). Sulfamethoxazole was identified with two variants, six s*ul2* and four *sul1. Aada2* encoding for aminoglycoside resistance was present in three isolates together with s*ul1*. In one isolate, q*nr-S1,* which encodes for quinolone resistance*, dfrA1*, which encodes for trimethoprim resistance and *catA1*, which encodes for resistance against chloramphenicol, were all identified.

Table 7. Overview of sequence types, resistance genes and MIC values in mg/l in *K. pneumoniae* for poultry.

Source (Isolate ID)	ST	Genotype	AMR phenotype	MIC above cut-off (Antimicrobial agent)
Turkey (2018-01-473)	ST1779	TetD	TE	>64 (TE)
Turkey (2018-01-2640)	ST1779	TetD	TE	>64 (TE)
Turkey (2018-01-2730)	ST1779	TetD	TE	>64 (TE)
Turkey (2018-01-3975)	ST1779	TetD	TE	>64 (TE)
Turkey (2018-01-1178)	ST1823	TetD	TE	>64 (TE)
Broiler (2018-01-582)	ST2441	Aada2**, Sul1	SUL*	>1024 (SUL)
Broiler (2018-01-798)	ST2441	Aada2**, Sul1	SUL*	>1024 (SUL)
Broiler (2018-01-3934)	ST2441	Aada2**, Sul1	SUL*	>1024 (SUL)
Broiler (2018-01-715)	ST2458	DfrA1, Sul1, TetA , Qnr-S1	TRI, SUL, TE	>32 (TRI) >1024 (SUL) >64 (TE) 0.5 (CIP)
Turkey (2018-01-1022)	ST35	Sul2	SUL*	>1024 (SUL)
Turkey (2018-01-1584)	ST35	Sul2	SUL*	>1024 (SUL)
Turkey (2018-01-1593)	ST35	Sul2	SUL*	>1024 (SUL)
Turkey (2018-01-3030)	ST35	TetB, Sul2	TE, SUL*	>64 (TE) >1024 (SUL)
Turkey (2018-01-3692)	ST35	Sul2	SUL*	>1024 (SUL)
Turkey (2018-01-4107)	ST35	Sul2	SUL*	>1024 (SUL)
Turkey	ST39	CatA1	C	>128 (C)

(2018-01-4682)				
Broiler	ST463	TetA	TE	>64 (TE)
(2018-01-1445)			TGC	1 (TGC)
Broiler	ST463	TetA	TE	>64 (TE)
(2018-01-4639)				
Turkey	ST550-1LV	TetB	TE	>64 (TE)
(2018-01-1116)				
Turkey	ST550-1LV	TetB	TE	>64 (TE)
(2018-01-1685)				
Turkey	ST550-1LV	TetB	TE	>64 (TE)
(2018-01-2939)				
Turkey	ST550-1LV	TetB	TE	>64 (TE)
(2018-01-3163)				
Turkey	ST550-1LV	TetB	TE	>64 (TE)
(2018-01-3590)				

** Identified by MIC test, ** Streptomycin is not included in the MIC panel*

C: chloramphenicol, TE: tetracycline, TGC: tigecycline, TRI: trimethoprim, SUL: sulfamethoxazole.

Beta-lactamase broad InhR was found in 39 isolates, 14 originating from broiler, and was only coded for by the *SHV-26* and *TEM-30* genes, and there were no outstanding sequence types correlating to these two genes.

According to the Kleborate and ResFinder, 20 samples contained ESBL- genes, SHV-101, SHV-13, SHV-41 and SHV-27, but none of the strains showed any indication of phenotypic resistance against broad-spectrum cephalosporins during AST. The zone diameter was checked with the EUCAST database for all the antimicrobial agents tested and concurred with being sensitive.

3.3.2 Screening for virulence genes; aerobactin, salmochelin and yersiniabactin

The following three virulence genes; aerobactin, salmochelin and yersiniabactin, were screened for by using Kleborate. Of the 203-screened isolates, 46 (22.66%) isolates encoded for one or more of the virulence genes. Yersiniabactin (*ybt*) was identified in 43 (21.18%) of the isolates, salmochelin (*iro5*) in 15 (7.39%) and aerobactin (*iuc5*) in 16 (7.88%) isolates. Among the yersiniabactin positive isolates, the sequence type (ST) diversity was the greatest among the three virulence genes

with several different sequence types (Table 8). When comparing the occurrence between the two poultry sources, the virulence genes are mostly found in turkey isolates.

Table 8. Summarises the relations between Yersiniabactin virulence genes, sequence type and source of identification in *K. pneumoniae*.

Yersiniabactin	ST	Source
ybt 14; ICEKp5	ST1229	Turkey
ybt 14; ICEKp5	ST1229	Turkey
ybt 14; ICEKp5	ST1229	Turkey
ybt 14; ICEKp5	ST1229	Turkey
ybt 14; ICEKp5	ST1229	Turkey
ybt 16; ICEKp12	ST1630	Broiler
ybt 10; ICEKp4	ST17	Turkey
ybt 10; ICEKp4	ST17	Broiler
ybt 10; ICEKp4	ST17	Turkey
ybt 14; ICEKp5	ST290	Broiler
ybt 14; ICEKp5	ST290	Broiler
ybt 14; ICEKp5	ST290	Broiler
ybt 14; ICEKp5	ST290	Broiler
ybt 14; ICEKp5	ST290	Broiler
ybt 14; ICEKp5	ST290	Broiler
ybt 14; ICEKp5	ST290	Broiler
ybt 16; ICEKp12	ST290	Turkey
ybt 16; ICEKp12	ST290	Turkey
ybt 16; ICEKp12	ST290	Turkey
ybt 16; ICEKp12	ST290	Turkey
ybt 16; ICEKp12	ST290	Turkey
ybt 16; ICEKp12	ST290	Turkey
ybt 16; ICEKp12	ST290	Turkey

ybt 16; ICEKp12	ST290	Turkey
ybt 16; ICEKp12	ST290	Turkey
ybt 16; ICEKp12	ST290	Turkey
ybt 16; ICEKp12	ST290	Turkey
ybt 16; ICEKp12	ST290	Turkey
ybt 8; ICEKp3	ST35	Broiler
ybt 8; ICEKp3	ST35	Broiler
ybt 15; ICEKp11	ST37	Turkey
ybt 15; ICEKp11	ST37	Turkey
ybt 15; ICEKp11	ST37	Turkey
ybt 15; ICEKp11	ST37	Turkey
ybt 15; ICEKp11	ST37	Turkey
ybt 15; ICEKp11	ST37	Turkey
ybt 10; ICEKp4	ST45	Turkey
ybt 10; ICEKp4	ST45	Broiler
ybt 10; ICEKp4	ST45	Turkey
ybt 5; ICEKp6	ST636	Turkey
ybt 5; ICEKp6	ST636	Broiler

*ICEKp is an integrative conjugative element for the ybt locus and encodes the biosynthesis of yersiniabactin (Lam et al, 2018).

The 15 isolates that had both aerobactin and salmochelin, all had the same sequence type (ST290-1LV) and all isolates originated from turkey. One additional salmochelin positive isolates originated from broiler, but this isolate had a different sequence type (ST969-1LV.) The geographical distribution of the 15 isolates grouping into ST290-1LV and containing both salmochelin and aerobactin was widespread. The poultry came from four different Norwegian counties, Østfold, Hedmark, Trøndelag and Vestfold (Figure 14). Nine of the isolates are from Østfold, but are distributed within five different area codes. Three samples originated from Hedmark, two from Trøndelag and one from Vestfold.

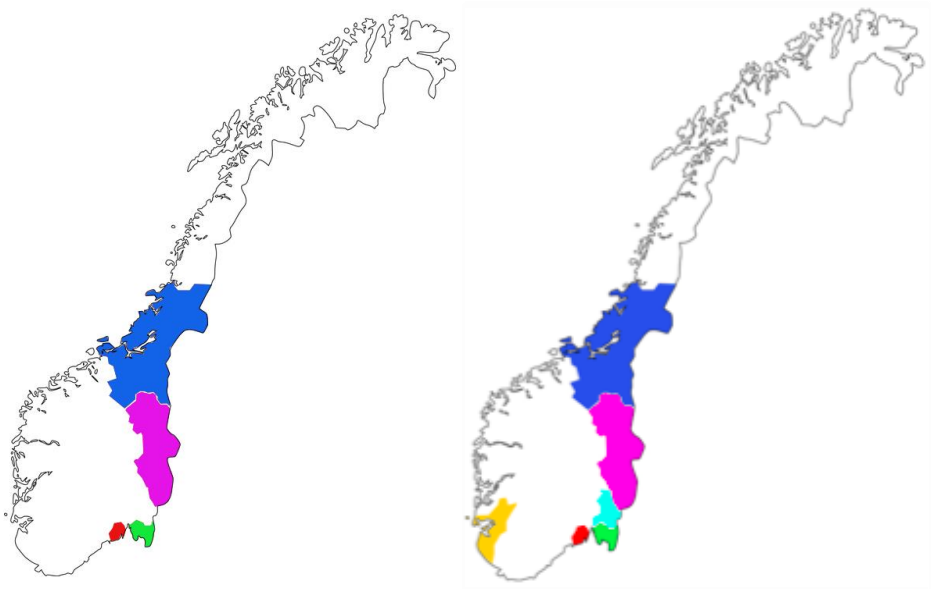

Figure 14. Geographical distribution of detection of virulence genes in poultry. Left: aerobactin and salmochelin, right: yersiniabactin

By running Mobsuite, Plasmidfinder and Blast in NCBI it seemed that aerobactin and salmochelin were located on an IncFII-FIB-replicon type plasmid, and the same was found in all 15 isolates. The isolates also clustered together in the phylogenetic analyses, indicating close genetic relationship (clonal relatedness).
The virulence score for the isolates containing all three virulence genes was four, the isolates only containing aerobactin and salmochelin had a score of three, and the isolates that only had yersiniabactin got a virulence score of one.

3.3.3 Sequence- and capsule type distribution within 203 *Klebsiella pneumoniae* isolates originating from poultry

There were 64 different sequence types among the 203 *K. pneumonia* isolates from poultry. ST35 was identified in 14.29%, followed by ST290-1LV with 7.39%. When separating the broiler and turkey isolates, the dominating sequence type among the broiler was ST1877 and ST2441 both with 8.79%, followed by ST290 and ST22-1LV with 7.69% (Figure 15).

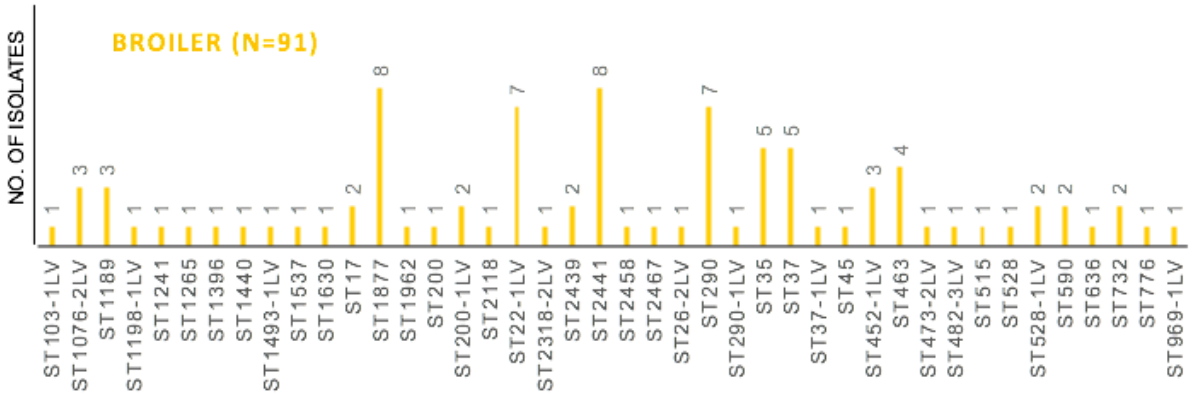

Figure 15. ST distribution in broiler isolates.

For turkey, ST35 dominates with 21.43% and ST290-1LV with 12.5% (Figure 16). With regards to the diversity of sequence types, broiler had a higher absolute and relative diversity than turkey, with 42 different sequence types distributed among the 91 different isolates. Turkey had 33 different sequence types amongst the 112 isolates.

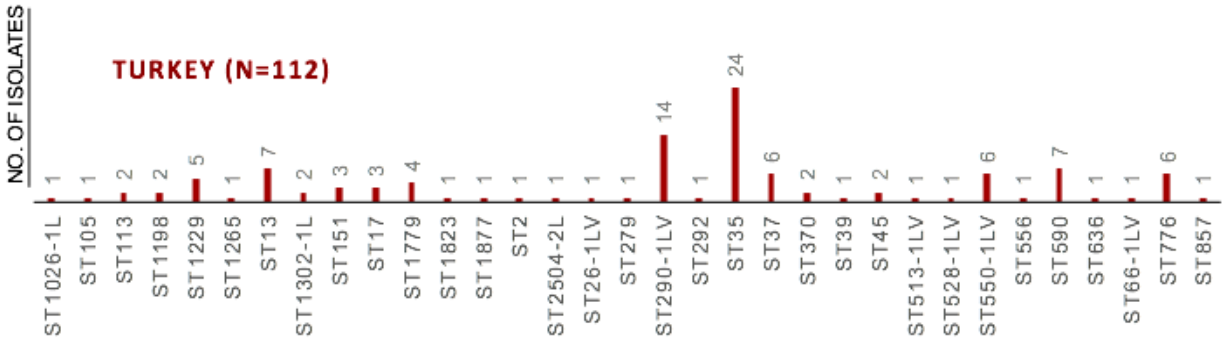

Figure 16. ST distribution in turkey isolates.

In the 203 poultry isolates, 43 different capsule types were identified, with KL21 found in 16.26%, KL22 in 14.29%, KL14 in 9.85% and KL30 and 38 in 4.93%. The rest of the capsule types were found in < 3% of the isolates. Among the 91 broiler isolates, 30 different capsule types were identified, with KL14 being identified in 20 isolates, KL21 in 13, KL64 in seven, KL22 in five, and the remaining varied from one to four isolates per capsule type (Figure 17). In turkey, KL22 is the dominant capsule type found in 21.43% of isolates, KL21 in 17.86%, KL30 and KL38 both

occurred in 8.93%, KL47 in 5.36% and the rest distributed with < 5% in each capsule type.

In three out of the 64 different sequence types there are two different capsule types found in the same sequence type, and within one ST five different capsule types were found. The remaining 61 sequence types had a one-to-one ratio with a capsule type.

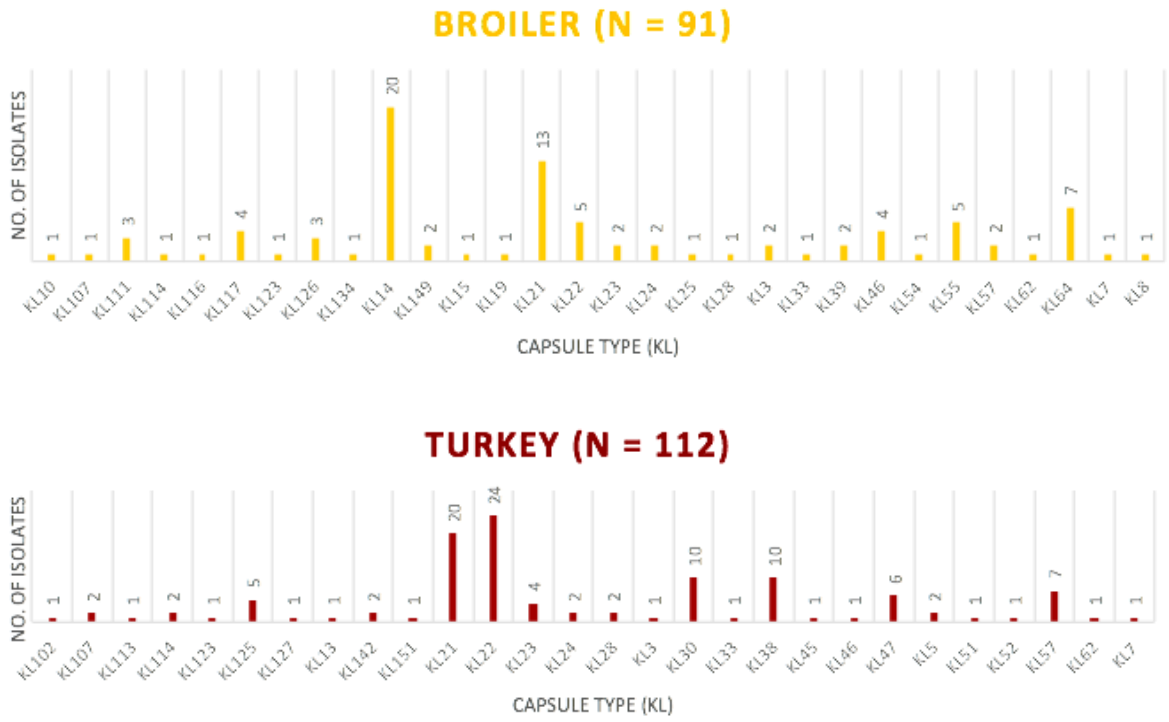

Figure 17. Capsule types identified in poultry isolates divided into origin of the source, broiler and turkey.

3.3.4 Phylogenetic tree based on core gene alignment

Roary is used to analyse species wide distribution of *K. pneumoniae* strains and the phylogenetic tree was generated using core genome alignment created using this program (Figure 18). A total of 193 strains are included based on alignment of 3928 core genes to display species wide distribution. Similar ST strains, e.g. ST13, ST22-2LV, ST35, ST37, ST290, ST290-1LV, ST590, ST1776, ST2441 are clustered together and further SNP analysis is required to define their clonality. Exceptions are ST1776 and ST37, as they differ only at one loci (tonB). One strain, ST35, has relocated out of the ST35 clustering, so further analysis is required to determine the cause.

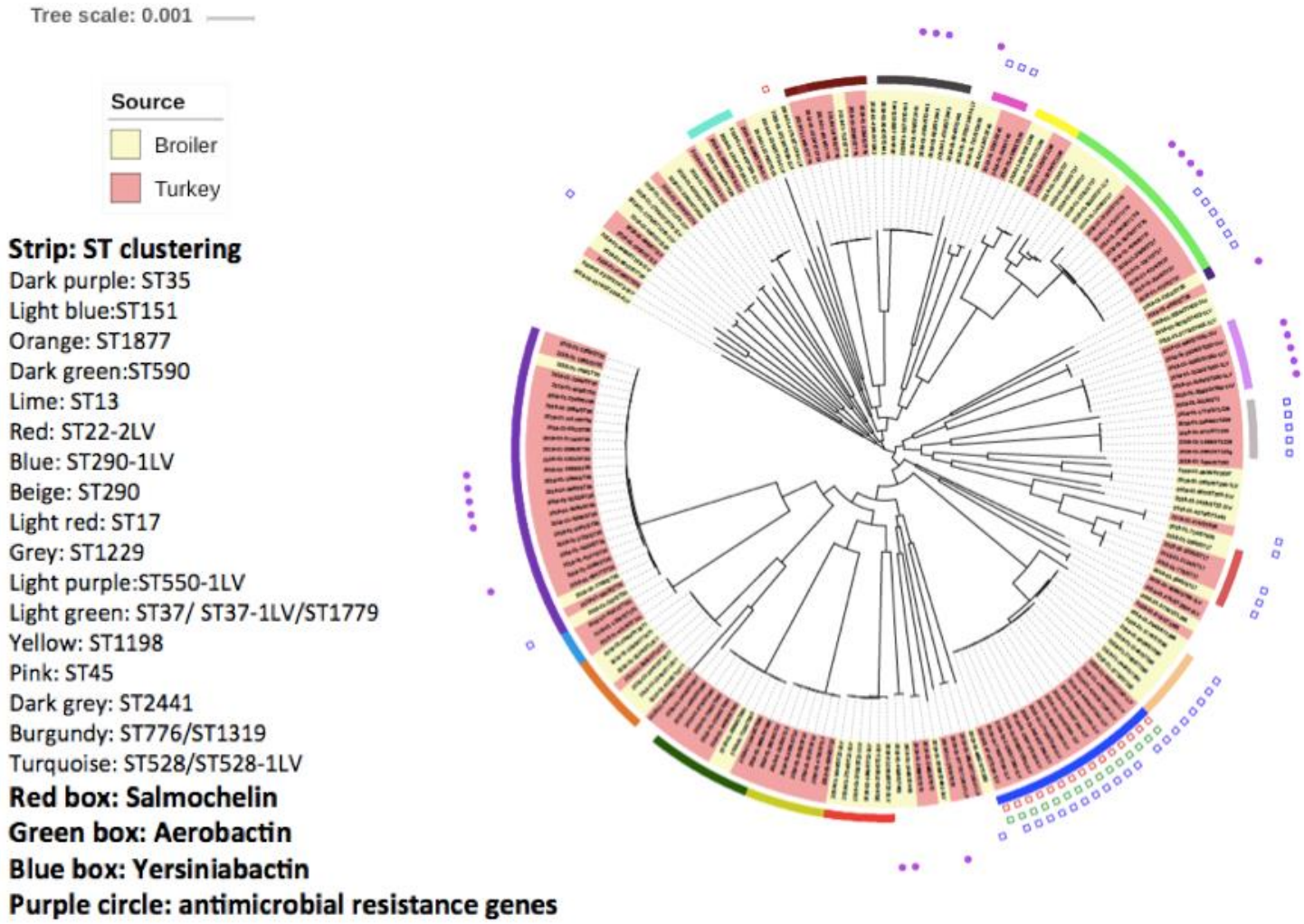

Figure 18. Phylogenetic tree of 193 *K. pneumoniae* isolated from poultry, displaying ST clustering, virulence genes and antimicrobial resistance genes.

3.4 Determination of biofilm forming abilities

3.4.1 The biofilm forming abilities of *Klebsiella pneumonia*

Most of the *K. pneumoniae* isolated (n= 339) were classified as good biofilm producers. There were two dominating categories; the largest was with OD_{595} 0.6-0.7 containing 105 isolates, and OD_{595} 0.7-0.8 with 90 isolates (figure 19). The average OD_{595} for *K. pneumoniae* was OD_{595} 0.66 (STDEV: 0.52-0.80). However, it had a wide distribution from the lowest category OD_{595} 0.0-0.1 up to OD_{595} 1.0. Only eight isolates were classified as "poor or non-biofilm producers" being in the OD_{595} 0.0-0.1 category.

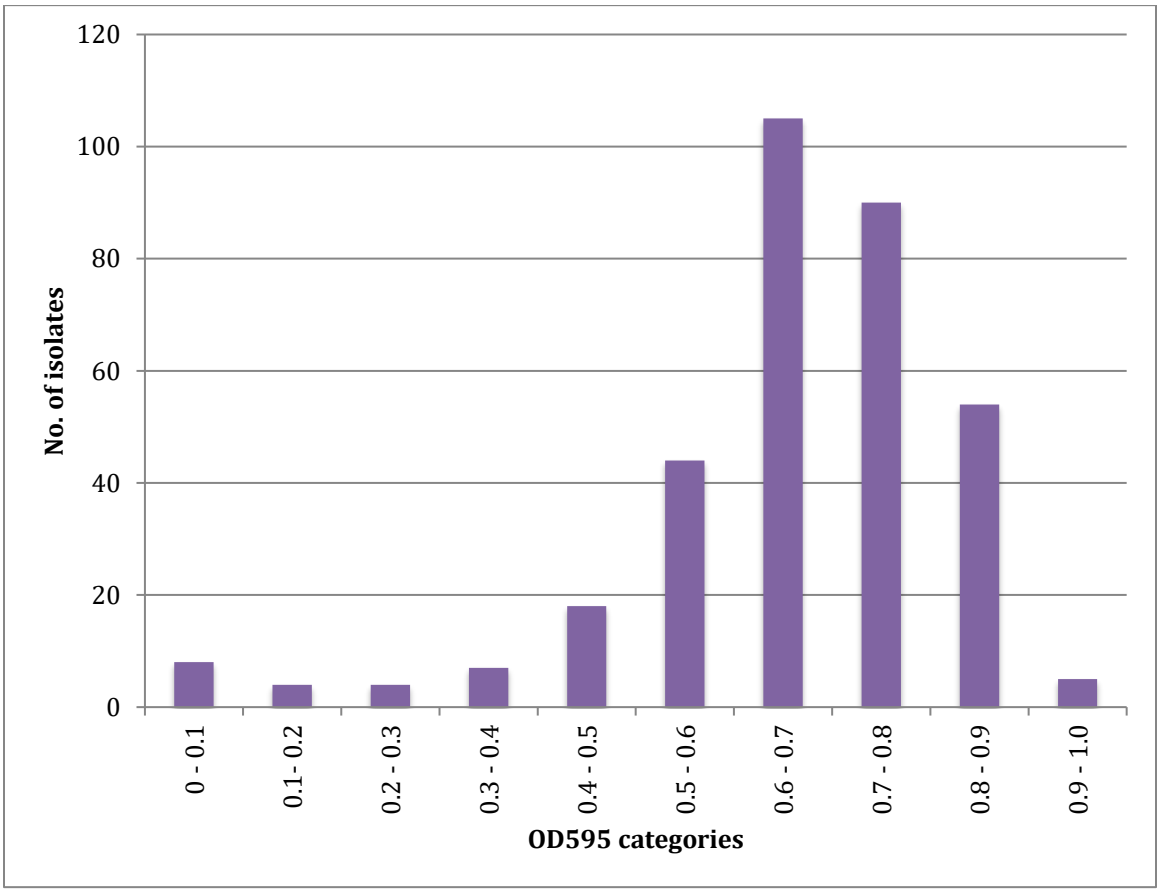

Figure 19. Categorization of *K. pneumoniae* isolates (n= 339) by ability to produce biofilm.

3.4.2 The biofilm forming abilities of *Klebsiella oxytoca, Klebsiella variicola* and *Klebsiella aerogenes*

K. oxytoca, containing 18 isolates, had the highest average OD_{595} with 0.61 (STDEV: 0.50-0.72), and the average biofilm forming ability for the 20 *K. variicola* isolates showed they had an OD_{595} of 0.56 (STDEV: 0.20-0.91) (Figure 20). When comparing the two species there was no significant difference in biofilm forming abilities between them (p-value=0.82).

There were also three *K. aerogenes* with OD_{595} of 0.23, but this result is not admissible for further analyses given that its only three samples.

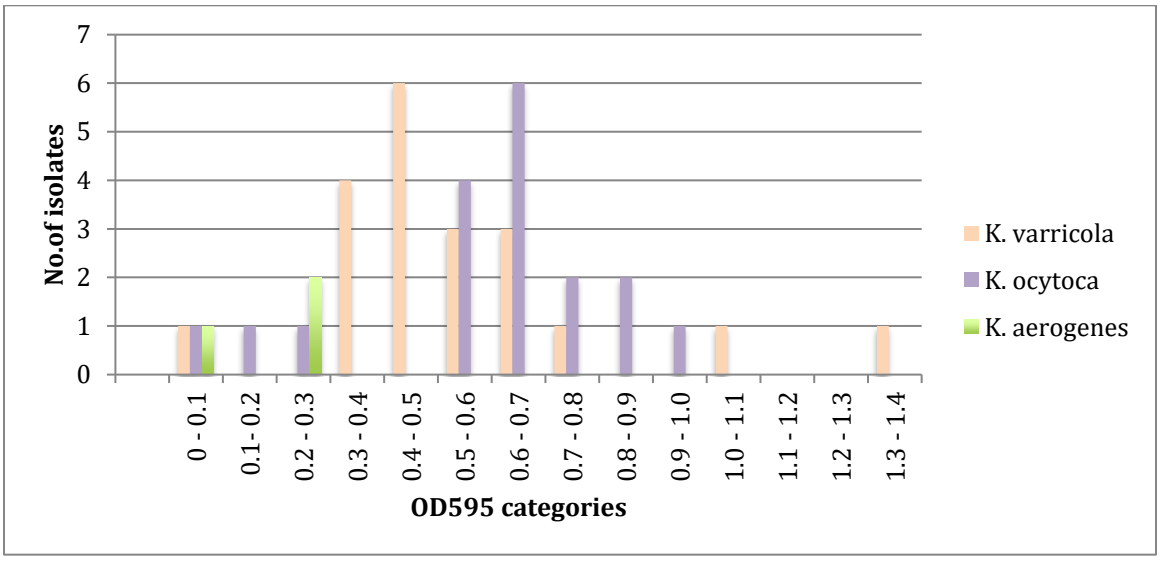

Figure 20. The percentage of isolates in each *Klebsiella* spp. showing their distribution in biofilm forming abilities (OD_{595}). *K. varricola* (n=20), *K. oxytoca* (n= 18) and *K. aerogenes* (n= 3).

3.4.3 General comparison of biofilm production amongst the five animal species

When comparing the biofilm forming abilities between the animals, there was only a statistically significant difference when comparing turkey to broiler (p value: 0.009), swine (p value: 0.001) dog (p value: 0.030), and cattle (p value: 0.041). All other comparisons resulted in a p value < 0.05 when performing the t-test.

Isolates from dogs had an average OD_{595} of 0.77 (STDEV: 0.57-0.96), followed by cattle with an average OD_{595} of 0.74 (STDEV: 0.59-0.89) (Figure 21). Swine had an

OD_{595} of 0.70 (STDEV: 0.49-0.91), turkey had OD_{595} 0.69 (STDEV: 0.56-0.83) and broiler had the lowest with an average OD_{595} of 0.64 (STDEV 0.50-0.78). But all five animals had isolates with average OD_{595} results being over 0.500, which means they all are considered good biofilm producers, as their average values are within the OD_{595} 0.5 - 0.9 category.

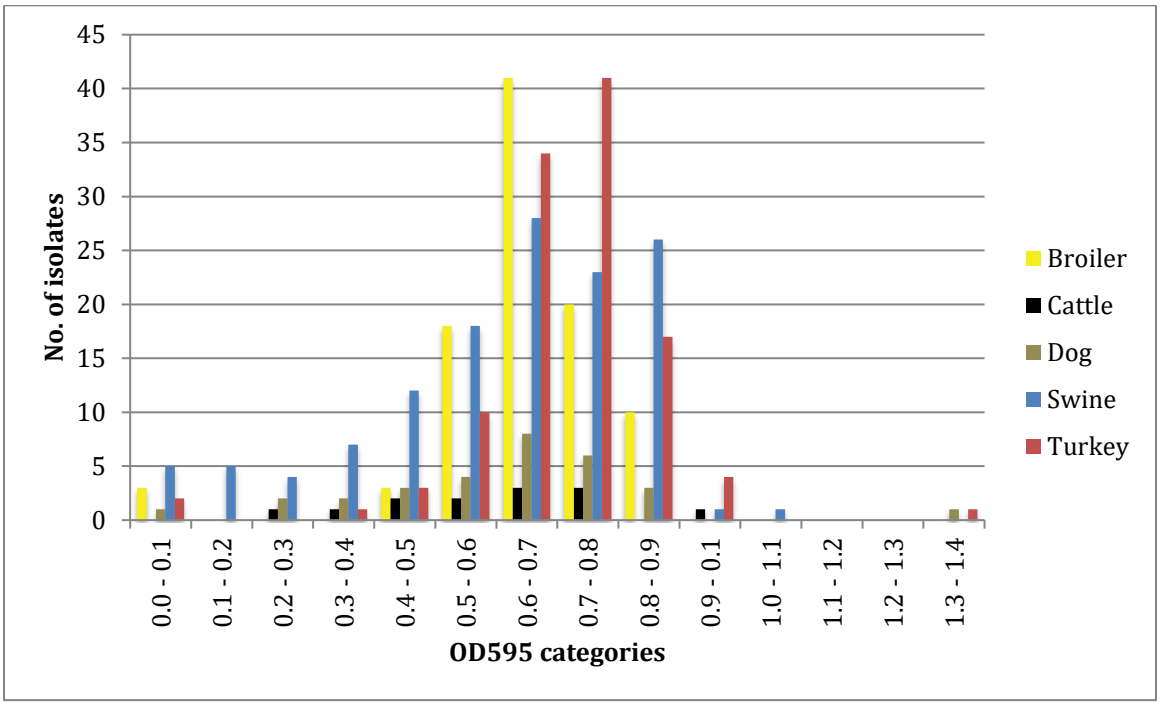

Figure 21. Number of isolates in each OD $_{595}$ category for all of the five animals in-dependent on the *Klebsiella* species.

3.4.4 Identifying the biofilm morphology

When examining the biofilm morphology, six previously confirmed good biofilm producers were selected, but only one isolate produced a bright pink biofilm, the five other isolates produced a larger, grey, mucoid colony (Figure 22).

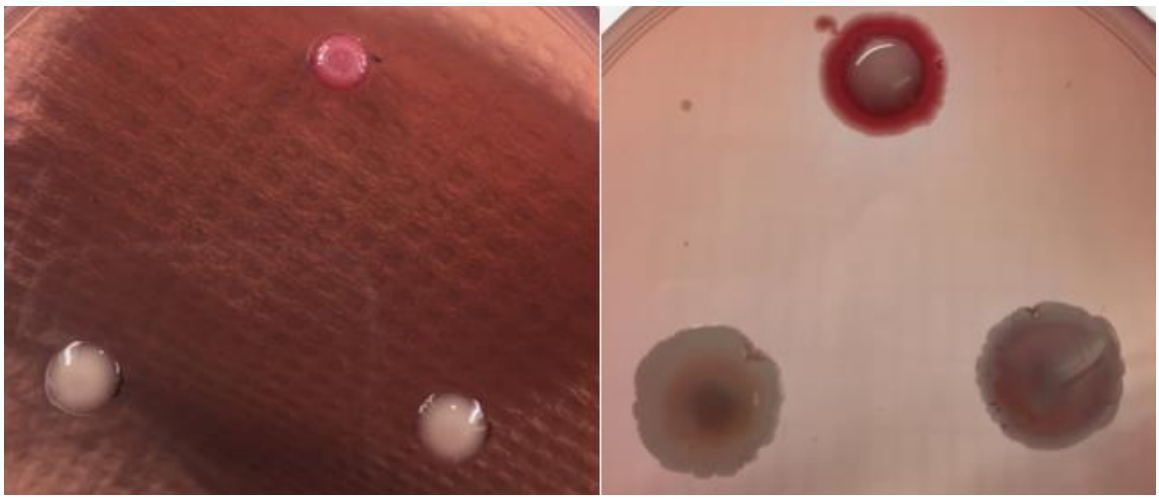

Figure 22. Picture taken of *K. pneumonia* on congo red agar trying to produce biofilm. Left: three days after incubation. Right: after two weeks incubation.

3.4.5 The biofilm forming abilities based on sequence type or capsule type

Another comparison conducted was identifying the relationship between ST and biofilm forming ability to identify if there were any correlation between the 64 different STs identified among the *K. pneumoniae* isolates from turkey and broiler and the bacteria´s ability to produce biofilm (Figure 23). ST292 and ST556 ranked highest with an OD_{595} of 0.820. The remaining sequence types varied, with OD_{595} from 0.095 to 0.820, and with a median of 0.658.

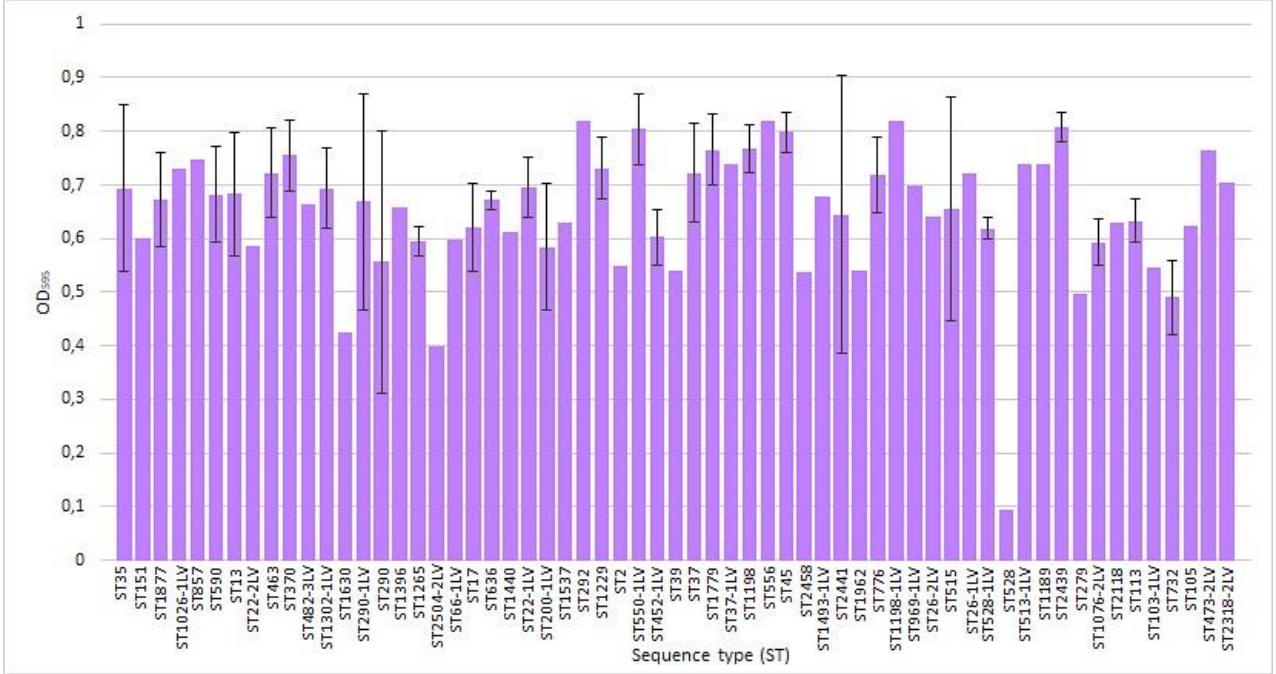

Figure 23. Correlation between ST and biofilm forming ability (OD_{595}). ST without standard deviation bar is due to the ST only containing one isolate.

The sequence types that had the highest prevalence in turkey and broiler isolates where chosen for comparisons, and to identify the distribution for biofilm forming abilities within each sequence type (Figure 24).

In turkey, the following sequence types ST13, ST290-1LV, ST1887, ST2441, ST290, ST35, ST 37, ST590 were the most prominent. Broiler also had ST35 and ST37, but here the remaining sequence types were ST1877, ST22-2LV, ST2441 and ST290. The distribution of the isolates within each of the previously mentioned sequence type is displayed in the figure below. Outliers are found in ST2441, ST290, ST290-1LV and ST35, with isolates in the <0.1 OD_{595} category, implying very poor or no biofilm producing abilities. The OD_{595} variation between and within the different STs does not vary greatly.

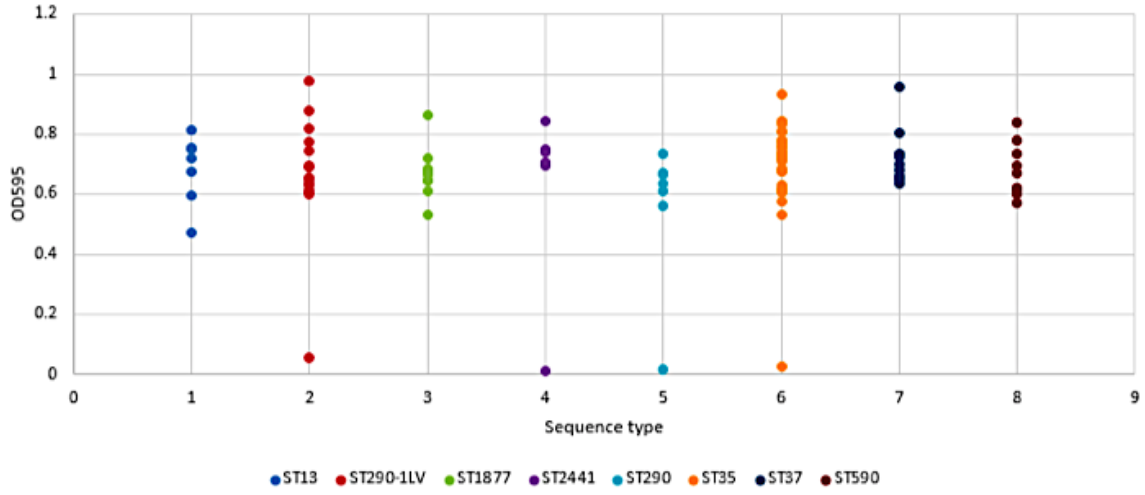

Figure 24. Distribution of biofilm forming abilities (OD_{595}) within the most dominant STs in poultry isolates.

Of the 44 different capsule types, none of them indicate being superior biofilm producers when compared to each other (Figure 25). The lowest OD_{595} value was observed for capsule type 45 (OD_{595}=0.399) and highest value for capsule type 51 (OD_{595}=0.820.) The median was 0.662.

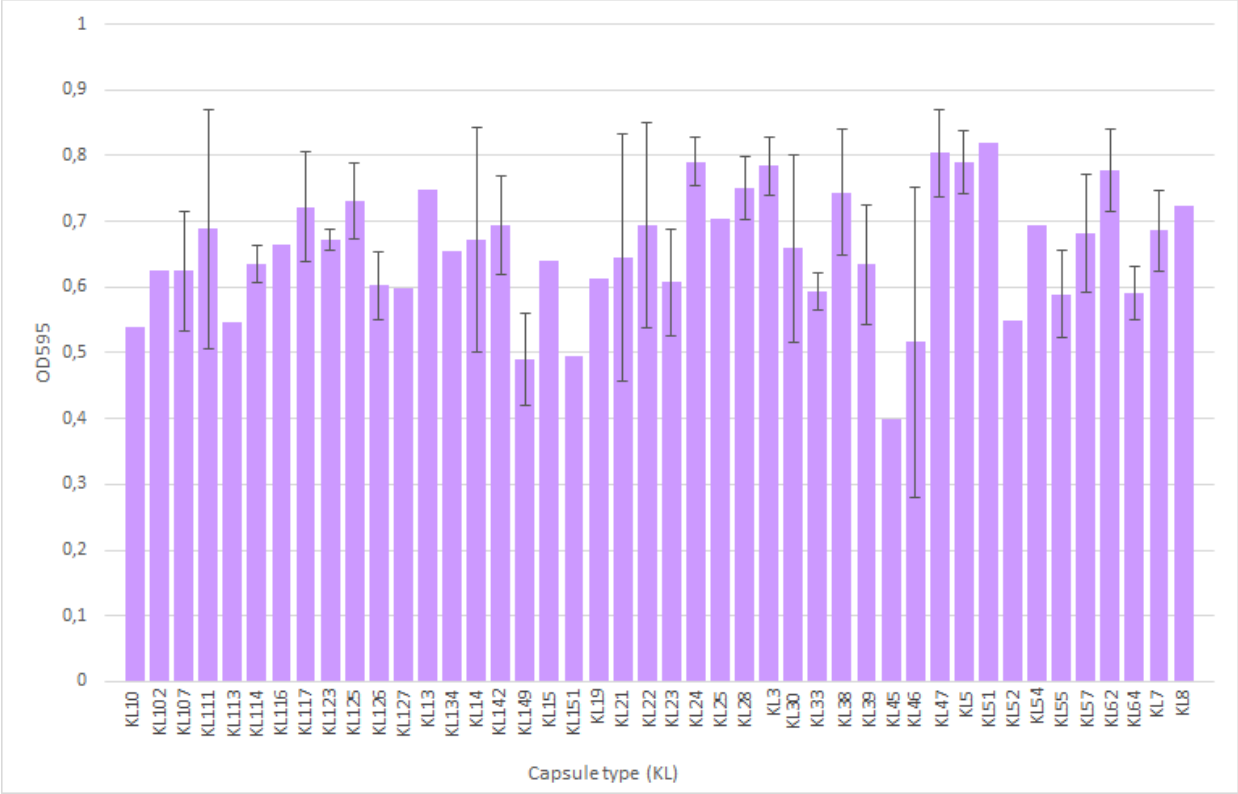

Figure 25. Correlation between capsule type and biofilm producing ability (OD_{595}) in poultry isolates. Bars without a standard deviation bar is due to the KL only containing one isolate.

3.4.6 Biofilm forming abilities in the isolates from swine in Thailand

The 46 *K. pneumoniae* isolates had an average biofilm forming ability of 0.41 (STDEV: 0.15-0.67) (Figure 26), but 10 isolates were in the 0.5-0.6 category and 11 in the 0.6-0.7. However, since there are 12 isolates that are poor or non-biofilm producers in the < 0.1 category, this might give a false representation of the actual biofilm forming abilities for the swine isolates.

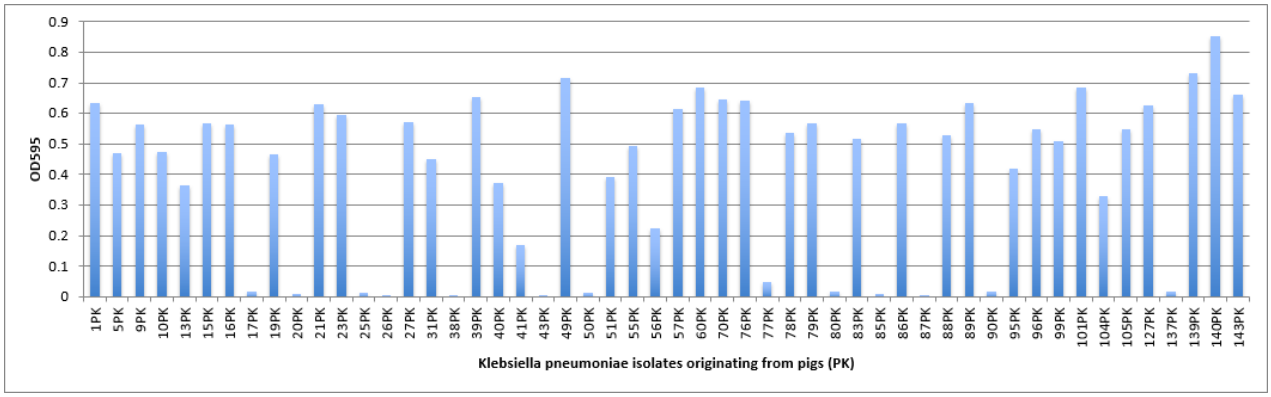

Figure 26. Biofilm forming abilities (OD_{595}) in swine isolates from Thailand.

The same problem was identified in *K. variicola*, there three of the isolates fall in the 0.5-0.6 category, one with 0.3-0.4, but because there was one isolate that had an OD_{595} of 0.004, the average value dropped to 0.43.

Thirteen (23.08%) of the isolates had OD results under 0.1, which indicate that they are poor biofilm producers (Figure 27). 13 (25.00%) isolates belong in the 0.5-0.6 category, and 11 (21.15%) in the 0.6-0.7 category. 11 of the isolates fall within the OD_{595} categories between 0.1- 0.5, two in 0.7-0.8 and one isolate being the best biofilm producer with an OD_{595} of 0.85. Comparing the Norwegian swine isolates with the swine isolates from Thailand, the distribution in the different categories had to be transformed to percentages to be able to conduct comparisons, due to the large sample size difference. The isolates originating from Norway have a higher

ability to produce biofilm based on both the clustering and of the number of isolates in each OD_{595} category.

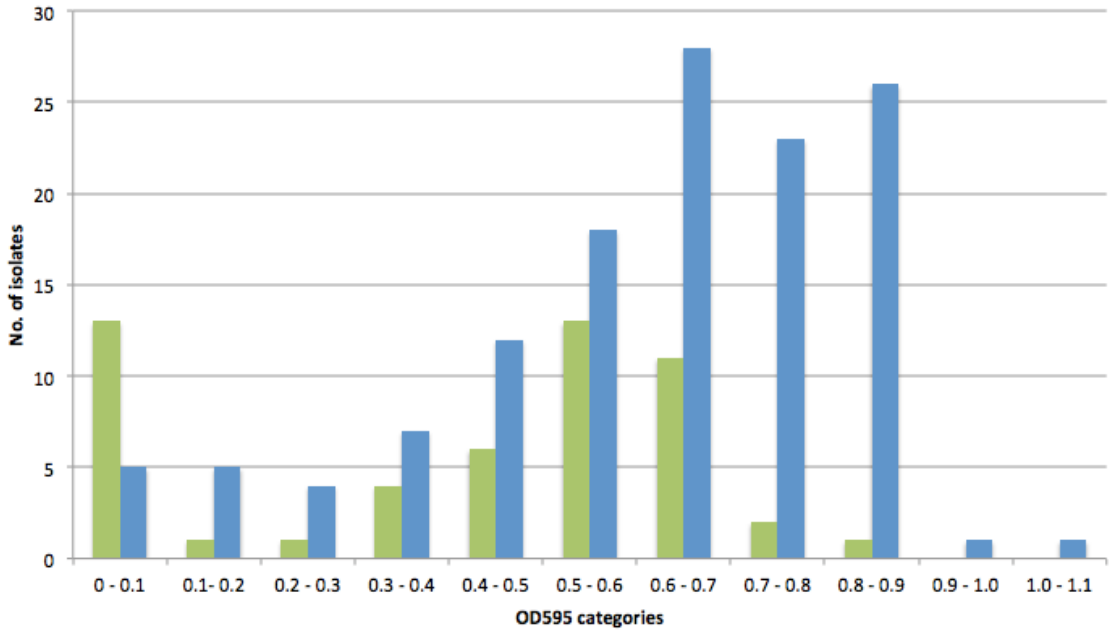

Figure 27. Number of isolates per OD_{595} category, comparing the Norwegian swine isolates to the Thailand isolates. Blue: Norwegian, green: Thailand.

4. Discussion

In this thesis, different culturing methods for detection and isolation of *Klebsiella* spp. have been investigated. The 343 faecal samples that were tested over the five-month period with three different isolation methods, had a significant difference between LB broth with amoxicillin and the two other methods (table 3). LB broth w/ amoxicillin proved a superior way to extract *Klebsiella* spp. from faecal matter with a detection rate of 45.52% [38-55%]. Enrichment in peptone water had the second highest detection rate with 25.56% [21- 30%] and lastly direct out plating had the lowest detection rate of *klebsiella* spp. with 19.53% [15-24%]. However, there was no significant difference in detection rates between direct out plating and enrichment in peptone water. None of these methods were without limitations, however. A potential drawback of both enrichment methods (peptone and LB/amoxicillin) was pointed out by the Pasteur Institute (Brisse, pers.com., 2019); by enriching the sample, a single *klebsiella* spp. strain could potentially become dominant in the sample, reducing the ability to identify all strains in the sample. One should consider what is more important; to isolate the *klebsiella* spp. or investigating the diversity of *Klebsiella* spp. in the faecal sample. For this thesis, the aim was mainly to identify *Klebsiella* spp. from the faecal matter from each animal species. To that end, the enrichment methods proved the preferred isolation method. But for a microbiota study focusing on a possible strain diversity, enrichment would not be a preferred choice. Another potential drawback is the risk of contamination from the environment in the laboratory. Direct out plating reduces the exposure to the environment in the laboratory where other bacteria or microbes are present compared to enrichment methods. With the enrichment methods, the two steps of inoculation double the risk of contamination and increases the risk of a false positive.

The *Klebsiella* spp. detection rate among the five animals ranged from 69.57% (turkey), 51.79% (swine), 23.51% (broiler), 15.87% (dog) and 4.56% (cattle) positive isolates. Indicating that turkey and swine are a more common source for carrying Klebsiella spp. than the other animals. This makes specially turkey an interesting reservoir for further study regarding bacterial transmission and AMR.

In the US, the estimated prevalence of *K. pneumoniae* in dairy cattle is 44% when sampling from the environment, faecal matter and feed (Zadoks *et al*., 2011.) However, this depends on which part of the animal the sample has been taken from, because *Klebsiella* spp. may be more likely to be found in the udders, 59.8%, (where they can cause mastitis) then in the faecal matter in cattle (Munoz et al, 2008; Olde Riekerink et al, 2008). The higher detection rate of *Klebsiella* spp. in udders is most likely due to transfer from the environment, such as hay and soil as the udders are highly exposed. A plausible reason for the difference in *Klebsiella* spp. detection rate between the animal species could be, in part, due to different sample collection techniques used between the different animals. From the dogs (and Thailand swine), the samples are collected with rectal swaps, while for the poultry, cattle and Norwegian swine the samples were collected from fresh caecum samples taken during/after the slaughtering process. If the *Klebsiella* spp. prefers different parts of the gastrointestinal (GI) tract depending on the animal, this could lead to different detection rates depending on the sampling site. In healthy humans, *Klebsiella* spp. seems to prefer the colon with a detection rate of up to 35% (Pomakova et al., 2011). The difference in sampling site could give an unrepresentative picture of what the actual microbial diversity is in the GI-tract in the different animals.

There are many contributing factors that effects the gut microbiota, such as diet, environment, genetics and use of antibiotics (Xiaofan *et al*, 2019). Another factor that could influence the presence of *Klebsiella* spp. could be that the different animals have different pH in their GI-tract. The mean GI- pH in swine is 4.4, and pH in the small intestine is 6.1-6.7 (Merchant *et al*., 2011), dogs have a mean GI- pH of 2.05 (Sagawa K et al., 98). *Klebsiella* spp. prefers pH 7.2 (Ristuccia *et al.*, 1984) and the pH in the animals can therefore be a contributing factor to the variations in prevalence of *klebsiella* spp. identified between animals in this thesis. The variations in pH along the GI- tract could also affect *Klebsiella* spp.´s ability to colonise the different parts of the GI tract. According to the isolation results, the detection rate was higher in swine than in dogs, which makes sense due to the higher GI-pH in swine. Despite *Klebsiella* spp. preference for an almost pH neutral environment, the

fact that strains were isolated from all five animal species indicates that they do have a certain tolerance for variations in pH, given the right conditions. *Klebsiella* spp. is an opportunistic pathogen which means that it can be present and harmless in a healthy host. But when the host`s immune system is compromised or other competing bacteria are reduced or removed, such as with the use of antibiotics, it can cause an infection (Selden *et al.*, 1971). It is therefore important to know if the animals used in this thesis have been subjected to antibiotic treatments previously, and if this antibiotic treatment has been intramuscular or oral (Simonsen et al, 2010). If the antibiotic has been given orally this increases the risk of other bacteria that are part of the gut microbiota being reduced, which could give *Klebsiella* spp. an opportunity to cause an infection (Palleja et al., 2018). In Norway the use of antibiotics in livestock is strictly controlled and monitored, but in other countries it is used more liberally, not just to combat infections but also as a growth enhancer (Ryan, 2019). For instance, in Norway broiler was given the antiparasitic Naracin and turkey were given Monensin as a preventive measure to reduce parasites in the intestines (forskning.no, 2014). Monensin it is still given to turkeys, but broiler is no longer treated with Narasin, and this might have an effect on their gut microbiota. Since the detection rate of *Klebsiella* spp. is much higher in turkey (69.57%) versus broiler (23.51%), the difference might be because of the use of such preventative compounds.

Animals have different microbiomes in their GI-tract which could possibly affect the prevalence of *Klebsiella* spp. Broiler, turkey, swine, cattle, have mostly *Firmicutes* and *Bacteroidetes* in their GI- tract, followed by *Proteobacteria, and Actinobacteria* (Mohammad *et al.*, 2018; Oakley *et al.*, 2014; Wilkinson *et al.*, 2017; Xiaofan *et al.*, 2019). Dogs on the other hand also have *Fusobacteria* as part of their core microbiota (Coelho *et al*., 2018). Although these bacteria belong to the same phylum for all five animals, a closer look at which species are present might give insight into whether they could cause the variation of *Klebsiella* spp. presence and abundance. This would also depend on our understanding of the interactions between these bacteria and *Klebsiella* spp. since it has been shown that nutrition has an effect on

shaping the microbiota (Carmody *et al.*, 2015). Nutrients in milk, which swine, dogs and cattle are given from birth, such as oligosaccharides, amino acids, and fat activate digestive enzymes and chemical secretions, which help establish a certain gut microbiota (Everaert *et al.*, 2017). Later in life dogs mostly eat dogfood, consisting of mostly animal protein while cattle eat grass, hay or silage. Swine are omnivores, but as a production animal they are fed corn, soybean with added vitamins and minerals and poultry have a high protein diet to quickly gain weight (Patience *et al*., 2015; Ravindran, 2013). These different diets could influence the intestinal microbiota and thus also have an effect on the prevalence and abundance of *Klebsiella* spp.

There were also large variations in which *Klebsiella* spp. were most successfully isolated. In turkey there was only *K. pneumoniae*, and collectively from all the five animals 94.21% of the *Klebsiella* spp. isolated where *K. pneumoniae*. The MALDI-TOF version used distinguished between *K. pneumoniae, K. variicola, K. oxytoca* and *K. aerogenes* so this could not be the reason for why *K. pneumoniae* showed such predominance. All four *Klebsiella* spp. that were identified during this thesis have previously been detected in faecal matter. One theory could be that *K. variicola, K. oxytoca* and *K. aerogenes* have a slightly different metabolism than *K. pneumoniae*, making faecal matter not the favourable environment.

PCR was used to investigate if this was an adequate method to identify *Klebsiella* spp. directly from the faecal matter. To establish this, pure DNA extracted from *K. pneumoniae* isolates from swine samples from the Thailand project (REDUCEAMU) was first used. When using extracted *K. pneumoniae* DNA the CT values ranged from 11.13 to 20.71 depending on dilution. However, when using pure culture *K. pneumoniae* colonies (DNA extracted by the boil lysis method), the CT level increased to 21.04, which could be caused by improper cell lysis or the presence of cell debris. This “noise” could distort the PCR results. Also, prior to the PCR, the bacterial solution was vortexed rather than centrifuged, after the DNA extraction, causing a mixture of DNA and cell debris rather than separating the components. There was also a trial with faecal matter from swine, but this

procedure needs large alterations, because simply boiling and washing the faecal matter twice with SDV was not adequate to obtain usable results. This protocol has previously been tested at the Pasteur Institute on environmental samples such as soil and vegetables, but there is a big difference in the components of the sample from soil, vegetable and faecal matter. Boiling does not only cause lysis of *Klebsiella,* but also of other bacterium and components in the faecal matter that may interfere with the PCR primers and the amplification process.
With pure DNA (extracted using a commercial kit) however, the method works to identify *Klebsiella*. But the primer used for detecting the *Klebsiella* was not for 16S rRNA. Instead, they were designed to attach prior to the gene hemolysin and the bacteria must then contain this gene for it to be detected. Since the PCR detection method is designed to only identify *K. pneumoniae* in a sample*,* then the presence of other *Klebsiella* strains would not be detected. Another important aspect of the PCR method is that, unlike culture-based detection methods, which require that the bacteria are alive and can grow into colonies on the agar, PCR works as long as the DNA is present in the sample. Hence, you run the risk of trying to grow cultures from a sample where the PCR indicates the presence of *Klebsiella* spp. but where no live bacteria are present to use for further analysis.

All the *Klebsiella* spp. isolates underwent susceptibility testing by disk diffusion and the overall results indicate that only 7.37% of the 380 isolates were resistant to additional antibiotics other than ampicillin. In addition, 0.79% were MDR. Across the board, all the animal species tested from both Norway and Thailand indicated highest resistance against tetracycline. An interesting observation was that 9.82% of the isolates from turkey showed resistance to this antimicrobial agent, but only 4.21% from broiler. The living conditions for turkey and broiler is relatively similar, they are both poultry, and their physiology is relatively similar. So why is there such a big difference?
In this thesis, all isolates were subject to disc diffusion tests, but only the isolates indicating resistance to more than ampicillin was further tested with MIC. Because of this, it was not until analysis of the WGS data, where the genes for *sul1* and *sul2*

were identified, that the isolates that first showed susceptibility to the Sulfamethoxazole-Trimethoprim compound were retested on the MIC plates and there the resistance was confirmed. This resulted in 3.16% of the isolates showing resistance to Sulfamethoxazole. Another weakness with this test is that the MIC plates do not include streptomycin, nitrofurantoin and fosfomycin. Thereby losing a quality control test to identify the degree of resistance. As a result, the isolates that may have been resistant against these two compounds where not uncovered by MIC testing and confirmed.

When testing on the MIC plates, tetracycline, sulfamethoxazole and chloramphenicol had growth in all the MIC- plate wells. Tetracycline had a MIC concentration of more than 64 mg/l, sulfamethoxazole more than 1024mg/l and, and chloramphenicol more than 128 mg/l. These results indicate that when the gene causing resistance to these antimicrobial agents are present, they cause high-level resistance to their antimicrobial agent once the gene is transcribed and translated.

Among the swine isolates from Thailand, 48% showed resistance against tetracycline. Comparatively, the swine samples from Norway had very low resistance rates. The question then becomes, -is this due to the environment, diet, use of antibiotics or something else entirely? It is easy to assume that the discrepancy is caused by different environments as the climate and living conditions greatly differ from Thailand and Norway. Also, the use of antimicrobial agent in Thailand (and several other countries) is less controlled than in Norway and several antibiotics are easier to come by (Nhung et al., 2016). A similar trend of high levels of antimicrobial resistance in *Klebsiella* spp. has also been identified in humans in India (Mondal, *et al.,* 2016). The next step would be to look at the transmission between animals and people. Because swine is such ubiquitous source of protein around the world and the fact that a large amount of people travel all over the globe, it is important to investigate the transmission of antibiotic resistance between swine and humans. This has not been conducted in this thesis but could be done in the future. When double checking the 20 isolates that contained ESBL genes, the ResFinder showed that they were all highly similar and with only one or two SNP in difference. According to the different types of mutations, one SNP can in theory

change the entire effect of the protein (Watson *et al*.,2014). If the promotor region is altered the gene would be registered as present, but not be expressed, but further research is needed to determine the cause of this result.

A comparison of antimicrobial resistance was done with *E. coli* isolates collected from the same poultry samples in 2018 through the NORM-VET screening program (table 6). Overall in this sampling the prevalence of AMR was fairly similar for *E. coli* and *Klebsiella* spp., for the six selected antimicrobial agents, except for ciprofloxacin. In *E. coli* the prevalence was ten times higher than in *Klebsiella* spp. This is an unsettling find since ciprofloxacin is a last resort antimicrobial agent (Statens legemiddelverk, 2019). These samples originated from healthy poultry, and since *Klebsiella* spp. is an opportunistic pathogen, it would be interesting to investigate differences in antimicrobial resistance in healthy and sick animals. Another interesting aspect is that the detection rate for *E. coli* in the poultry samples were 99% in both broiler and turkey. Taking that into consideration, since *E. coli* should raise a slightly higher concern than *Klebsiella* spp., for the time being.
The WGS displayed that the 46 isolates encoding for yersiniabactin were greatly distributed among the STs. Thirteen of the yersiniabactin isolates grouping within ST290-1LV also encoded aerobactin and salmochelin, making them highly virulent isolates, with a virulence score of 4. Yersiniabactin was also identified in ST290 that is related to ST290-1LV which are in the same clustering (figure 17). In the 15 isolates that encoded for them, both aerobactin and salmochelin was the only STs where virulence genes overlapped. There is no other overlap with either antimicrobial resistance genes or with the three different virulence genes in the remainder of the STs. This could be because when the bacterium inhabits either virulence or AMR genes, they have a stronger defence mechanism and a higher chance of survival. However, carrying both gene types could be an unnecessary genetic weight in most environments. There was one additional strain positive for the gene encoding salmochelin, identified as ST-969, but in the phylogenetic tree this ST is highly diverse from the 15 other isolates with salmochelin. A possible explanation could be a misidentification during assembly, placing the isolate in the

wrong ST group. Since the MLST placing the isolates into the different STs is based on seven housekeeping genes, the results from the annotation based on over 2000 genes might give a more accurate result, at least in theory. There is another possible flaw in the displayed tree, as one isolate of ST35 is not grouped together with all the other ST35 and not even in the same branch. Instead, it is closer to ST37.
According to the MLST results, many of the STs have previously been identified in humans and geographically in Asia, Africa, Europe, Oceania and North- and South America (Pasteur inst./MLST). In July 2019 there was a paper from Wang *et al*. stating that *K. pneumoniae* isolates with ST290 was the cause of a hospital outbreak in China. This makes *Klebsiella* spp. a bit scarier, because that means the same ST can be identified in both humans and animals, which increases the chance of zoonotic transfer. But even if a bacterium is found in animals, the microbe still must adapt to a different host, so it is not certain that the bacteria will be pathogenic in humans. There are several factors that need to be taken into consideration, such as host temperature, mucosal membranes or epithelial cells in the respiratory tract and GI-tract, as these can vary between different hosts.

The phylogenetic tree showed that there are several STs that cluster together that originate from both broiler and turkey even though the poultry are from different farms. Among the 15 isolates belonging to ST290-1LV that all carried salmochelin and aerobactin genes, 13 of them also carried yersiniabactin genes and the strains originated only from Turkey. Geographically, the turkey farms were distributed across different counties. The turkey farms in Norway get their breeding animals (grandparents) from the United Kingdom (Mo, *et al.*, 2016) This could be a reason for the widespread geographical distribution, since they all originate from the same source in the UK. Further analysis of the plasmid, identified in these 15 isolates, which contains aerobactin and salmochelin was identified as Inc-F plasmid. According to research by Kat Holt research group, the virulence genes aerobactin (*iuc 5*) and salmochelin (*iro 5*) both originate from *E. coli* Inc-F plasmid and are often found together.

K. pneumoniae has the ability to take up genes via horizontal gene transfer. Therefore, the isolates ability to produce biofilm was tested since this can be an environment for DNA/ plasmid exchange (Nesse et al., 2014). Comparing the biofilm forming abilities between the animals, there was only a statistically significant difference when comparing turkey to broiler (p value: 0.009), swine (p value: 0.001), dog (p value: 0.030), and cattle (p value:0.041). All other comparisons resulted in a p value < 0.05 when performing the t-test. These results could be caused by the large discrepancy in number of isolates between the animals, especially cattle and dogs.

When comparing the biofilm forming abilities of isolates from swine in Norway and Thailand. The isolates from the Norwegian swine had a better biofilm production (p value: 0.041) than their Thai counterparts, which is very interesting given the fact that the isolates from Thailand have a higher antimicrobial resistance rate than the Norwegian swine. This could be caused by the fact that biofilm is a mode of survival under subpar conditions (Nesse, *et al.,* 2014). Since the bacteria contains one or several antimicrobial genes it could be better suited to survive in conditions were competing microbes cannot, and therefore the need to be a decent biofilm producer decreases. To properly answer this question, WGS data on the isolates from Thailand would be required.

Identification of the biofilm morphology needs more testing, because only one out of six isolates tested produced biofilm on the agar plate. The five other isolates seemed to need more stimulation to initiate biofilm production than were given in this thesis. One explanation could be that the isolate producing biofilm on the congo red agar has the ability to utilize cellulose and encode for curly fimbriae, which can be associated with the pink morphology identified (Zogaj *et al.*, 2003). Or perhaps by changing the pH in the agar it can trigger biofilm production. The pH in LB agar usually lies around 7.0. Since *Klebsiella* has an optimum of pH 7.2, it is possible that the pH of the agar needs to be changed in order to stress the bacteria further. Another possible way to stress the bacteria is by starving it. The congo red agar plate might contain too many nutrients and is not a stressful enough environment to

kick start the biofilm production. A simple test would then be to remove NaCl from the agar and see if that triggers the biofilm production. A third option could be to incubate at a higher temperature. According to the literature, *E. coli* has a difference in biofilm forming abilities when being incubated at 20°C versus 37°C (Zogaj *et al.*, 2003). In this thesis, all the isolates were incubated at 20°C to try and simulate external environments, since biofilm is a way of surviving in suboptimal conditions. Since some of the *Klebsiella* had the ability to produce biofilm at 20°C, then perhaps an option could be to increase the temperature to match the core temperature, 42°C, of some animals, such as dogs or poultry (Purswell et al., 2012; Sousa et al., 2011). When comparing the biofilm forming ability to either STs or capsule types, there were no clear pattern. This could be because there were only 44 different capsule types and 65 different STs tested against the biofilm production, and several of the both capsule types and STs only had one or a few isolates. Because of this, a larger sample size both in variating STs and capsule types, and number of isolates per type should be tested further to be able to obtain a concluding result.

5. Conclusion

In conclusion, the overall antimicrobial resistance occurrence among *Klebsiella* spp. from Norwegian domestic animals was low. Among the resistant isolates resistance against tetracycline and sulfamethoxazole was most commonly observed. Virulence genes encoding, yersiniabactin, salmochelin and aerobactin, were detected in some of the isolates, with salmochelin and aerobactin encoding genes located on incF plasmids. Further analysis would be recommended in regards to ST290 and ST290-1LV and the detected virulence genes. Lastly, *Klebsiella* spp. originating from Norwegian samples are generally good biofilm producers.

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Appendix

Breakpoint table extracted from EUCAST

Antimicrobial agent		*Klebsiella spp.*		ATCC *E. coli*
		Disk size resistant	MIC concentration	Disk size
		mm	mg/l	mm
AMP	Ampicillin	14	16	15-22
C	Chloramphenicol	17	32	21-27
CAZ	Ceftazidime	19	1	23-29
CIP	Ciprofloxacin	17	0.12	29-37
CTX	Cefotaxime	17	0.5	25-31
COL	Colestin		4	
F	Nitrofurantoin	11		17-23
FEP	Cefepime	24	0.125	31-37
FOS	Fosfomycin	24		26-34
NA	Nalidixic acid	16	32	22-28
GEN	Gentamicin		4	
MEM	Meropenem	16	0.25	28-35
STR*	Streptomycin			
SXT	Trimethoprim/ sulfamethoxazole	11		23-29
SMX	Sulfamethoxazole		128	
TE	Tetracycline	19	16	19
TGC	Tigecycline	18	1	20-27
W	Trimethoprim	15	4	21-28
AZM	Azithromycin		32	

Disc size (mm): clinical breakpoints. MIC (mg/l): epidemiological breakpoint

* NVI iin-house breakpoint.

Disc diffusion results from broiler and turkey. Only showing isolates with resistance.

Sample ID	C 30 S ≥17 R≤17	CIP 5 S ≥17 R≤17	W 5 S ≥18 R≤15	CTX 5 S ≥20 R≤17	FEP 30 S ≥27 R≤ 24	TE 30 S ≥19 R≤19	AMP 10 S ≥14 R≤14	MEM 10 S ≥22 R≤16	CAZ 10 S ≥22 R≤19	CN 10 S ≥17 R≤14	NA 30 S ≥16 R≤16	AZM 15	TGC 15 S ≥18 R≤18	FOS 50 S ≥24 R≤24	F 100 S ≥11 R≤11	STR 10	SXT 25 S ≥14 R≤11
2018-01-473	27	29	23	27	33	0	0	31	27	19	21	11	20	24	11	13	ND
2018-01- 715	23	24	0	29	31	0	0	30	27	17	17	10	19	24	11	20	0
2018-01- 1116	24	29	26	29	32	0	0	33	27	19	25	15	21	24	16	20	29

2018-01- 1178	25	30	26	29	31	0	9	31	25	20	23	14	20	24	15	20	25
2018-01-1445	25	29	30	30	35	0	0	33	29	21	25	15	20	26	13	20	29
2018-01-1585	27	30	29	29	33	23	10	32	28	21	27	16	21	25	0	20	29
2018-01-1685	27	32	28	27	32	0	0	31	26	23	25	16	22	25	17	20	32
2018-01-2359	27	32	27	31	33	22	11	33	27	22	25	15	20	24	0	21	30
2018-01-2640	23	28	27	26	30	0	0	30	26	20	25	14	21	24	12	19	30
2018-01-2716	26	27	28	27	31	25	9	32	27	21	26	15	20	24	0	19	29
2018-01-2730	23	29	26	26	33	0	0	30	26	21	24	15	22	25	11	20	30
2018-01-2939	23	31	27	27	30	0	0	31	25	21	25	13	20	25	14	19	30
2018-01-3030	23	30	28	28	33	0	0	33	26	23	24	15	20	24	16	20	20
2018-01-3163	25	31	26	27	31	0	0	29	25	19	23	14	20	24*	16	19	29
2018-01-3590	26	31	26	26	33	0	9	31	27	20	25	14	21	24	16	ND	30
2018-01-3975	23	28	26	29	29	0	0	30	27	20	25	14	20	24	11	18	29
2018-01-4113	25	31	26	26	33	0	0	29	30	19	26	14	20	25	22	17	29
2018-01-4639	26	29	27	25	31	0	0	30	25	22	24	14	19	24	11	19	28
2018-01-4682	0	29	25	25	33	22	0	29	26	20	24	13	20	24	15	18	29
2018-01-4736	25	29	25	28	34	25	0	30	25	19	23	12	20	15	14	17	27

Disc diffusion results on isolates originating from swine, dog and cattle. Only resistant isolates displayed.

Sample ID	C 30 S ≥17 R≤17	CIP 5 S ≥17 R≤17	W 5 S ≥18 R≤15	CTX 5 S ≥20 R≤17	FEP 30 S ≥27 R≤ 24	TE 30 S ≥19 R≤19	AMP 10 S ≥14 R≤14	MEM 10 S ≥22 R≤16	CAZ 10 S ≥22 R≤19	CN 10 S ≥17 R≤14	NA 30 S ≥16 R≤16	AZM 15	TGC 15 S ≥18 R≤18	FOS 50 S ≥24 R≤24	F 100 S ≥11 R≤11	STR 10	SXT 25 S ≥14 R≤11
2019-01-794	6	29	29	29	34	24	0	31	27	21	24	14	20	24	21	17	20
2019-01-865	27	30	31	31	32	23	0	33	27	25	21	18	21	0	8	20	30
2019-01-1961	25	30	27	29	ND	24	0	31	27	21	22	15	19	23	17	0	28
2019-01-3159	25	30	0	30	33	0	0	30	29	22	24	13	21	25	14	0	0
2019-01-3179	25	30	25	28	34	24	0	31	26	21	24	14	20	0	17	19	18
2019-01-3588	23	29	25	27	33	0	0	31	24	21	23	12	20	23	15	21	28

Disc diffusion results from isolates originating from swine in Thailand. Raw data table made by Thongpan Leangpigchart

Sample ID	AMP 10	FEP 30	CAZ 10	CTX 5	MEM 10	CN 10	AK	TE 30	CIP 5	SXT 25	C 30	FOS 50	TGC 15	CT
	S ≥14 R≤14	S ≥27 R≤ 24	S ≥22 R≤19	S ≥20 R≤17	S ≥22 R≤16	S ≥17 R≤14	S ≥18 R≤14	S ≥19 R≤19	S ≥17 R≤17	S ≥14 R≤11	S ≥17 R≤17	S ≥24 R≤24	S ≥18 R≤18	
1PK	R	S	S	S	S	S	S	S	S	S	S	21	20	17
5PK	R	S	S	S	S	S	S	S	S	S	S	25	20	18
9PK	R	S	S	S	S	S	S	S	S	S	S	24	19	17
10PK	R	R	R	R	S	S	S	R	S	S	R	23	21	17
13PK	R	S	S	S	S	S	S	R	S	R	R	23	19	17
15PK	R	S	S	S	S	S	S	S	S	S	S	22	19	17
16PK	R	S	S	S	S	S	S	S	S	S	S	25	22	18
17PK	R	S	S	S	S	S	S	R	S	R	S	23	20	18
19PK	R	S	S	S	S	S	S	R	S	R	S	23	19	16
20PK	R	S	S	S	S	S	S	S	S	S	S	22	21	17
21PK	R	S	S	S	S	S	S	R	S	S	S	0	22	17
23PK	R	S	S	S	S	S	S	R	S	S	S	23	17	17
25PK	R	S	S	S	S	S	S	S	S	S	S	24	20	17
26PK	R	S	S	S	S	S	S	S	S	S	S	22	21	17
27PK	R	S	S	S	S	S	S	R	R	R	S	25	18	17
31PK	R	S	S	S	S	S	S	S	S	S	S	24	21	18
38PK	R	S	S	S	S	S	S	R	S	R	S	25	20	17
39PK	R	S	S	S	S	S	S	R	S	R	R	23	19	10
40PK	R	S	S	S	S	S	S	S	S	S	S	26	21	18
41PK	R	S	S	S	S	S	S	S	S	S	S	21	20	0
43PK	R	S	S	S	S	S	S	S	S	S	S	0	20	17
49PK	R	S	S	S	S	S	S	S	S	S	S	24	18	18
50PK	R	S	S	S	S	S	S	R	S	R	R	22	16	17
51PK	R	S	S	S	S	S	S	S	S	S	S	0	20	17

55PK	R	S	S	S	S	S	S	S	S	S	S	0	18	17
56PK	R	S	S	S	S	S	S	R	S	S	S	21	19	17
57PK	R	S	S	S	S	S	S	R	S	S	S	24	18	17
60PK	R	S	S	S	S	S	S	S	S	S	S	25	18	17
70PK	R	S	S	S	S	S	S	S	S	S	S	22	19	18
76PK	R	S	S	S	S	S	S	S	S	S	S	24	20	17
77PK	R	S	S	S	S	S	S	S	S	S	S	25	21	17
78PK	R	S	S	S	S	S	S	S	S	S	S	22	19	19
79PK	R	S	S	S	S	S	S	S	S	S	S	22	20	17
80PK	R	S	S	S	S	S	S	S	S	S	S	23	17	17
83PK	R	S	S	S	S	S	S	R	S	R	S	21	19	17
85PK	R	S	S	S	S	S	S	S	S	R	S	23	21	16
86PK	R	S	S	S	S	S	S	S	S	S	S	21	18	17
87PK	R	S	S	S	S	S	S	S	S	S	S	21	23	17
88PK	R	S	S	S	S	S	S	S	S	S	S	21	19	17
89PK	R	S	S	S	S	S	S	R	S	S	S	22	19	17
90PK	R	S	S	S	S	S	S	S	S	S	S	27	20	17
95PK	R	S	S	S	S	S	S	S	S	S	S	21	18	15
96PK	R	S	S	S	S	S	S	S	S	S	S	21	19	17
99PK	R	S	S	S	S	S	S	S	S	S	S	20	19	17
101PK	R	S	S	S	S	S	S	R	S	R	S	22	21	10
104PK	R	S	S	S	S	S	S	R	S	S	S	23	20	17
105PK	R	S	S	S	S	S	S	S	S	S	S	21	19	16
127PK	R	S	S	S	S	S	S	R	S	R	S	18	19	16
137PK	R	S	S	S	S	S	S	R	S	S	S	25	17	21
139PK	R	S	S	S	S	S	S	S	S	S	S	18	19	17
140PK	R	S	S	S	S	S	S	S	S	S	S	22	19	17
143PK	R	S	S	S	S	S	S	S	S	S	S	22	17	0

Disc diffusion results for control strains E. coli ATCC 22925 and K. pneumoniae AMR isolate

Sample ID	C 30	CIP 5	W 5	CTX 5	FEP 30	TE 30	AMP 10	MEM 10	CAZ 10	CN 10	NA 30	AZM 15	TGC 15	FOS 50	F 100	STR 10	SXT 25
	21-27	29-37	21-28	25-31	31-37	19	15-22	28-35	23-29	19-26	22-28		20-27	26-34	17-23		23-29
25922	23	33	23	25	33	21	15	31	25	21	23	13	19	33	21	13	ND
25922	21	31	23	25	31	21	23	33	27	21	23	15	21	35	15	21	27
25922	23	33	25	27	31	23	20	33	23	19	25	18	21	31	21	19	25
25922	27	33	29	29	36	26	20	35	27	22	28	17	25	33	24	19	29
25922	23	32	27	27	33	23	16	32	25	22	28	15	21	31	22	18	27
25922	23	35	28	30	33	23	18	33	26	21	28	16	26	31	22	18	29
25922	21	33	28	27	33	23	20	33	26	23	28	18	24	30	26	17	29
25922	25	31	27	27	32	22	16	31	23	22	28	15	23	28	22	17	29
25922	25	33	27	27	33	23	17	33	25	21	28	16	25	29	23	19	29
25922	21	30	26	27	33	24	15	32	25	19	28	16	24	32	21	17	29
25922	22	29	28	28	31	24	15	33	25	22	27	17	24	32	22	19	29
25922	23	33	28	27	33	24	16	33	25	22	27	15	24	32	23	19	28
25922	23	34	26	28	36	26	17	33	26	21	28	15	22	30	23	ND	29
25922	25	25	27	27	33	26	17	33	25	21	27	15	25	31	22	ND	29
25922	24	30	25	26	34	23	16	32	25	21	28	15	24	31	22	18	29
25922	23	35	26	27	33	24	16	31	26	21	28	15	24	31	22	17	30
25922	22	33	26	28	32	24	16	31	27	20	27	17	24	32	22	16	28
25922	21	29	28	27	31	22	16	33	25	20	26	14	25	31	22	15	29
25922	21	33	25	26	31	25	16	30	25	22	26	14	23	30	19	16	28
25922	25	31	27	27	32	22	16	31	23	22	28	15	23	28	22	17	29
25922	25	33	27	27	33	23	17	33	25	21	28	16	25	29	23	19	29
25922	21	30	26	27	33	24	15	32	25	19	28	16	24	32	21	17	29
25922	24	30	25	26	34	23	16	32	25	21	28	15	24	31	22	18	29
25922	22	33	26	28	32	24	16	31	27	20	27	17	24	32	22	16	28

25922	21	29	28	27	31	22	16	33	25	20	26	14	25	31	22	15	29
25922	21	33	25	26	31	25	16	30	25	22	26	14	23	30	19	16	28
25922	21	33	25	26	31	25	16	30	25	22	26	14	23	30	19	16	29
25922	24	31	27	28	31	23	ND	34	28	22	27	16	24	32	21	16	28
25922	24	30	27	29	34	24	17	33	26	21	26	17	23	31	22	18	29
25922	23	33	28	30	33	25	17	34	27	22	27	16	23	31	22	18	28
25922	23	35	28	30	34	23	17	33	26	22	ND	17	25	31	22	16	29
25922	21	34	27	28	31	23	17	31	28	24	27	16	23	31	21	17	28
25922	23	33	28	30	ND	25	17	34	28	23	28	17	25	32	22	17	29
25922	22	33	28	27	ND	25	16	33	26	22	28	15	23	33	23	18	29
25922	24	35	27	28	31	23	17	35	27	21	27	16	23	30	22	16	28
25922	24	33	26	29	31	24	18	33	28	21	27	15	23	30	22	16	28
25922	22	32	28	27	33	24	17	31	ND	22	27	17	24	32	22	17	29
25922	23	32	27	27	32	22	17	30	26	20	27	13	23	30	22	17	28
25922	23	33	27	26	34	23	ND	33	25	21	28	14	25	24	23	19	31
25922	23	33	30	28	33	25	18	31	26	19	26	14	22	30	22	17	28
2013-01-5243	28	0	0	0	26	0	0	31	13	0	0	15	18	26	15	0	0
2013-01-5243	26	0	0	0	19	0	0	31	12	0	0	16	20	25	15	0	0
2013-01-5243	25	0	0	0	18	0	0	33	13	0	0	ND	19	26	17	0	0
2013-01-5243	25	0	0	0	18	0	0	32	13	0	0	14	19	25	15	0	0
2013-01-5243	25	0	0	0	15	0	0	30	12	0	0	14	19	25	15	0	0
2013-01-5243	24	0	0	0	17	0	0	29	13	0	0	14	19	24	16	0	0
2013-01-5243	27	0	0	0	14	0	0	30	12	0	0	13	18	24	15	0	0
2013-01-5243	28	0	0	0	19	0	ND	31	13	0	0	14	20	25	17	0	0
2013-01-5243	27	0	0	0	20	0	ND	33	ND	6	0	14	19	25	16	0	0
2013-01-	28	0	0	0	26	0	0	31	13	0	0	15	18	26	15	0	0

5243																
2013-01-5243	26	0	0	0	19	0	0	31	12	0	0	16	20	25	15	0	0
2013-01-5243	25	0	0	0	18	0	0	33	13	8	0	ND	19	26	17	0	0

MIC results from isolates originating from broiler & turkey

Sample	SMX	TMP	CIP	TET	MERO	AZI	NAL	FOT	CHL	TGC	TAZ	COL	AMP	GEN
2018-01- 1116	≤ 8	0.5	0.03	> 64	≤ 0.03	8	≤ 4	≤ 0.25	≤ 8	0.5	≤ 0.5	≤ 1	32	≤ 0.5
2018-01- 1178	16	≤ 0.25	0.03	> 64	≤ 0.03	16	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	32	≤ 0.5
2018-01- 715	> 1024	> 32	0.5	> 64	≤ 0.03	16	8	≤ 0.25	≤ 8	0.5	≤ 0.5	≤ 1	64	≤ 0.5
2018-01-1022	> 1024	≤ 0.25	≤ 0.015	≤ 2	≤ 0.03	8	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	32	≤ 0.5
2018-01-1319	≤ 8	≤ 0.25	0.06	≤2	0.06	16	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	2	32	≤ 0.5
2018-01-1445	≤ 8	≤ 0.25	0.03	> 64	≤ 0.03	8	≤ 4	≤ 0.25	≤ 8	1	≤ 0.5	≤ 1	32	≤ 0.5
2018-01-1584	> 1024	0.5	0.03	≤ 2	≤ 0.03	8	≤ 4	≤ 0.25	≤ 8	0.5	≤ 0.5	≤ 1	32	≤ 0.5
2018-01-1585	16	1	0.03	> 64	≤ 0.03	8	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	16	≤ 0.5
2018-01-1593	> 1024	1	0.03	≤ 2	≤ 0.03	8	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	64	≤ 0.5
2018-01-1685	≤ 8	0.5	0.03	> 64	≤ 0.03	16	≤ 4	≤ 0.25	≤ 8	0.5	≤ 0.5	≤ 1	32	≤ 0.5
2018-01-2359	16	0.5	0.03	≤ 2	≤ 0.03	8	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 0.5	32	≤ 0.5
2018-01-2640	≤ 8	0.5	0.06	> 64	≤ 0.03	16	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	> 64	≤ 0.5
2018-01-2643	16	0.5	0.03	≤ 2	≤ 0.03	16	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	32	≤ 0.5
2018-01-2716	≤ 8	0.5	0.03	≤ 2	≤ 0.03	8	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	16	≤ 0.5
2018-01-2730	16	0.5	0.03	> 64	≤ 0.03	16	≤ 4	≤ 0.25	≤ 8	0.5	≤ 0.5	≤ 1	> 64	≤ 0.5
2018-01-2939	≤ 8	0.5	0.03	> 64	≤ 0.03	16	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	32	≤ 0.5
2018-01-3030	> 1024	1	0.06	> 64	≤ 0.03	16	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	32	≤ 0.5
2018-01-3163	32	0.5	0.03	> 64	≤ 0.03	16	≤ 4*	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	32	≤ 0.5
2018-01-3590	32	0.5	0.06	> 64	≤ 0.03	16	≤ 4	≤ 0.25	16	≤ 0.25	≤ 0.5	≤ 1	32	≤ 0.5
2018-01-3692	> 1024	≤ 0.25	≤ 0.015	≤ 2	≤ 0.03	8	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	32	≤ 0.5

2018-01-3934	> 1024	≤ 0.25	0.03	≤ 2	≤ 0.03	16	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	64	≤ 0.5
2018-01-3975	16	0.5	≤ 0.015	> 64	≤ 0.03	16	≤ 4	≤ 0.25	≤ 8	0.5	≤ 0.5	≤ 1	> 64	≤ 0.5
2018-01-4107	> 1024	≤ 0.25	≤ 0.015	≤ 2	≤ 0.03	8	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	32	≤ 0.5
2018-01-4113	16	0.5	≤ 0.015	64	≤ 0.03	16	≤ 4	≤ 0.25	≤ 8	0.5	≤ 0.5	≤ 1	16	≤ 0.5
2018-01-4248	32	0.5	0.03	> 64	≤ 0.03	32	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	64	≤ 0.5
2018-01-4639	16	0.5	0.06	> 64	≤ 0.03	8	≤ 4	≤ 0.25	≤ 8	0.5	≤ 0.5	≤ 1	64	≤ 0.5
2018-01-4682	≤ 8	0.5	0.06	≤ 2	≤ 0.03	16	≤ 4	≤ 0.25	> 128	≤ 0.25	≤ 0.5	≤ 1	32	≤ 0.5
2018-01-473	≤ 8	≤ 0.25	0.03	> 64	≤0.03	16	≤4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	> 64	≤ 0.5
2018-01-4736	32	0.5	0.06	≤ 2	≤ 0.03	16	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	64	≤ 0.5
2018-01-582	> 1024	0.5	0.06	≤ 2	≤ 0.03	16	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	32	≤ 0.5
2018-01-798	> 1024	≤ 0.25	0.03	≤ 2	≤ 0.03	16	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	32	≤ 0.5
ATCC 25922	16	0.5	≤ 0.015	≤ 2	≤ 0.03	4	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	4	≤ 0.5
ATCC 25922	32	≤ 0.25	≤ 0.015	≤ 2	≤ 0.03	4	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	4	≤ 0.5
ATCC 25922	32	0.5	≤ 0.015	≤ 2	≤ 0.03	4	≤ 4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	2	≤ 0.5

MIC results for isolates originating from swine and dog samples

Sample	SMX	TMP	CIP	TET	MERO	AZI	NAL	FOT	CHL	TGC	TAZ	COL	AMP	GEN
2019-01-794	>1024	1	0.06	≤ 2	≤0.03	16	≤4	≤ 0.25	128	0.5	≤ 0.5	≤ 1	> 64	≤ 0.5
2019-01-865	≤ 8	≤ 0.25	≤ 0.015	≤ 2	≤0.03	4	≤4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	64	≤ 0.5
2019-01-1961	16	0.5	0.03	≤ 2	≤0.03	8	≤4	≤ 0.25	≤ 8	0.5	≤ 0.5	≤ 1	32	≤ 0.5
2019-01-3159	>1024	>32	0.03	>64	≤0.03	16	≤4	≤ 0.25	≤ 8	0.5	≤ 0.5	≤ 1	> 64	≤ 0.5
2019-01-3179	16	1	0.03	≤ 2	≤0.03	16	≤4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	16	≤ 0.5
2019-01-3588	32	0.5	0.03	>64	≤0.03	16	≤4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	> 64	≤ 0.5
ATCC 25922	32	0.5	≤ 0.015	≤ 2	≤0.03	4	≤4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	8	≤ 0.5
ATCC 25922	16	0.5	≤ 0.015	≤ 2	≤0.03	4	≤4	≤ 0.25	≤ 8	≤ 0.25	≤ 0.5	≤ 1	4	≤ 0.5

WGS data extracted from Kleborate results of isolates originating from poultry

Sample ID	Source	Species match	Contig count	N50	Largest contig	ST
2018-01- 1000	Turkey	strong	62	323024	420632	ST113
2018-01- 1001	Turkey	strong	67	337942	619081	ST35
2018-01- 1007	Turkey	strong	76	301373	799888	ST37
2018-01- 1022	Turkey	strong	65	373508	618818	ST35
2018-01- 1024	Turkey	strong	80	456386	1255920	ST590
2018-01- 1097	Turkey	strong	96	261292	479588	ST290-1LV
2018-01- 1116	Turkey	strong	87	392135	688987	ST550-1LV
2018-01- 1117	Broiler	strong	79	317389	812630	ST2441
2018-01- 1148	Broiler	strong	73	322327	519103	ST290
2018-01- 1176	Broiler	strong	62	328646	1320170	ST290
2018-01- 1178	Turkey	strong	88	316804	1294582	ST1823
2018-01- 1214	Broiler	strong	66	378033	922004	ST528-1LV
2018-01- 1256	Turkey	strong	50	265646	1276841	ST151
2018-01- 706	Turkey	strong	79	456386	1255918	ST590
2018-01- 707	Broiler	strong	98	261287	479588	ST290-1LV
2018-01- 709	Broiler	strong	90	274027	604930	ST35
2018-01- 710	Broiler	strong	58	648080	924350	ST590
2018-01- 711	Broiler	strong	64	441882	1045661	ST776
2018-01- 714	Broiler	strong	34	415948	1507489	ST636
2018-01- 715	Broiler	strong	98	273086	715982	ST2458
2018-01- 723	Broiler	strong	46	428384	960872	ST37
2018-01- 798	Broiler	strong	101	317389	812630	ST2441
2018-01- 802	Turkey	strong	99	261287	479588	ST290-1LV
2018-01- 810	Turkey	strong	63	297230	812034	ST45
2018-01- 873	Turkey	strong	85	374331	1332984	ST1265
2018-01- 874	Turkey	strong	98	261292	469459	ST290-1LV
2018-01- 877	Turkey	strong	97	214392	523351	ST1229
2018-01- 878	Turkey	strong	71	369165	718130	ST776
2018-01- 879	Turkey	strong	75	456386	1255922	ST590
2018-01- 880	Turkey	strong	64	392135	769238	ST550-1LV
2018-01-1095	Turkey	strong	100	261291	479588	ST290-1LV
2018-01-1221	Broiler	strong	72	316910	784725	ST37
2018-01-1319	Turkey	strong	67	369162	718160	ST776
2018-01-1320	Turkey	strong	61	297230	812034	ST45
2018-01-1417	Turkey	strong	75	285199	618871	ST35
2018-01-1418	Broiler	strong	49	450610	1250625	ST22-1LV
2018-01-1439	Turkey	strong	83	378880	866197	ST1229
2018-01-1440	Turkey	strong	64	493295	914480	ST590

2018-01-1445	Broiler	strong	89	314903	1294182	ST463
2018-01-1446	Broiler	strong	69	337521	727458	ST1189
2018-01-1475	Broiler	strong	152	214756	689471	ST2467
2018-01-1584	Turkey	strong	111	172451	400724	ST35
2018-01-1585	Turkey	strong	73	285199	960017	ST35
2018-01-1593	Turkey	strong	79	350098	579451	ST35
2018-01-1594	Turkey	strong	40	481879	776090	ST292
2018-01-1649	Broiler	strong	117	321050	535686	ST22-2LV
2018-01-1658	Broiler	strong	65	321377	535666	ST22-2LV
2018-01-1683	Broiler	strong	96	294613	578334	ST2441
2018-01-1685	Turkey	strong	90	376084	688986	ST550-1LV
2018-01-1768	Broiler	strong	66	350190	622913	ST35
2018-01-1769	Broiler	strong	78	297574	780185	ST1076-2LV
2018-01-1774	Turkey	strong	126	214392	866213	ST1229
2018-01-1775	Turkey	strong	108	261292	479588	ST290-1LV
2018-01-1802	Broiler	strong	49	385863	991464	ST200-1LV
2018-01-1803	Turkey	strong	73	350190	956403	ST35
2018-01-1933	Turkey	strong	79	373494	542620	ST35
2018-01-1945	Turkey	strong	66	387943	718160	ST776
2018-01-1946	Turkey	strong	97	274025	604828	ST35
2018-01-2050	Broiler	strong	68	321068	779727	ST22-2LV
2018-01-2051	Turkey	strong	71	374985	618817	ST35
2018-01-2084	Turkey	strong	80	285199	645073	ST35
2018-01-2085	Turkey	strong	72	337764	645073	ST35
2018-01-2124	Turkey	strong	56	323862	1525191	ST17
2018-01-2125	Turkey	strong	111	261292	479588	ST290-1LV
2018-01-215	Broiler	strong	52	380351	654058	ST35
2018-01-2151	Broiler	strong	71	374985	618817	ST35
2018-01-2242	Broiler	strong	63	326366	709194	ST37
2018-01-2248	Turkey	strong	73	337764	618618	ST35
2018-01-2279	Broiler	strong	110	271913	968057	ST515
2018-01-2280	Broiler	strong	64	260295	580971	ST2439
2018-01-2295	Broiler	strong	122	353814	618484	ST1877
2018-01-2297	Broiler	strong	69	303186	729915	ST1198
2018-01-2353	Broiler	strong	68	380277	740908	ST26-2LV
2018-01-2359	Turkey	strong	86	337764	604930	ST35
2018-01-2360	Turkey	strong	60	388370	718405	ST776
2018-01-2372	Broiler	strong	84	339894	494808	ST473-2LV
2018-01-2406	Broiler	strong	82	245227	462797	ST37
2018-01-2485	Turkey	strong	111	261292	479159	ST290-1LV
2018-01-2486	Turkey	strong	81	378880	890200	ST1229
2018-01-2503	Turkey	strong	61	358862	746888	ST13
2018-01-2544	Broiler	strong	72	372446	922004	ST528-1LV

2018-01-2545	Broiler	strong	126	293257	506158	ST1877
2018-01-2615	Broiler	strong	56	372707	1910726	ST1198
2018-01-2639	Turkey	strong	61	437335	1446105	ST279
2018-01-2640	Turkey	strong	89	331362	580006	ST1779
2018-01-2643	Broiler	strong	65	328140	638318	ST1396
2018-01-2699	Broiler	strong	69	427352	1034643	ST17
2018-01-2716	Turkey	strong	80	287628	604929	ST35
2018-01-2723	Turkey	strong	60	373482	618818	ST35
2018-01-2729	Turkey	strong	57	405450	1010478	ST105
2018-01-2730	Turkey	strong	92	326346	544368	ST1779
2018-01-2734	Broiler	strong	118	321050	535666	ST22-2LV
2018-01-2776	Broiler	strong	65	297573	585548	ST1076-2LV
2018-01-2782	Broiler	strong	68	328603	881266	ST290
2018-01-2809	Turkey	strong	45	717113	1507446	ST13
2018-01-2837	Broiler	strong	68	339520	814601	ST1493-1LV
2018-01-2839	Broiler	strong	119	364239	506158	ST1877
2018-01-2860	Turkey	strong	65	473614	1106132	ST528-1LV
2018-01-2861	Turkey	strong	81	378880	866197	ST1229
2018-01-2926	Turkey	strong	55	371538	746887	ST13
2018-01-2939	Turkey	strong	88	465078	688987	ST550-1LV
2018-01-2940	Broiler	strong	128	342602	506158	ST1877
2018-01-3012	Broiler	strong	87	345128	925438	ST732
2018-01-3029	Broiler	strong	112	362577	1520708	ST290
2018-01-3030	Turkey	strong	93	322473	542729	ST35
2018-01-3042	Broiler	strong	78	317991	812629	ST2441
2018-01-3046	Turkey	strong	59	430823	1188698	ST37
2018-01-3094	Turkey	strong	66	229089	770075	ST513-1LV
2018-01-3095	Turkey	strong	34	629680	815889	ST66-1LV
2018-01-3096	Turkey	strong	70	384415	799888	ST37
2018-01-3115	Broiler	strong	69	320423	1000945	ST1076-2LV
2018-01-3125	Turkey	strong	78	348793	704789	ST1198
2018-01-3163	Turkey	strong	75	477621	1164426	ST550-1LV
2018-01-3218	Turkey	strong	85	473214	1019106	ST2
2018-01-3247	Broiler	strong	57	812606	940301	ST45
2018-01-3254	Turkey	strong	65	414239	718152	ST776
2018-01-3269	Broiler	strong	44	452553	980659	ST482-3LV
2018-01-3295	Turkey	strong	73	427352	886001	ST17
2018-01-3296	Turkey	strong	139	350098	619074	ST35
2018-01-3297	Turkey	strong	44	644020	781379	ST13
2018-01-3305	Turkey	strong	59	348799	744180	ST13
2018-01-3334	Broiler	strong	129	293257	506269	ST1877
2018-01-3408	Turkey	strong	48	427364	1412763	ST857
2018-01-3447	Broiler	strong	121	342602	618485	ST1877

2018-01-3448	Broiler	strong	89	328604	483028	ST290
2018-01-3463	Broiler	strong	44	968443	1934939	ST103-1LV
2018-01-3464	Broiler	strong	76	228022	919146	ST528
2018-01-3465	Turkey	strong	86	375551	928885	ST370
2018-01-3545	Broiler	strong	51	323862	1525195	ST17
2018-01-3554	Broiler	strong	75	305321	809732	ST452-1LV
2018-01-3576	Broiler	strong	71	305321	809732	ST452-1LV
2018-01-358	Broiler	strong	64	372951	536857	ST37
2018-01-3590	Turkey	strong	85	392135	561007	ST550-1LV
2018-01-3596	Broiler	strong	86	456386	1255922	ST590
2018-01-3650	Turkey	strong	51	457981	1100487	ST1302-1LV
2018-01-3688	Turkey	strong	91	375551	882615	ST370
2018-01-3692	Turkey	strong	86	350098	579451	ST35
2018-01-3723	Broiler	strong	159	265953	676504	ST969-1LV
2018-01-3773	Broiler	strong	69	325196	809732	ST452-1LV
2018-01-3776	Broiler	strong	96	374318	747333	ST1265
2018-01-3777	Turkey	strong	49	457981	1100487	ST1302-1LV
2018-01-3779	Broiler	strong	100	261284	511441	ST290
2018-01-3780	Broiler	strong	120	321050	541684	ST22-2LV
2018-01-3781	Broiler	strong	125	321050	535686	ST22-2LV
2018-01-3805	Broiler	strong	33	439784	983942	ST1537
2018-01-3863	Turkey	strong	54	515141	912258	ST590
2018-01-3864	Turkey	strong	96	244585	704823	ST1198
2018-01-388	Broiler	strong	70	291720	472967	ST732
2018-01-389	Broiler	strong	52	380351	1118740	ST35
2018-01-3892	Broiler	strong	75	238480	495897	ST1241
2018-01-3893	Broiler	strong	129	321545	618485	ST1877
2018-01-3925	Broiler	strong	71	362578	800280	ST290
2018-01-3926	Broiler	strong	75	391096	779811	ST463
2018-01-3928	Broiler	strong	82	435932	1419052	ST200
2018-01-3929	Broiler	strong	74	391096	1294178	ST463
2018-01-3934	Broiler	strong	115	210924	567778	ST2441
2018-01-3935	Turkey	strong	128	293257	506158	ST1877
2018-01-3975	Turkey	strong	91	248084	544368	ST1779
2018-01-4019	Turkey	strong	51	393377	817217	ST151
2018-01-4046	Turkey	strong	95	327627	665359	ST1026-1LV
2018-01-4107	Turkey	strong	178	285944	473836	ST35
2018-01-4108	Turkey	strong	75	286218	761708	ST556
2018-01-4174	Broiler	strong	70	361782	964787	ST2318-2LV
2018-01-4175	Broiler	strong	64	281177	587150	ST1198-1LV
2018-01-4196	Turkey	strong	54	388476	982032	ST26-1LV
2018-01-4197	Turkey	strong	59	332409	815311	ST151
2018-01-4203	Broiler	strong	67	260303	434387	ST2439

2018-01-4247	Turkey	strong	115	231426	479373	ST290-1LV
2018-01-4248	Turkey	strong	69	456007	746887	ST13
2018-01-4306	Turkey	strong	81	285199	542620	ST35
2018-01-4341	Broiler	strong	98	289680	549406	ST1962
2018-01-4379	Broiler	strong	38	425729	1426241	ST1440
2018-01-4390	Turkey	strong	87	301154	467776	ST37
2018-01-4458	Turkey	strong	91	425092	1255778	ST590
2018-01-4459	Turkey	strong	163	126601	323572	ST290-1LV
2018-01-4460	Turkey	strong	58	456639	1255922	ST590
2018-01-4487	Turkey	strong	111	247841	479588	ST290-1LV
2018-01-4514	Turkey	strong	67	369162	761936	ST776
2018-01-4639	Broiler	strong	74	369775	926349	ST463
2018-01-4652	Broiler	strong	68	352734	591551	ST1630
2018-01-4653	Turkey	strong	111	247841	479588	ST290-1LV
2018-01-4682	Turkey	strong	127	211931	554788	ST39
2018-01-473	Turkey	strong	130	227285	429368	ST1779
2018-01-4734	Turkey	strong	72	301121	521896	ST37
2018-01-4735	Turkey	strong	60	384415	1200141	ST37
2018-01-4736	Turkey	strong	61	340850	746886	ST13
2018-01-4739	Broiler	strong	92	294613	812630	ST2441
2018-01-474	Turkey	strong	32	415948	1498641	ST636
2018-01-475	Turkey	strong	105	247841	479588	ST290-1LV
2018-01-476	Turkey	strong	111	415753	1013108	ST2504-2LV
2018-01-4766	Turkey	strong	116	247841	479588	ST290-1LV
2018-01-4847	Turkey	strong	71	285200	898150	ST35
2018-01-507	Broiler	strong	50	385863	991464	ST200-1LV
2018-01-531	Turkey	strong	74	317331	618871	ST35
2018-01-580	Broiler	strong	68	371344	880905	ST2118
2018-01-582	Broiler	strong	98	213345	812630	ST2441
2018-01-583	Broiler	strong	92	207375	519854	ST37-1LV
2018-01-598	Turkey	strong	83	310511	450559	ST113
2018-01-609	Turkey	strong	73	317325	618873	ST35
2018-01-610	Broiler	strong	127	293257	506158	ST1877
2018-01-694	Broiler	strong	84	294613	812630	ST2441
2018-01-778	Turkey	strong	50	323862	1525191	ST17
2018-01-779	Turkey	strong	115	261292	469459	ST290-1LV

Resistance genes identified in isolates originating from poultry

Sample-ID	AGly	Flq	Phe	Sul	Tet	Tmt	Bla	ESBL	Bla-broad	Bla-broad-inhR
2018-01-1000	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-1001	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-1007	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-1022	-	-	-	SulII	-	-	AmpH*	-	SHV-33	-
2018-01-1024	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-1095	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-1097	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-1116	-	-	-	-	TetB*	-	AmpH*	-	SHV-60	-
2018-01-1117	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-1148	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-1176	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-1178	-	-	-	-	TetD	-	AmpH*;SHV-187*	-	-	-
2018-01-1214	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-1221	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-1256	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-1319	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-1320	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-1417	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-1418	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-1439	-	-	-	-	-	-	SHV-172*;AmpH*	-	-	-
2018-01-1440_	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-1445	-	-	-	-	TetA	-	AmpH*	-	SHV-1*?	-
2018-01-1446	-	-	-	-	-	-	AmpH*	-	SHV-11*	-
2018-01-1475	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-1584	-	-	-	SulII	-	-	AmpH*	-	SHV-33	-
2018-01-1585	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-1593	-	-	-	SulII	-	-	AmpH*	-	SHV-33	-
2018-01-1594	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-1649	-	-	-	-	-	-	SHV-28*;AmpH*	-	-	-
2018-01-1658	-	-	-	-	-	-	SHV-28*;AmpH*	-	-	-
2018-01-	-	-	-	-	-	-	AmpH*	-	SHV-	-

1683									77*	
2018-01-1685	-	-	-	-	TetB*	-	AmpH*	-	SHV-60	-
2018-01-1768	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-1769.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-1774.	-	-	-	-	-	-	SHV-172*;AmpH*	-	-	-
2018-01-1775.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-1802.	-	-	-	-	-	-	AmpH*	SHV-41	-	-
2018-01-1803.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-1933.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-1945.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-1946.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-2050.	-	-	-	-	-	-	SHV-28*;AmpH*	-	-	-
2018-01-2051.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-2084.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-2085.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-2124.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-2125.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-2151.	-	-	-	-	-	-	AmpH*	SHV-27	-	-
2018-01-215.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-2242.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-2248.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-2279.	-	-	-	-	-	-	SHV-173*;AmpH*	-	-	-
2018-01-2280.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-2295.	-	-	-	-	-	-	AmpH*	SHV-13*	-	-
2018-01-2297.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-2353.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-2359.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-2360.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-2372.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-2406.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-2485.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-2486.	-	-	-	-	-	-	SHV-172*;AmpH*	-	-	-
2018-01-2503.	-	-	-	-	-	-	AmpH*	SHV-101*	-	-
2018-01-2544.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-

2018-01-2545.	-	-	-	-	-	-	AmpH*	SHV-13*	-	-
2018-01-2615.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-2639.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-2640.	-	-	-	-	TetD	-	AmpH*	-	SHV-77*	TEM-30*
2018-01-2643.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-2699.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-2716.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-2723.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-2729.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-2730.	-	-	-	-	TetD	-	AmpH*	-	SHV-77*	TEM-30*
2018-01-2734.	-	-	-	-	-	-	SHV-28*;AmpH*	-	-	-
2018-01-2776.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-2782.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-2809.	-	-	-	-	-	-	AmpH*	SHV-101*	-	-
2018-01-2837.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-2839.	-	-	-	-	-	-	AmpH*	SHV-13*	-	-
2018-01-2860.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-2861.	-	-	-	-	-	-	SHV-172*;AmpH*	-	-	-
2018-01-2926.	-	-	-	-	-	-	AmpH*	SHV-101*	-	-
2018-01-2939.	-	-	-	-	TetB*	-	AmpH*	-	SHV-60	-
2018-01-2940.	-	-	-	-	-	-	AmpH*	SHV-13*	-	-
2018-01-3012.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-3029.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-3030.	-	-	-	SulII	TetB*	-	AmpH*	-	SHV-33	-
2018-01-3042.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-3046.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-3094.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-3095.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-3096.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-3115.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-3125.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-3163.	-	-	-	-	TetB*	-	AmpH*	-	SHV-60	-
2018-01-3218.	-	-	-	-	-	-	AmpH*;SHV-193	-	-	-
2018-01-	-	-	-	-	-	-	AmpH*	-	-	SHV-

3247.										26*
2018-01-3254.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-3269.	-	-	-	-	-	-	SHV-108*;AmpH*	-	-	-
2018-01-3295.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-3296.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-3297.	-	-	-	-	-	-	AmpH*	SHV-101*	-	-
2018-01-3305.	-	-	-	-	-	-	AmpH*	SHV-101*	-	-
2018-01-3334.	-	-	-	-	-	-	AmpH*	SHV-13*	-	-
2018-01-3408.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-3447.	-	-	-	-	-	-	AmpH*	SHV-13*	-	-
2018-01-3448.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-3463.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-3464.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-3465.	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-3545.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-3554.	-	-	-	-	-	-	AmpH*	-	SHV-61*	-
2018-01-3576.	-	-	-	-	-	-	AmpH*	-	SHV-61*	-
2018-01-358.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-3590.	-	-	-	-	TetB*	-	AmpH*	-	SHV-60	-
2018-01-3596.	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-3650.	-	-	-	-	-	-	AmpH*	-	-	-
2018-01-3688.	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-3692.	-	-	-	SulII	-	-	AmpH*	-	SHV-33	-
2018-01-3723.	-	-	-	-	-	-	AmpH*	SHV-27	-	-
2018-01-3773.	-	-	-	-	-	-	AmpH*	-	SHV-61*	-
2018-01-3776.	-	-	-	-	-	-	AmpH*	-	SHV-82*	-
2018-01-3777.	-	-	-	-	-	-	AmpH*	-	-	-
2018-01-3779.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-3780.	-	-	-	-	-	-	SHV-28*;AmpH*	-	-	-
2018-01-3781.	-	-	-	-	-	-	SHV-28*;AmpH*	-	-	-
2018-01-3805.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-3863.	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-3864.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-388.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-

2018-01-3892.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-3893.	-	-	-	-	-	-	AmpH*	SHV-13*	-	-
2018-01-389.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-3925.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-3926.	-	-	-	-	-	-	AmpH*	-	SHV-1*?	-
2018-01-3928.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-3929.	-	-	-	-	-	-	AmpH*	-	SHV-1*?	-
2018-01-3934.	AadA2	-	-	SulI	-	-	AmpH*	-	SHV-77*	-
2018-01-3935.	-	-	-	-	-	-	AmpH*	SHV-13*	-	-
2018-01-3975.	-	-	-	-	TetD	-	AmpH*	-	SHV-77*	TEM-30*
2018-01-4019.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-4046.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-4107.	-	-	-	SulII	-	-	AmpH*	-	SHV-33	-
2018-01-4108.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-4174.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-4175.	-	-	-	-	-	-	AmpH*	-	SHV-11*	-
2018-01-4196.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-4197.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-4203.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-4247.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-4248.	-	-	-	-	-	-	AmpH*	SHV-101*	-	-
2018-01-4306.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-4341.	-	-	-	-	-	-	SHV-193*;AmpH*	-	-	-
2018-01-4379.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-4390.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-4458.	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-4459.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-4460.	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-4487.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-4514.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-4639.	-	-	-	-	TetA	-	AmpH*	-	SHV-1*?	-
2018-01-4652.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-4653.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-	-	-	CatA1*	-	-	-	AmpH*;SHV-	-	-	-

4682.							187*			
2018-01-4734.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-4735.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-4736.	-	-	-	-	-	-	AmpH*	SHV-101*	-	-
2018-01-4739.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-473.	-	-	-	-	TetD	-	AmpH*	-	SHV-77*	TEM-30*
2018-01-474.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-475.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-4766.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-476.	-	-	-	-	-	-	AmpH*;SHV-187*	-	-	-
2018-01-4847.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-507.	-	-	-	-	-	-	AmpH*	SHV-41	-	-
2018-01-531.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-580.	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-582.	AadA2	-	-	SulI	-	-	AmpH*	-	SHV-77*	-
2018-01-583.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-598.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-609.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-610.	-	-	-	-	-	-	AmpH*	SHV-13*	-	-
2018-01-694.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-706.	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-707.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-709.	-	-	-	-	-	-	AmpH*	-	SHV-33	-
2018-01-710.	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-711.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-714.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-715.	-	Qnr-S1	-	SulI	TetA	DfrA1	AmpH*;LAP-2	-	-	SHV-26*
2018-01-723.	-	-	-	-	-	-	AmpH*	-	SHV-77*	-
2018-01-778.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-779.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-798.	AadA2	-	-	SulI	-	-	AmpH*	-	SHV-77*	-
2018-01-802.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-810.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-873.	-	-	-	-	-	-	AmpH*	-	SHV-82*	-

2018-01-874.	-	-	-	-	-	-	AmpH*	-	-	SHV-26*
2018-01-877.	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-878.	-	-	-	-	-	-	SHV-187*;AmpH*	-	-	-
2018-01-879.	-	-	-	-	-	-	AmpH*;SHV-172*	-	-	-
2018-01-880.	-	-	-	-	-	-	AmpH*	-	SHV-60	-

Virulence genes identified in isolates originating from poultry samples

Sample-ID	virulence score	Yersiniabactin	YbST	Aerobactin	AbST	Salmochelin	SmST
2018-01-1000.	1	ybt unknown	0	-	0	-	0
2018-01-1007.	1	ybt 15; ICEKp11	230-1LV	-	0	-	0
2018-01-1095.	4	ybt 16; ICEKp12	277-3LV	iuc 5	62-1LV	iro 5	35-1LV
2018-01-1097.	4	ybt 16; ICEKp12	277-3LV	iuc 5	62-1LV	iro 5	35-1LV
2018-01-1148.	1	ybt 14; ICEKp5	145-2LV	-	0	-	0
2018-01-1176.	1	ybt 14; ICEKp5	145-2LV	-	0	-	0
2018-01-1320.	1	ybt 10; ICEKp4	78	-	0	-	0
2018-01-1439.	1	ybt 14; ICEKp5	132-4LV	-	0	-	0
2018-01-1774.	1	ybt 14; ICEKp5	132-4LV	-	0	-	0
2018-01-1775.	4	ybt 16; ICEKp12	277-3LV	iuc 5	62-1LV	iro 5	35-1LV
2018-01-2124.	1	ybt 10; ICEKp4	97-3LV	-	0	-	0
2018-01-2125.	4	ybt 16; ICEKp12	277-3LV	iuc 5	62-1LV	iro 5	35-1LV
2018-01-215.	1	ybt 8; ICEKp3	196-1LV	-	0	-	0
2018-01-2485.	4	ybt 16; ICEKp12	277-3LV	iuc 5	62-1LV	iro 5	35-1LV
2018-01-2486.	1	ybt 14; ICEKp5	132-4LV	-	0	-	0
2018-01-2782.	1	ybt 14; ICEKp5	145-2LV	-	0	-	0
2018-01-2861.	1	ybt 14; ICEKp5	132-4LV	-	0	-	0
2018-01-3029.	1	ybt 14; ICEKp5	145-2LV	-	0	-	0
2018-01-3046.	1	ybt 15; ICEKp11	230-1LV	-	0	-	0
2018-01-3096.	1	ybt 15; ICEKp11	230-1LV	-	0	-	0
2018-01-3247.	1	ybt 10; ICEKp4	78	-	0	-	0
2018-01-3448.	1	ybt 14; ICEKp5	145-2LV	-	0	-	0
2018-01-3545.	1	ybt 10; ICEKp4	97-3LV	-	0	-	0
2018-01-3723.	0	-	0	-	0	iro 5	34-2LV
2018-01-3779.	1	ybt 14; ICEKp5	145-2LV	-	0	-	0
2018-01-389.	1	ybt 8; ICEKp3	196-1LV	-	0	-	0
2018-01-3925.	1	ybt 14; ICEKp5	145-2LV	-	0	-	0
2018-01-4247.	3	-	0	iuc 5	62-1LV	iro 5	35-1LV
2018-01-4390.	1	ybt 15; ICEKp11	230-1LV	-	0	-	0
2018-01-4459.	4	ybt 16; ICEKp12	277-3LV	iuc 5	62-1LV	iro 5	35-1LV
2018-01-4487.	4	ybt 16; ICEKp12	277-3LV	iuc 5	62-1LV	iro 5	35-1LV
2018-01-4652.	1	ybt 16; ICEKp12	280-2LV	-	0	-	0
2018-01-4653.	4	ybt 16; ICEKp12	277-3LV	iuc 5	62-1LV	iro 5	35-1LV
2018-01-4734.	1	ybt 15; ICEKp11	230-1LV	-	0	-	0
2018-01-4735.	1	ybt 15; ICEKp11	230-1LV	-	0	-	0
2018-01-474.	1	ybt 5; ICEKp6	10	-	0	-	0
2018-01-475.	4	ybt 16; ICEKp12	277-3LV	iuc 5	62-1LV	iro 5	35-1LV
2018-01-4766.	4	ybt 16; ICEKp12	277-3LV	iuc 5	62-1LV	iro 5	35-1LV
2018-01-707.	4	ybt 16; ICEKp12	277-3LV	iuc 5	62-1LV	iro 5	35-1LV
2018-01-714.	1	ybt 5; ICEKp6	10	-	0	-	0
2018-01-778.	1	ybt 10; ICEKp4	97-3LV	-	0	-	0
2018-01-779.	4	ybt unknown	0	iuc 5	62-1LV	iro 5	35-1LV
2018-01-802.	4	ybt 16; ICEKp12	277-3LV	iuc 5	62-1LV	iro 5	35-1LV
2018-01-810.	1	ybt 10; ICEKp4	78	-	0	-	0
2018-01-874.	3	-	0	iuc 5	62-1LV	iro 5	35-1LV
2018-01-877.	1	ybt 14; ICEKp5	132-4LV	-	0	-	0

Results from PlasmidFinder of ST290-1LV isolates

Plasmid	ColRNAI	Col(MG828)	InFIB (AP001918)	InFIC (FII)
2018-01-475.	100	95.68	97.36	95.79
2018-01-707.	100	95.68	97.36	95.79
2018-01-779.	100	95.41	97.36	95.79
2018-01-802.	100	95.68	97.36	95.79
2018-01-874.	100		97.36	95.79
2018-01-1095.	100	95.41	97.36	95.79
2018-01-1097.	100	95.68	97.36	95.79
2018-01-1775.	100	95.41	97.36	95.79
2018-01-2125.	100	95.68	97.36	95.79
2018-01-2485.	100	95.41	97.36	95.79
2018-01-3723	98.66		98.68	96.56
2018-01-4247.	100	95.56	97.36	95.79
2018-01-4459.	100		97.36	95.79
2018-01-4487.	100	95.26	97.36	95.79
2018-01-4653.	100		97.36	95.79
2018-01-4766.	100	95.26	97.36	95.79

Results from NCBI BLAST of 290-1LV isolates

Sample-ID	Contig nr	length	Host strain	Plasmid
2018-01-1095.	22	84090	E. coli HS13-1	pHS 13-1-IncF
2018-01-1097.	22	84090	E. coli HS13-1	pHS 13-1-IncF
2018-01-475.	26	45973	E. coli HS13-1	pHS 13-1-IncF
2018-01-4766.	27	45973	E. coli HS13-1	pHS 13-1-IncF
2018-01-4459.	38	45973	E. coli HS13-1	pHS 13-1-IncF
2018-01-4487.	26	45973	E. coli HS13-1	pHS 13-1-IncF
2018-01-707.	24	75283	E. coli HS13-1	pHS 13-1-IncF
2018-01-802.	25	60838	E. coli HS13-1	pHS 13-1-IncF
2018-01-4247.	25	45973	E. coli HS13-1	pHS 13-1-IncF
2018-01-1775.	24	45973	E. coli HS13-1	pHS 13-1-IncF
2018-01-2485.	25	60838	E. coli HS13-1	pHS 13-1-IncF
2018-01-4653.	23	69225	E. coli HS13-1	pHS 13-1-IncF
2018-01-2125.	25	45973	E. coli HS13-1	pHS 13-1-IncF
2018-01-779.	24	69307	E. coli HS13-1	pHS 13-1-IncF
2018-01-874.	23	84090	E. coli HS13-1	pHS 13-1-IncF
2018-01-3723	32	27047	E.Coli	pCOV8 clone COV8-c2

MLST

strain	gapA	infB	mdh	pgi	phoE	rpoB	tonB
2018-01-1000.	14	1	2	1	21	1	23
2018-01-1001.	2	1	2	1	10	1	19
2018-01-1007.	2	9	2	1	13	1	16
2018-01-1022.	2	1	2	1	10	1	19
2018-01-1024.	2	1	13	3	7	4	25
2018-01-1095.	2	1	1	37	10	1*	86
2018-01-1097.	2	1	1	37	10	1*	86
2018-01-1116.	4	1	1	2	12*	4	100
2018-01-1117.	2	1	2	1	12	4	9
2018-01-1148.	2	1	1	37	10	1	86
2018-01-1176.	2	1	1	37	10	1	86
2018-01-1178.	2	1	1	1	1	1	19
2018-01-1214.	55*	5	1	1	9	4	18
2018-01-1221.	2	9	2	1	13	1	16
2018-01-1256.	4	1	32	1	7	4	10
2018-01-1319.	2	1	2	1	2	1	2
2018-01-1320.	2	1	1	6	7	1	12
2018-01-1417.	2	1	2	1	10	1	19
2018-01-1418.	2	3	2*	1	1	4	4

2018-01-1439.	4	1	1	1	20	1	218
2018-01-1440.	2	1	13	3	7	4	25
2018-01-1445.	2	1	4	1	7	6	19
2018-01-1446.	3	5	95	1	16	1	12
2018-01-1475.	2	3	1	4	6	2	38
2018-01-1584.	2	1	2	1	10	1	19
2018-01-1585.	2	1	2	1	10	1	19
2018-01-1593.	2	1	2	1	10	1	19
2018-01-1594.	2	1	2	1	1	1	4
2018-01-1649.	2	3	1	1	1	7	25
2018-01-1658.	2	3	1	1	1	7	25
2018-01-1683.	2	1	2	1	12	4	9
2018-01-1685.	4	1	1	2	12*	4	100
2018-01-1768.	2	1	2	1	10	1	19
2018-01-1769.	2	3	2	4*	12	1	91
2018-01-1774.	4	1	1	1	20	1	218
2018-01-1775.	2	1	1	37	10	1*	86
2018-01-1802.	2	1	2	1	186*	1	68
2018-01-1803.	2	1	2	1	10	1	19
2018-01-1933.	2	1	2	1	10	1	19
2018-01-1945.	2	1	2	1	2	1	2
2018-01-1946.	2	1	2	1	10	1	19
2018-01-2050.	2	3	1	1	1	7	25
2018-01-2051.	2	1	2	1	10	1	19
2018-01-2084.	2	1	2	1	10	1	19
2018-01-2085.	2	1	2	1	10	1	19
2018-01-2124.	2	1	1	1	4	4	4
2018-01-2125.	2	1	1	37	10	1*	86
2018-01-2151.	2	1	19	4	9	4	34
2018-01-215.	2	1	2	1	10	1	19
2018-01-2242.	2	9	2	1	13	1	16
2018-01-2248.	2	1	2	1	10	1	19
2018-01-2279.	2	1	1	1	1	1	4
2018-01-2280.	2	5	2	1	26	4	2
2018-01-2295.	1	1	2	1	10	24	19
2018-01-2297.	2	9	2	1	9	1	4
2018-01-2353.	2	2	1	1	9	1	38
2018-01-2359.	2	1	2	1	10	1	19
2018-01-2360.	2	1	2	1	2	1	2
2018-01-2372.	2	1	169	12	9	4	40
2018-01-2406.	2	9	2	1	13	1	16
2018-01-2485.	2	1	1	37	10	1*	86
2018-01-2486.	4	1	1	1	20	1	218
2018-01-2503.	2	3	1	1	10	1	19
2018-01-2544.	55*	5	1	1	9	4	18
2018-01-2545.	1	1	2	1	10	24	19
2018-01-2615.	2	9	2	1	9	1	4
2018-01-2639.	2	2	1	47	1	4	43
2018-01-2640.	2	9	2	1	13	1	296
2018-01-2643.	2	1	1	1	10	1	86
2018-01-2699.	2	1	1	1	4	4	4
2018-01-2716.	2	1	2	1	10	1	19
2018-01-2723.	2	1	2	1	10	1	19
2018-01-2729.	2	3	2	1	1	4	18
2018-01-2730.	2	9	2	1	13	1	296
2018-01-2734.	2	3	1	1	1	7	25
2018-01-2776.	2	3	2	4*	12	1	91

2018-01-2782.	2	1	1	37	10	1	86
2018-01-2809.	2	3	1	1	10	1	19
2018-01-2837.	2	1	1	6	10	1	9*
2018-01-2839.	1	1	2	1	10	24	19
2018-01-2860.	55*	5	1	1	9	4	18
2018-01-2861.	4	1	1	1	20	1	218
2018-01-2926.	2	3	1	1	10	1	19
2018-01-2939.	4	1	1	2	12*	4	100
2018-01-2940.	1	1	2	1	10	24	19
2018-01-3012.	2	3	2	1	7	4	56
2018-01-3029.	2	1	1	37	10	1	86
2018-01-3030.	2	1	2	1	10	1	19
2018-01-3042.	2	1	2	1	12	4	9
2018-01-3046.	2	9	2	1	13	1	16
2018-01-3094.	2	1	1	6	2	1	129
2018-01-3095.	2	3	2	1	10	1	2
2018-01-3096.	2	9	2	1	13	1	16
2018-01-3115.	2	3	2	4*	12	1	91
2018-01-3125.	2	9	2	1	9	1	4
2018-01-3163.	4	1	1	2	12*	4	100
2018-01-3218.	3	4	1	1	9	4	17
2018-01-3247.	2	1	1	6	7	1	12
2018-01-3254.	2	1	2	1	2	1	2
2018-01-3269.	2	10	1	26	1	31	39
2018-01-3295.	2	1	1	1	4	4	4
2018-01-3296.	2	1	2	1	10	1	19
2018-01-3297.	2	3	1	1	10	1	19
2018-01-3305.	2	3	1	1	10	1	19
2018-01-3334.	1	1	2	1	10	24	19
2018-01-3408.	2	35	2	35	56	24	19
2018-01-3447.	1	1	2	1	10	24	19
2018-01-3448.	2	1	1	37	10	1	86
2018-01-3463.	4	1	1	1	7	1	352*
2018-01-3464.	32	5	1	1	9	4	18
2018-01-3465.	41	47	1	14	1	1	12
2018-01-3545.	2	1	1	1	4	4	4
2018-01-3554.	3	1	2	2	12	4	121
2018-01-3576.	3	1	2	2	12	4	121
2018-01-358.	2	9	2	1	13	1	16
2018-01-3590.	4	1	1	2	12*	4	100
2018-01-3596.	2	1	13	3	7	4	25
2018-01-3650.	3	1	1	1	1	4	39
2018-01-3688.	41	47	1	14	1	1	12
2018-01-3692.	2	1	2	1	10	1	19
2018-01-3723.	4	1	2	36	9	1	14
2018-01-3773.	3	1	2	2	12	4	121
2018-01-3776.	2	1	1	1	10	4	12
2018-01-3777.	3	1	1	1	1	4	39
2018-01-3779.	2	1	1	37	10	1	86
2018-01-3780.	2	3	1	1	1	7	25
2018-01-3781.	2	3	1	1	1	7	25
2018-01-3805.	2	1	2	1	12	1	46
2018-01-3863.	2	1	13	3	7	4	25
2018-01-3864.	2	9	2	1	9	1	4
2018-01-388.	2	3	2	1	7	4	56
2018-01-3892.	4	3	1	1	1	4	223
2018-01-3893.	1	1	2	1	10	24	19

2018-01-389.	2	1	2	1	10	1	19
2018-01-3925.	2	1	1	37	10	1	86
2018-01-3926.	2	1	4	1	7	6	19
2018-01-3928.	2	1	2	1	12	1	68
2018-01-3929.	2	1	4	1	7	6	19
2018-01-3934.	2	1	2	1	12	4	9
2018-01-3935.	1	1	2	1	10	24	19
2018-01-3975.	2	9	2	1	13	1	296
2018-01-4019.	4	1	32	1	7	4	10
2018-01-4046.	2	1	2	35	10	24	135
2018-01-4107.	2	1	2	1	10	1	19
2018-01-4108.	2	1	1	26	12	15	138
2018-01-4174.	4	3	1	1	274	1	110*
2018-01-4175.	2	5	2	1	9	1	4
2018-01-4196.	2	1	1	1	9	1	25*
2018-01-4197.	4	1	32	1	7	4	10
2018-01-4203.	2	5	2	1	26	4	2
2018-01-4247.	2	1	1	37	10	1*	86
2018-01-4248.	2	3	1	1	10	1	19
2018-01-4306.	2	1	2	1	10	1	19
2018-01-4341.	2	1	65	1	240	4	43
2018-01-4379.	2	3	1	1	4	1	4
2018-01-4390.	2	9	2	1	13	1	16
2018-01-4458.	2	1	13	3	7	4	25
2018-01-4459.	2	1	1	37	10	1*	86
2018-01-4460.	2	1	13	3	7	4	25
2018-01-4487.	2	1	1	37	10	1*	86
2018-01-4514.	2	1	2	1	2	1	2
2018-01-4639.	2	1	4	1	7	6	19
2018-01-4652.	2	94	5	1	1	1	4
2018-01-4653.	2	1	1	37	10	1*	86
2018-01-4682.	2	1	2	4	9	1	14
2018-01-4734.	2	9	2	1	13	1	16
2018-01-4735.	2	9	2	1	13	1	16
2018-01-4736.	2	3	1	1	10	1	19
2018-01-4739.	2	1	2	1	12	4	9
2018-01-473.	2	9	2	1	13	1	296
2018-01-474.	2	5	1	1	4	1	4
2018-01-475.	2	1	1	37	10	1*	86
2018-01-4766.	2	1	1	37	10	1*	86
2018-01-476.	2	20	1	2	4	4	38
2018-01-4847.	2	1	2	1	10	1	19
2018-01-507.	2	1	2	1	186*	1	68
2018-01-531.	2	1	2	1	10	1	19
2018-01-580.	5	1	1	1	9	1	34
2018-01-582.	2	1	2	1	12	4	9
2018-01-583.	2	9*	2	1	13	1	16
2018-01-598.	14	1	2	1	21	1	23
2018-01-609.	2	1	2	1	10	1	19
2018-01-610.	1	1	2	1	10	24	19
2018-01-694.	2	1	2	1	12	4	9
2018-01-706.	2	1	13	3	7	4	25
2018-01-707.	2	1	1	37	10	1*	86
2018-01-709.	2	1	2	1	10	1	19
2018-01-710.	2	1	13	3	7	4	25
2018-01-711.	2	1	2	1	2	1	2
2018-01-714.	2	5	1	1	4	1	4

2018-01-715.	2	1	1	1	12	1	38
2018-01-723.	2	9	2	1	13	1	16
2018-01-778.	2	1	1	1	4	4	4
2018-01-779.	2	1	1	37	10	1*	86
2018-01-798.	2	1	2	1	12	4	9
2018-01-802.	2	1	1	37	10	1*	86
2018-01-810.	2	1	1	6	7	1	12
2018-01-873.	2	1	1	1	10	4	12
2018-01-874.	2	1	1	37	10	1*	86
2018-01-877.	4	1	1	1	20	1	218
2018-01-878.	2	1	2	1	2	1	2
2018-01-879.	2	1	13	3	7	4	25
2018-01-880.	4	1	1	2	12*	4	100

Capsule type and O-type

strain	K locus	K locus identity	O locus	O locus identity
2018-01-1000.	KL114	99.24%	O3/O3a	97.87%
2018-01-1001.	KL22	99.49%	O1v1	98.12%
2018-01-1007.	KL38	99.68%	O3b	99.30%
2018-01-1022.	KL22	99.49%	O1v1	98.12%
2018-01-1024.	KL57	98.78%	O2v2	98.50%
2018-01-1095.	KL21	98.57%	O1v1	98.12%
2018-01-1097.	KL21	98.57%	O1v1	98.12%
2018-01-1116.	KL47	97.40%	OL101	91.92%
2018-01-1117.	KL14	99.87%	O3b	99.73%
2018-01-1148.	KL21	98.57%	O1v1	98.12%
2018-01-1176.	KL21	98.57%	O1v1	98.12%
2018-01-1178.	KL113	96.69%	O1v2	99.18%
2018-01-1214.	KL46	98.80%	O3b	99.20%
2018-01-1221.	KL14	99.92%	O3b	99.25%
2018-01-1256.	KL30	99.15%	O1v2	99.20%
2018-01-1319.	KL21	99.47%	O3b	99.42%
2018-01-1320.	KL5	99.21%	O3b	99.40%
2018-01-1417.	KL22	99.49%	O1v1	98.12%
2018-01-1418.	KL54	99.96%	O1v2	99.17%
2018-01-1439.	KL125	90.26%	O5	94.32%
2018-01-1440.	KL57	98.78%	O2v2	98.50%
2018-01-1445.	KL117	100.00%	O2v2	98.52%
2018-01-1446.	KL28	99.39%	O2v1	98.85%
2018-01-1475.	KL111	99.74%	O3b	98.87%
2018-01-1584.	KL22	99.49%	O1v1	98.12%
2018-01-1585.	KL22	99.49%	O1v1	98.12%
2018-01-1593.	KL22	99.49%	O1v1	98.12%
2018-01-1594.	KL51	100.00%	O3b	99.09%
2018-01-1649.	KL64	99.91%	O2v1	98.66%
2018-01-1658.	KL64	99.91%	O2v1	98.66%
2018-01-1683.	KL14	99.87%	O3b	99.72%
2018-01-1685.	KL47	97.41%	OL101	91.92%
2018-01-1768.	KL22	99.45%	O1v1	98.12%
2018-01-1769.	KL55	96.21%	O3/O3a	98.10%
2018-01-1774.	KL125	90.25%	O5	94.32%
2018-01-1775.	KL21	98.57%	O1v1	98.12%
2018-01-1802.	KL55	96.25%	O3/O3a	98.52%
2018-01-1803.	KL22	99.49%	O1v1	98.12%

2018-01-1933.	KL22	99.32%	O1v1	98.12%
2018-01-1945.	KL21	99.47%	O3b	99.40%
2018-01-1946.	KL22	99.49%	O1v1	98.12%
2018-01-2050.	KL64	99.91%	O2v1	98.66%
2018-01-2051.	KL22	99.49%	O1v1	98.12%
2018-01-2084.	KL22	99.49%	O1v1	98.12%
2018-01-2085.	KL22	99.48%	O1v1	98.12%
2018-01-2124.	KL23	99.94%	O2v2	98.44%
2018-01-2125.	KL21	98.57%	O1v1	98.12%
2018-01-2151.	KL18	98.68%	O1v2	98.76%
2018-01-215.	KL22	99.50%	O1v1	98.10%
2018-01-2242.	KL21	99.87%	O3b	99.29%
2018-01-2248.	KL22	99.48%	O1v1	98.12%
2018-01-2279.	KL134	99.94%	O3/O3a	98.88%
2018-01-2280.	KL3	99.96%	O1v1	98.66%
2018-01-2295.	KL14	99.71%	O3b	98.97%
2018-01-2297.	KL24	99.01%	O2v1	98.76%
2018-01-2353.	KL15	99.81%	O4	99.67%
2018-01-2359.	KL22	99.49%	O1v1	98.12%
2018-01-2360.	KL21	99.47%	O3b	99.42%
2018-01-2372.	KL111	99.62%	O3b	98.76%
2018-01-2406.	KL114	98.03%	O3/O3a	99.28%
2018-01-2485.	KL21	98.57%	O1v1	98.12%
2018-01-2486.	KL125	90.26%	O5	94.32%
2018-01-2503.	KL30	99.13%	O1v1	99.90%
2018-01-2544.	KL46	98.81%	O3b	99.20%
2018-01-2545.	KL14	99.73%	O3b	98.95%
2018-01-2615.	KL8	99.67%	O2v2	98.45%
2018-01-2639.	KL151	99.99%	O5	94.30%
2018-01-2640.	KL38	99.70%	O3b	99.29%
2018-01-2643.	KL21	98.57%	O1v1	98.12%
2018-01-2699.	KL23	99.93%	O2v2	98.45%
2018-01-2716.	KL22	99.48%	O2v1	98.12%
2018-01-2723.	KL22	99.49%	O1v1	98.12%
2018-01-2729.	KL102	99.24%	O2v2	98.44%
2018-01-2730.	KL38	99.70%	O3b	99.30%
2018-01-2734.	KL64	99.91%	O2v1	98.66%
2018-01-2776.	KL55	96.21%	O3/O3a	98.10%
2018-01-2782.	KL21	98.57%	O1v1	98.12%
2018-01-2809.	KL30	99.13%	O1v1	99.90%
2018-01-2837.	KL14	99.87%	O3b	99.15%
2018-01-2839.	KL14	99.72%	O3b	98.92%
2018-01-2860.	KL46	98.81%	O3b	99.20%
2018-01-2861.	KL125	90.26%	O5	94.32%
2018-01-2926.	KL30	99.13%	O1v1	99.90%
2018-01-2939.	KL47	97.40%	OL101	91.92%
2018-01-2940.	KL14	99.73%	O3b	98.94%
2018-01-3012.	KL149	99.97%	O4	99.74%
2018-01-3029.	KL21	98.57%	O1v1	98.12%
2018-01-3030.	KL22	99.30%	O1v1	98.12%
2018-01-3042.	KL14	99.88%	O3b	99.72%
2018-01-3046.	KL38	99.68%	O3b	99.30%
2018-01-3094.	KL3	99.97%	O2v2	98.73%
2018-01-3095.	KL127	99.76%	OL101	95.11%
2018-01-3096.	KL38	99.68%	O3b	99.30%

2018-01-3115.	KL55	96.21%	O3/O3a	98.10%
2018-01-3125.	KL24	99.01%	O2v1	98.76%
2018-01-3163.	KL47	97.40%	OL101	91.92%
2018-01-3218.	KL52	97.88%	OL103	94.50%
2018-01-3247.	KL24	99.02%	O2v1	98.76%
2018-01-3254.	KL21	99.48%	O3b	99.42%
2018-01-3269.	KL116	98.55%	O2v1	98.81%
2018-01-3295.	KL23	99.93%	O2v2	98.45%
2018-01-3296.	KL22	99.49%	O1v1	98.12%
2018-01-3297.	KL30	99.13%	O1v1	99.90%
2018-01-3305.	KL30	99.13%	O1v1	99.90%
2018-01-3334.	KL14	99.70%	O3b	98.97%
2018-01-3408.	KL13	98.77%	O3b	99.15%
2018-01-3447.	KL14	99.72%	O3b	98.93%
2018-01-3448.	KL21	98.57%	O1v1	98.12%
2018-01-3463.	KL21	99.83%	O3/O3a	97.93%
2018-01-3464.	KL46	98.79%	O3b	99.18%
2018-01-3465.	KL28	98.94%	O3b	99.23%
2018-01-3545.	KL23	99.94%	O2v2	98.44%
2018-01-3554.	KL126	99.23%	OL101	91.94%
2018-01-3576.	KL126	99.23%	OL101	91.89%
2018-01-358.	KL14	99.94%	O3b	99.27%
2018-01-3590.	KL47	97.40%	OL101	91.92%
2018-01-3596.	KL57	98.78%	O2v2	98.50%
2018-01-3650.	KL142	99.31%	O1v2	98.83%
2018-01-3688.	KL28	98.94%	O3b	99.21%
2018-01-3692.	KL22	99.49%	O1v1	98.12%
2018-01-3723.	KL39	99.97%	O3b	99.16%
2018-01-3773.	KL126	99.23%	OL101	91.90%
2018-01-3776.	KL33	80.08%	O3b	98.98%
2018-01-3777.	KL142	99.31%	O1v2	98.83%
2018-01-3779.	KL21	98.57%	O1v1	98.12%
2018-01-3780.	KL64	99.91%	O2v1	98.66%
2018-01-3781.	KL64	99.91%	O2v1	98.66%
2018-01-3805.	KL64	99.86%	O2v1	98.65%
2018-01-3863.	KL57	98.79%	O2v2	98.50%
2018-01-3864.	KL24	99.01%	O2v1	98.76%
2018-01-388.	KL149	99.96%	O4	99.74%
2018-01-3892.	KL7	98.44%	O2v2	98.35%
2018-01-3893.	KL14	99.71%	O3b	98.94%
2018-01-389.	KL22	99.49%	O1v1	98.10%
2018-01-3925.	KL21	98.57%	O1v1	98.12%
2018-01-3926.	KL117	100.00%	O2v2	98.52%
2018-01-3928.	KL39	99.20%	O3b	99.10%
2018-01-3929.	KL117	100.00%	O2v2	98.52%
2018-01-3934.	KL14	99.86%	O3b	99.76%
2018-01-3935.	KL107	88.71%	O3b	98.97%
2018-01-3975.	KL38	99.68%	O3b	99.30%
2018-01-4019.	KL30	99.14%	O1v2	99.20%
2018-01-4046.	KL7	98.50%	O2v2	98.34%
2018-01-4107.	KL22	99.49%	O1v1	98.12%
2018-01-4108.	KL62	99.94%	O2v1	98.87%
2018-01-4174.	KL25	99.83%	O3/O3a	98.67%
2018-01-4175.	KL111	99.43%	O3b	98.86%
2018-01-4196.	KL107	87.15%	O12	98.10%

2018-01-4197.	KL30	98.99%	O1v2	99.20%
2018-01-4203.	KL3	99.96%	O1v1	98.66%
2018-01-4247.	KL21	98.73%	OL102	74.03%
2018-01-4248.	KL30	99.13%	O1v1	99.90%
2018-01-4306.	KL22	99.49%	O1v1	98.12%
2018-01-4341.	KL107	88.69%	O3b	99.90%
2018-01-4379.	KL19	94.93%	O2v2	98.44%
2018-01-4390.	KL38	99.61%	O3b	99.30%
2018-01-4458.	KL57	98.78%	O2v2	98.50%
2018-01-4459.	KL21	98.57%	O1v1	98.12%
2018-01-4460.	KL57	98.78%	O2v1	94.15%
2018-01-4487.	KL21	98.57%	O1v1	98.12%
2018-01-4514.	KL21	99.48%	O3b	99.42%
2018-01-4639.	KL117	100.00%	O2v2	98.52%
2018-01-4652.	KL21	99.97%	O3b	99.19%
2018-01-4653.	KL21	98.57%	O1v1	98.12%
2018-01-4682.	KL23	99.92%	O1v2	98.49%
2018-01-4734.	KL38	99.68%	O3b	99.30%
2018-01-4735.	KL38	99.68%	O3b	99.30%
2018-01-4736.	KL30	99.13%	O1v1	99.89%
2018-01-4739.	KL14	99.87%	O3b	99.73%
2018-01-473.	KL38	99.70%	O3b	99.30%
2018-01-474.	KL123	94.98%	O5	94.21%
2018-01-475.	KL21	98.54%	O1v1	98.12%
2018-01-4766.	KL21	98.57%	O1v1	98.12%
2018-01-476.	KL45	94.94%	O2v2	98.38%
2018-01-4847.	KL22	99.48%	O1v1	98.12%
2018-01-507.	KL55	96.25%	O3/O3a	98.52%
2018-01-531.	KL22	99.48%	O1v1	98.12%
2018-01-580.	KL46	99.07%	O3b	99.27%
2018-01-582.	KL14	99.86%	O3b	99.75%
2018-01-583.	KL14	99.99%	O3b	99.17%
2018-01-598.	KL114	99.24%	O3/O3a	97.86%
2018-01-609.	KL22	99.48%	O1v1	98.12%
2018-01-610.	KL14	99.72%	O3b	98.93%
2018-01-694.	KL14	99.87%	O3b	99.74%
2018-01-706.	KL57	98.78%	O2v2	98.50%
2018-01-707.	KL21	98.57%	O1v1	98.12%
2018-01-709.	KL22	99.49%	O1v1	98.12%
2018-01-710.	KL57	98.78%	O2v2	98.50%
2018-01-711.	KL21	99.49%	O3b	99.42%
2018-01-714.	KL123	94.98%	O5	94.21%
2018-01-715.	KL10	96.70%	O5	94.29%
2018-01-723.	KL62	99.94%	O2v1	98.87%
2018-01-778.	KL23	99.94%	O2v2	98.44%
2018-01-779.	KL21	98.57%	O1v1	98.12%
2018-01-798.	KL14	99.87%	O3b	99.73%
2018-01-802.	KL21	98.57%	O1v1	98.12%
2018-01-810.	KL5	99.21%	O3b	99.40%
2018-01-873.	KL33	80.08%	O3b	98.99%
2018-01-874.	KL21	98.57%	O1v1	98.12%
2018-01-877.	KL125	90.72%	O5	94.32%
2018-01-878.	KL21	99.49%	O3b	99.44%
2018-01-879.	KL57	98.78%	O2v2	98.50%
2018-01-880.	KL47	97.40%	OL101	91.92%

Klebsiella spp. positive isolates from swine, dog and cattle samples

Sample number	Source	MALDI-TOF score	Species
2019-01-61	Swine	2.34	*Klebsiella pneumonia*
2019-01-63	Swine	2.4	*Klebsiella pneumonia*
2019-01-78	Swine	2.4	*Klebsiella pneumonia*
2019-01-154	Swine	2.44	*Klebsiella pneumoniae*
2019-01-156	Swine	2.41	*Klebsiella pneumoniae*
2019-01-204	Swine	2.52	*Klebsiella pneumoniae*
2019-01-205	Swine	2.33	*Klebsiella pneumonia*
2019-01-206	Swine	2.48	*Klebsiella variicola*
2019-01-324	Swine	2.4	*Klebsiella pneumoniae*
2019-01-326	Swine	2.39	*Klebsiella pneumoniae*
2019-01-375	Swine	2.29	*Klebsiella pneumoniae*
2019-01-376	Swine	2.34	*Klebsiella pneumoniae*
2019-01-448	Cattle	2.33	*Klebsiella oxytoca*
2019-01-476	Swine	2.42	*Klebsiella pneumoniae*
2019-01-587	Swine	2.42	*Klebsiella pneumoniae*
2019-01-588	Swine	2.41	*Klebsiella pneumoniae*
2019-01-589	Swine	2.35	*Klebsiella pneumoniae*
2019-01-591	Swine	2.33	*Klebsiella pneumoniae*
2019-01-604	Swine	2.4	*Klebsiella pneumoniae*
2019-01-605	Swine	2.41	*Klebsiella pneumoniae*
2019-01-606	Swine	2.36	*Klebsiella pneumoniae*
2019-01-608	Swine	2.41	*Klebsiella pneumoniae*
2019-01-655	Swine	1.96	*Klebsiella pneumoniae*
2019-01-658	Swine	2.36	*Klebsiella pneumoniae*
2019-01-792	Dog	2.38	*Klebsiella variicola*
2019-01-794	Dog	2.33	*Klebsiella pneumoniae*
2019-01-796	Dog	2.45	*Klebsiella pneumoniae*
2019-01-798	Dog	2.4	*Klebsiella pneumoniae*
2019-01-802	Dog	2.31	*Klebsiella pneumoniae*
2019-01-803	Dog	2.31	*Klebsiella oxytoca*
2019-01-804	Dog	2.42	*Klebsiella pneumoniae*
2019-01-810	Cattle	2.31	*Klebsiella pneumoniae*
2019-01-858	Dog	2.39	*Klebsiella pneumoniae*
2019-01-862	Dog	2.34	*Klebsiella pneumoniae*
2019-01-863	Dog	2.4	*Klebsiella pneumoniae*
2019-01-865	Dog	2.35	*Klebsiella pneumoniae*
2019-01-895	Swine	2.29	*Klebsiella pneumoniae*

2019-01-896	Swine	2.18	*Klebsiella pneumoniae*
2019-01-897	Swine	2.42	*Klebsiella pneumoniae*
2019-01-901	Swine	2.35	*Klebsiella pneumoniae*
2019-01-902	Swine	2.34	*Klebsiella pneumoniae*
2019-01-1007	Dog	1.96	*Klebsiella oxytoca*
2019-01-1065	Swine	2.45	*Klebsiella aerogenes*
2019-01-1066	Swine	2.36	*Klebsiella pneumoniae*
2019-01-1068	Swine	2.24	*Klebsiella pneumoniae*
2019-01-1635	Swine	2.53	*Klebsiella pneumoniae*
2019-01-1336	Swine	2.43	*Klebsiella pneumoniae*
2019-01-1338	Swine	2.39	*Klebsiella pneumoniae*
2019-01-1343	Swine	2.39	*Klebsiella pneumoniae*
2019-01-1363	Swine	2.39	*Klebsiella pneumoniae*
2019-01-1364	Swine	2.34	*Klebsiella pneumoniae*
2019-01-1378	Swine	2.38	*Klebsiella pneumoniae*
2019-01-1379	Swine	2.35	*Klebsiella pneumoniae*
2019-01-1380	Swine	2.44	*klebsiella variicola*
2019-01-1381	Swine	2.29	*klebsiella pneumoniae*
2019-01-1382	Swine	2.43	*Klebsiella pneumoniae*
2019-01-1383	Swine	2.47	*Klebsiella pneumoniae*
2019-01-1384	Swine	2.39	*Klebsiella pneumoniae*
2019-01-1635	Swine	2.53	*Klebsiella pneumoniae*
2019-01-1638	Swine	2.42	*Klebsiella pneumoniae*
2019-01-1672	Swine	2.33	*klebsiella variicola*
2019-01-1675	Swine	2.44	*Klebsiella pneumoniae*
2019-01-1676	Swine	2.48	*klebsiella variicola*
2019-01-1699	Swine	2.28	*Klebsiella pneumoniae*
2019-01-1700	Swine	2.33	*Klebsiella pneumoniae*
2019-01-1701	Swine	2.34	*Klebsiella pneumoniae*
2019-01-1759	Swine	2.4	*Klebsiella pneumoniae*
2019-01-1760	Swine	2.44	*Klebsiella pneumoniae*
2019-01-1761	Swine	2.46	*Klebsiella pneumoniae*
2019-01-1792	Cattle	2.39	*Klebsiella pneumoniae*
2019-01-1793	Cattle	2.32	*Klebsiella pneumoniae*
2019-01-1794	Cattle	2.41	*Klebsiella pneumoniae*
2019-01-1803	Swine	2.37	*Klebsiella pneumoniae*
2019-01-1805	Swine	2.45	*Klebsiella pneumoniae*
2019-01-1806	Swine	2.45	*Klebsiella pneumoniae*
2019-01-1807	Swine	2.43	*Klebsiella pneumoniae*
2019-01-1844	Swine	2.49	*Klebsiella pneumoniae*
2019-01-1847	Swine	2.48	*Klebsiella pneumoniae*

2019-01-1848	Swine	2.42	*Klebsiella pneumoniae*
2019-01-1960	Swine	2.2	*klebsiella pneumoniae*
2019-01-1961	Swine	2.45	*Klebsiella pneumoniae*
2019-01-1964	Swine	2.33	*Klebsiella pneumoniae*
2019-01-1986	Swine	2.36	*Klebsiella variicola*
2019-01-2018	Dog	2.12	*Klebsiella pneumoniae*
2019-01-2024	Dog	2.24	*Klebsiella oxytoca*
2019-01-2112	Swine	2.4	*Klebsiella pneumonia*
2019-01-2113	Swine	2.42	*Klebsiella pneumonia*
2019-01-2114	Swine	2.53	*Klebsiella variicola*
2019-01-2116	Swine	3.37	*Klebsiella variicola*
2019-01-2163	Swine	2.42	*Klebsiella pneumoniae*
2019-01-2164	Swine	2.3	*Klebsiella pneumoniae*
2019-01-2166	Swine	2.47	*Klebsiella pneumonia*
2019-01-2200	Swine	2.39	*Klebsiella pneumonia*
2019-01-2201	Swine	2.29	*Klebsiella pneumoniae*
2019-01-2218	Swine	2.24	*Klebsiella oxytoca*
2019-01-2219	Swine	2.18	*Klebsiella pneumoniae*
2019-01-2221	Swine	2.43	*Klebsiella pneumoniae*
2019-01-2222	Swine	2.49	*Klebsiella pneumoniae*
2019-01-2264	Dog	2.31	*Klebsiella pneumoniae*
2019-01-2303	Swine	2.07	*Klebsiella pneumoniae*
2019-01-2304	Swine	2.44	*Klebsiella variicola*
2019-01-2305	Swine	2.42	*Klebsiella variicola*
2019-01-2308	Swine	2.44	*Klebsiella pneumoniae*
2019-01-2309	Swine	2.36	*Klebsiella pneumoniae*
2019-01-2310	Swine	2.22	*Klebsiella pneumoniae*
2019-01-2311	Swine	2.43	*Klebsiella pneumoniae*
2019-01-2853	Dog	2.49	*Klebsiella pneumoniae*
2019-01-2869	Swine	2.38	*Klebsiella pneumoniae*
2019-01-2870	Swine	2.44	*Klebsiella pneumoniae*
2019-01-2871	Swine	2.35	*Klebsiella pneumoniae*
2019-01-2872	Swine	2.42	*Klebsiella pneumoniae*
2019-01-2873	Swine	2.38	*Klebsiella pneumoniae*
2019-01-2904	Swine	2.44	*Klebsiella pneumoniae*
2019-01-2929	Swine	2.42	*Klebsiella variicola*
2019-01-2930	Swine	2.32	*Klebsiella pneumoniae*
2019-01-2931	Swine	2.22	*Klebsiella oxytoca*
2019-01-2932	Swine	2.42	*Klebsiella pneumoniae*
2019-01-2933	Swine	2.43	*Klebsiella pneumoniae*
2019-01-2934	Swine	2.39	*Klebsiella pneumoniae*

2019-01-2949	Swine	2.21	*Klebsiella pneumoniae*
2019-01-2950	Swine	2.14	*Klebsiella pneumoniae*
2019-01-2951	Swine	2.36	*Klebsiella aerogenes*
2019-01-2955	Cattle	2.23	*Klebsiella pneumoniae*
2019-01-3120	Cattle	2.14	*Klebsiella pneumoniae*
2019-01-3125	Cattle	2.3	*Klebsiella pneumoniae*
2019-01-3127	Swine	2.4	*Klebsiella pneumoniae*
2019-01-3128	Swine	2.42	*Klebsiella pneumoniae*
2019-01-3129	Swine	2.15	*Klebsiella oxytoca*
2019-01-3130	Swine	2.23	*Klebsiella pneumoniae*
2019-01-3131	Swine	2.22	*Klebsiella oxytoca*
2019-01-3158	Swine	2.39	*Klebsiella variicola*
2019-01-3159	Swine	2.29	*Klebsiella pneumoniae*
2019-01-3160	Swine	2.4	*Klebsiella variicola*
2019-01-3161	Swine	2.26	*Klebsiella variicola*
2019-01-3162	Swine	2.35	*Klebsiella pneumoniae*
2019-01-3178	Swine	2.1	*Klebsiella oxytoca*
2019-01-3179	Swine	2.36	*Klebsiella variicola*
2019-01-3181	Swine	2.37	*Klebsiella pneumoniae*
2019-01-3183	Swine	2.3	*Klebsiella pneumoniae*
2019-01-3185	Swine	2.42	*Klebsiella pneumoniae*
2019-01-3186	Swine	2.28	*Klebsiella oxytoca*
2019-01-3187	Swine	2.44	*Klebsiella pneumoniae*
2019-01-3233	Dog	2.27	*Klebsiella pneumoniae*
2019-01-3465	Dog	2.37	*Klebsiella variicola*
2019-01-3472-1	Dog	2.39	*Klebsiella oxytoca*
2019-01-3472-4	Dog	2.26	*Klebsiella pneumoniae*
2019-01-3481	Dog	2.4	*Klebsiella oxytoca*
2019-01-3483	Dog	2.25	*Klebsiella oxytoca*
2019-01-3560	Dog	2.3	*Klebsiella aerogenes*
2019-01-3588	Dog	2.48	*Klebsiella pneumoniae*
2019-01-3618	Dog	2.27	*Klebsiella pneumoniae*
2019-01-3619	Dog	2.14	*Klebsiella pneumoniae*
2019-01-3793	Swine	2.41	*Klebsiella pneumoniae*
2019-01-3794	Swine	2.34	*Klebsiella pneumoniae*
2019-01-3796	Swine	2.37	*Klebsiella pneumoniae*
2019-01-3912	Dog	2.43	*Klebsiella pneumoniae*
2019-01-3922	Dog	2.37	*Klebsiella pneumoniae*
2019-01-3895	Cattle	2.23	*Klebsiella oxytoca*
2019-01-3928	Cattle	2.36	*Klebsiella variicola*
2019-01-3929	Cattle	2.42	*Klebsiella pneumoniae*

2019-01-3930	Cattle	2.25	*Klebsiella pneumoniae*
2019-01-3932	Cattle	2.43	*Klebsiella variicola*
2019-01-3971	Swine	2.38	*Klebsiella pneumoniae*
2019-01-3989	Dog	2.39	*Klebsiella pneumoniae*
2019-01-3992	Dog	2.36	*Klebsiella oxytoca*
2019-01-4118	Swine	2.17	*Klebsiella pneumoniae*
2019-01-4119	Swine	2.46	*Klebsiella variicola*
2019-01-4121	Swine	2.38	*Klebsiella variicola*
2019-01-4122	Swine	2.41	*Klebsiella pneumoniae*
2019-01-4123	Swine	2.43	*Klebsiella pneumoniae*
2019-01-4124	Swine	2.44	*Klebsiella pneumoniae*
2019-01-4125	Swine	2.39	*Klebsiella pneumoniae*
2019-01-4126	Swine	2.45	*Klebsiella pneumoniae*
2019-01-4127	Swine	2.35	*Klebsiella pneumoniae*

Biofilm results for isolates from broiler & turkey

Sample	1 st run	2nd run	3rd run	Average
2018-01-215	0.723	0.571	0.877	0.724
2018-01-358	0.641	0.563	0.783	0.662
2018-01-388	0.69	0.375	0.551	0.539
2018-01-389	0.585	0.4	0.861	0.615
2018-01-473	0.766	0.542	0.796	0.701
2018-01-474	0.786	0.446	0.749	0.66
2018-01-475	0.117	0.017	0.037	0.057
2018-01-476	0.741	0.404	0.0523	0.399
2018-01-507	0.767	0.458	0.776	0.667
2018-01-531	0.751	0.651	0.754	0.719
2018-01-580	0.76	0.405	0.722	0.629
2018-01-582	0.958	0.723	0.853	0.845
2018-01-583	0.965	0.52	0.728	0.738
2018-01-598	0.659	0.478	0.676	0.604
2018-01-609	0.906	0.533	0.787	0.742
2018-01- 707	1.049	0.618	0.975	0.881
2018-01- 709	0.759	0.438	0.641	0.613
2018-01- 710	0.952	0.461	0.674	0.696
2018-01- 711	0.797	0.475	0.772	0.681
2018-01- 714	0.824	0.488	0.741	0.684
2018-01- 715	0.654	0.319	0.642	0.538
2018-01- 723	1.025	0.446	0.727	0.733
2018-01-778	0.807	0.526	0.706	0.68
2018-01-779	0.789	0.498	0.797	0.695
2018-01- 798	0.874	0.428	0.795	0.699

2018-01- 802	0.929	1.136	0.871	0.979
2018-01- 810	0.842	0.88	0.746	0.823
2018-01- 873	0.489	0.682	0.551	0.574
2018-01- 874	0.718	0.702	0.65	0.69
2018-01- 877	0.81	0.859	0.691	0.787
2018-01- 878	0.782	0.895	0.841	0.839
2018-01- 879	0.761	0.619	0.639	0.673
2018-01- 880	0.77	1.043	0.937	0.917
2018-01- 1000	0.549	0.79	0.647	0.662
2018-01- 1001	0.023	0.031	0.022	0.025
2018-01- 1007	1.038	1.013	0.824	0.958
2018-01- 1022	0.779	1.109	0.917	0.935
2018-01-1024	0.472	0.705	0.671	0.616
2018-01- 1095	0.828	0.874	0.761	0.821
2018-01- 1097	0.817	0.731	0.774	0.774
2018-01- 1116	0.808	0.788	0.825	0.807
2018-01- 1117	0.538	0.774	0.808	0.707
2018-01- 1148	0.774	0.751	0.679	0.735
2018-01- 1176	0.743	0.673	0.588	0.668
2018-01- 1178	0.493	0.57	0.578	0.547
2018-01- 1214	0.578	0.677	0.664	0.64
2018-01-1221	0.831	0.791	0.794	0.805
2018-01- 1256	0.612	0.488	0.618	0.573
2018-01-1319	0.572	0.709	0.841	0.707
2018-01-1320	0.695	0.766	0.807	0.756
2018-01-1417	0.695	0.731	0.739	0.722
2018-01-1418	0.722	0.633	0.729	0.695
2018-01-1439	0.73	0.65	0.764	0.715
2018-01-1440	0.614	0.436	0.668	0.573
2018-01-1445	0.734	0.636	0.743	0.704
2018-01-1446	0.828	0.612	0.781	0.74
2018-01-1475	0.511	0.423	0.513	0.482
2018-01-1584	0.71	0.712	0.78	0.734
2018-01-1585	0.812	0.661	0.726	0.733
2018-01-1593	0.818	0.587	0.757	0.721
2018-01-1594	0.846	0.746	0.867	0.82
2018-01-1649	0.631	0.456	0.593	0.56
2018-01-1658	0.681	0.463	0.555	0.566
2018-01-1683	0.811	0.627	0.657	0.698
2018-01-1685	0.809	0.696	0.786	0.764
2018-01-1768	0.668	0.556	0.625	0.616
2018-01-1769	0.661	0.457	0.564	0.561
2018-01-1774	0.768	0.574	0.674	0.672

2018-01-1775	0.65	0.538	0.617	0.602
2018-01-1802	0.55	0.372	0.578	0.5
2018-01-1803	0.838	0.554	0.642	0.678
2018-01-1933	0.792	0.736	0.785	0.771
2018-01-1945	0.724	0.557	0.664	0.648
2018-01-1946	0.69	0.62	0.743	0.684
2018-01-2050	0.634	0.511	0.485	0.543
2018-01-2051	0.839	0.684	0.745	0.756
2018-01-2084	0.658	0.492	0.577	0.576
2018-01- 2085	0.683	0.539	0.598	0.607
2018-01-2124	0.726	0.558	0.578	0.621
2018-01-2125	0.7	0.576	0.551	0.609
2018-01-2151	0.679	0.443	0.472	0.531
2018-01-2242	0.678	0.7	0.807	0.728
2018-01-2248	0.781	0.771	0.732	0.761
2018-01-2279	0.596	0.718	0.65	0.655
2018-01-2280	0.723	0.883	0.873	0.826
2018-01-2295	0.529	0.728	0.743	0.667
2018-01-2297	0.672	0.757	0.806	0.745
2018-01-2353	0.519	0.734	0.67	0.641
2018-01-2359	0.601	0.773	0.757	0.71
2018-01-2360	0.578	0.824	0.865	0.756
2018-01-2372	0.51	0.82	0.966	0.765
2018-01-2406	0.58	0.68	0.655	0.638
2018-01-2485	0.541	0.645	0.781	0.656
2018-01-2486	0.732	0.757	0.899	0.796
2018-01-2503	0.678	0.764	0.825	0.756
2018-01-2544	0.597	0.536	0.674	0.602
2018-01-2545	0.564	0.505	0.53	0.533
2018-01-2615	0.655	0.704	0.814	0.724
2018-01-2639	0.42	0.475	0.592	0.496
2018-01-2640	0.754	0.678	0.823	0.752
2018-01-2643	0.607	0.55	0.818	0.658
2018-01-2699	0.444	0.469	0.703	0.539
2018-01-2716	0.565	0.679	0.643	0.629
2018-01-2723	0.625	0.85	0.863	0.779
2018-01-2729	0.452	0.742	0.677	0.624
2018-01-2730	0.521	0.838	0.896	0.752
2018-01-2734	0.468	0.545	0.749	0.587
2018-01-2776	0.449	0.532	0.747	0.576
2018-01-2782	0.519	0.569	0.744	0.611
2018-01-2809	0.464	0.689	0.879	0.677
2018-01-2836	0.509	0.646	0.865	0.673

2018-01-2837	0.444	0.737	0.85	0.677
2018-01-2839	0.535	0.566	0.841	0.647
2018-01-2860	0.426	0.58	0.84	0.615
2018-01-2861	0.569	0.577	0.916	0.687
2018-01-2912	0.602	0.682	0.98	0.755
2018-01-2926	0.584	0.611	0.969	0.721
2018-01-2939	0.682	0.632	1.083	0.799
2018-01-2940	0.557	0.581	0.893	0.677
2018-01-3012	0.303	0.385	0.633	0.44
2018-01-3029	0.441	0.462	0.782	0.562
2018-01-3030	0.624	0.648	1.071	0.781
2018-01-3042	0.013	0.007	0.022	0.014
2018-01-3046	0.573	0.595	0.873	0.68
2018-01-3094	0.671	0.619	0.925	0.738
2018-01-3095	0.569	0.511	0.712	0.597
2018-01-3096	0.616	0.563	0.785	0.655
2018-01-3115	0.574	0.63	0.72	0.641
2018-01-3125	0.748	0.644	0.932	0.775
2018-01-3163	0.596	0.663	0.9	0.72
2018-01-3218	0.455	0.524	0.669	0.549
2018-01-3247	0.607	1.048	0.787	0.814
2018-01-3254	0.586	0.852	0.843	0.76
2018-01-3269	0.637	0.622	0.735	0.665
2018-01-3295	0.423	0.524	0.673	0.54
2018-01-3296	0.524	0.654	0.862	0.68
2018-01-3297	0.358	0.457	0.606	0.474
2018-01-3305	0.528	0.591	0.666	0.595
2018-01-3334	0.616	0.584	0.744	0.648
2018-01-3408	0.592	0.78	0.868	0.747
2018-01-3447	0.527	0.648	0.886	0.687
2018-01-3448	0.508	0.635	0.764	0.636
2018-01-3463	0.454	0.498	0.689	0.547
2018-01-3464	0.066	0.072	0.147	0.095
2018-01-3465	0.624	0.762	1.021	0.802
2018-01-3545	0.654	0.665	0.849	0.723
2018-01-3554	0.542	0.527	0.57	0.546
2018-01-3576	0.523	0.549	0.865	0.646
2018-01-3590	0.613	0.674	1.171	0.819
2018-01-3596	0.478	0.558	0.825	0.62
2018-01-3650	0.577	0.694	0.967	0.746
2018-01-3688	0.556	0.651	0.913	0.707
2018-01-3692	0.634	0.783	1.016	0.811
2018-01-3723	0.606	0.641	0.847	0.698

2018-01-3773	0.505	0.541	0.797	0.614
2018-01-3776	0.491	0.523	0.826	0.613
2018-01-3777	0.307	0.654	0.965	0.642
2018-01-3779	0.475	0.632	0.903	0.67
2018-01-3780	0.409	0.583	0.783	0.592
2018-01-3781	0.495	0.65	0.837	0.661
2018-01-3805	0.551	0.533	0.801	0.628
2018-01-3863	0.627	0.778	0.935	0.78
2018-01-3864	0.618	0.756	1.101	0.825
2018-01-3892	0.484	0.642	0.8	0.642
2018-01-3893	0.532	0.698	0.925	0.718
2018-01-3925	0.012	0.016	0.018	0.015
2018-01-3926	0.471	0.718	1.035	0.741
2018-01-3928	0.491	0.509	0.708	0.569
2018-01-3929	0.545	0.563	0.754	0.621
2018-01-3934	0.757	0.583	0.784	0.708
2018-01-3935	0.511	0.577	0.742	0.61
2018-01-3975	0.832	0.793	0.948	0.858
2018-01-4019	0.365	0.369	0.484	0.406
2018-01-4046	0.753	0.704	0.732	0.73
2018-01-4107	0.673	1.021	0.839	0.844
2018-01-4108	0.57	1.106	0.783	0.82
2018-01-4113	0.66	0.945	0.856	0.82
2018-01-4174	0.541	0.806	0.767	0.705
2018-01-4175	0.736	0.756	0.962	0.818
2018-01-4196	0.72	0.661	0.781	0.721
2018-01-4197	0.779	0.851	0.832	0.821
2018-01-4203	0.609	0.913	0.843	0.788
2018-01-4247	0.6	0.865	0.765	0.743
2018-01-4248	0.618	0.948	0.875	0.814
2018-01-4278	0.637	0.996	0.885	0.839
2018-01-4306	0.58	1.028	0.892	0.833
2018-01-4341	0.369	0.736	0.515	0.54
2018-01-4379	0.397	0.7	0.741	0.613
2018-01-4390	0.568	0.796	0.593	0.652
2018-01-4458	0.468	0.692	0.653	0.604
2018-01-4459	0.565	0.735	0.607	0.636
2018-01-4460	0.541	0.971	0.689	0.734
2018-01-4487	0.508	0.742	0.639	0.63
2018-01-4514	0.452	0.806	0.665	0.641
2018-01-4639	0.673	0.853	0.936	0.821
2018-01-4652	0.329	0.431	0.515	0.425
2018-01-4653	0.495	0.66	0.678	0.611

2018-01-4682	0.51	0.515	0.592	0.539
2018-01-4734	0.692	0.677	0.738	0.702
2018-01-4735	0.678	0.712	0.79	0.727
2018-01-4736	0.676	0.651	0.916	0.748
2018-01-4739	0.716	0.596	0.902	0.738
2018-01-4766	0.525	0.573	0.85	0.649
2018-01-4847	0.584	0.958	0.883	0.808

Biofilm results for isolates from swine, dog & cattle

Sample ID	Batch 1	Batch 2	Batch 3	Average
2019-01-61	0.619	0.74	0.521	0.627
2019-01-63	0.737	0.929	0.646	0.771
2019-01-78	0.548	0.634	0.445	0.542
2019-01-154	0.635	0.657	0.568	0.62
2019-01-156	0.673	0.891	0.614	0.726
2019-01-204	0.584	0.642	0.517	0.581
2019-01-205	0.591	0.685	0.541	0.606
2019-01-206	0.53	0.694	0.562	0.595
2019-01-324	0.637	0.817	0.697	0.717
2019-01-326	0.686	0.858	0.726	0.757
2019-01-375	0.722	0.949	0.822	0.831
2019-01-376	0.648	0.943	0.796	0.796
2019-01-448	0.768	1.173	0.96	0.967
2019-01-476	0.582	0.705	0.609	0.632
2019-01-587	0.68	0.763	0.681	0.708
2019-01-588	0.769	0.962	0.812	0.848
2019-01-589	0.744	0.956	0.811	0.837
2019-01-591	0.745	1.102	0.827	0.891
2019-01-604	0.82	0.945	0.857	0.874
2019-01-605	0.315	0.364	0.365	0.348
2019-01-606	0.713	0.883	0.806	0.801
2019-01-608	0.694	0.679	0.636	0.67
2019-01-655	0.641	0.635	0.702	0.659
2019-01-658	0.549	0.577	0.538	0.555
2019-01-792	1.141	1.518	1.426	1.362
2019-01-794	0.447	0.614	0.557	0.539
2019-01-796	0.708	0.822	0.669	0.733
2019-01-798	0.892	0.942	0.843	0.892
2019-01-802	0.721	0.593	0.732	0.682
2019-01-803	0.725	0.642	0.61	0.659
2019-01-804	0.827	0.753	0.717	0.766
2019-01-810	0.682	0.635	0.661	0.659

2019-01-858	0.557	0.457	0.428	0.481
2019-01-862	0.881	0.844	0.819	0.848
2019-01-863	0.86	0.734	0.743	0.779
2019-01-865	0.919	0.838	0.724	0.827
2019-01-895	0.828	0.719	0.67	0.739
2019-01-896	0.77	0.658	0.653	0.694
2019-01-897	0.939	0.833	0.736	0.836
2019-01-901	0.018	0.024	0.06	0.034
2019-01-902	0.744	0.692	0.654	0.697
2019-01-1007	0.792	0.772	0.776	0.78
2019-01-1065	0.263	0.332	0.281	0.292
2019-01-1066	0.948	0.921	0.812	0.894
2019-01-1068	0.901	0.732	0.784	0.806
2019-01-1635	0.726	0.589	0.75	0.688
2019-01-1336	0.814	0.822	0.811	0.816
2019-01-1338	0.842	0.766	0.844	0.817
2019-01-1343	0.853	0.843	0.804	0.833
2019-01-1363	0.896	0.767	0.726	0.796
2019-01-1364	0.618	0.596	0.657	0.624
2019-01-1378	0.86	0.68	0.779	0.773
2019-01-1379	0.896	0.776	0.921	0.864
2019-01-1380	0.765	0.671	0.727	0.721
2019-01-1381	0.882	0.89	0.857	0.876
2019-01-1382	0.608	0.652	0.735	0.665
2019-01-1383	0.759	0.668	0.594	0.674
2019-01-1384	0.774	0.692	0.657	0.708
2019-01-1635	0.626	0.601	0.468	0.565
2019-01-1638	0.919	0.687	0.846	0.817
2019-01-1672	0.485	0.494	0.437	0.472
2019-01-1675	0.684	0.598	0.608	0.63
2019-01-1676	1.18	1.041	1.027	1.083
2019-01-1699	0.964	0.837	0.718	0.84
2019-01-1700	0.754	0.872	0.589	0.738
2019-01-1701	0.649	0.591	0.522	0.587
2019-01-1759	0.738	0.622	0.5	0.62
2019-01-1760	0.812	0.823	0.585	0.74
2019-01-1761	0.816	0.782	0.602	0.733
2019-01-1792	0.808	0.792	0.709	0.77
2019-01-1793	0.839	0.695	0.619	0.718
2019-01-1794	0.606	0.598	0.548	0.584
2019-01-1803	0.436	0.448	0.288	0.391
2019-01-1805	0.487	0.485	0.466	0.479
2019-01-1806	0.842	0.985	0.787	0.871

2019-01-1807	0.752	0.754	0.681	0.729
2019-01-1844	0.674	0.621	0.604	0.633
2019-01-1847	0.826	0.914	0.681	0.807
2019-01-1848	0.99	0.683	0.725	0.799
2019-01-1960	0.863	0.834	0.685	0.794
2019-01-1961	0.86	0.853	0.698	0.804
2019-01-1964	0.826	1.08	0.721	0.876
2019-01-1986	0.629	0.645	0.514	0.596
2019-01-2018	0.674	0.958	0.655	0.762
2019-01-2024	0.648	0.627	0.642	0.639
2019-01-2112	1.037	0.682	0.681	0.8
2019-01-2113	0.735	0.511	0.632	0.626
2019-01-2114	0.929	0.391	0.433	0.584
2019-01-2116	0.768	0.553	0.64	0.654
2019-01-2163	0.786	0.593	0.591	0.657
2019-01-2164	0.959	0.751	0.737	0.816
2019-01-2166	0.822	0.659	0.718	0.733
2019-01-2200	0.765	0.565	0.571	0.634
2019-01-2201	0.93	0.7	0.745	0.792
2019-01-2218	0.576	0.631	0.803	0.67
2019-01-2219	0.97	0.852	0.791	0.871
2019-01-2221	0.907	0.732	0.785	0.808
2019-01-2222	0.864	0.708	0.702	0.758
2019-01-2264	0.83	0.688	0.728	0.749
2019-01-2303	0.579	0.509	0.609	0.566
2019-01-2304	0.016	0.023	0.02	0.02
2019-01-2305	0.637	0.577	0.773	0.662
2019-01-2308	0.994	0.815	0.872	0.894
2019-01-2309	0.968	0.737	0.66	0.788
2019-01-2310	0.553	0.453	0.459	0.488
2019-01-2311	1.194	0.835	0.953	0.994
2019-01-2853	0.023	0.014	0.024	0.02
2019-01-2869	0.905	0.464	0.768	0.712
2019-01-2870	0.271	0.317	0.389	0.326
2019-01-2871	0.621	0.689	1.011	0.774
2019-01-2872	0.655	0.478	0.745	0.626
2019-01-2873	0.304	0.046	0.066	0.139
2019-01-2904	0.803	0.466	0.731	0.667
2019-01-2929	0.353	0.36	0.408	0.374
2019-01-2930	0.189	0.109	0.244	0.181
2019-01-2931	0.015	0.019	0.018	0.017
2019-01-2932	0.654	0.468	0.659	0.594
2019-01-2933	0.238	0.182	0.225	0.215

2019-01-2934	0.807	0.573	0.669	0.683
2019-01-2949	1.081	0.639	0.794	0.838
2019-01-2950	0.753	0.517	0.719	0.663
2019-01-2951	0.091	0.091	0.096	0.093
2019-01-2955	0.867	0.556	0.624	0.682
2019-01-3120	0.764	0.462	0.62	0.615
2019-01-3125	0.832	0.553	0.833	0.739
2019-01-3127	0.65	0.416	0.527	0.531
2019-01-3128	0.822	0.48	0.706	0.669
2019-01-3129	0.168	0.177	0.233	0.193
2019-01-3130	0.23	0.166	0.144	0.18
2019-01-3158	0.759	0.47	0.604	0.611
2019-01-3159	0.644	0.329	0.474	0.482
2019-01-3160	0.511	0.346	0.398	0.418
2019-01-3161	0.569	0.368	0.465	0.467
2019-01-3162	0.114	0.092	0.151	0.119
2019-01-3178	0.714	0.547	0.4	0.554
2019-01-3179	0.551	0.307	0.294	0.384
2019-01-3181	0.924	0.549	0.552	0.675
2019-01-3183	0.745	0.463	0.466	0.558
2019-01-3185	0.729	0.393	0.307	0.476
2019-01-3186	0.394	0.268	0.22	0.294
2019-01-3187	0.796	0.519	0.468	0.594
2019-01-3233	0.67	0.437	0.36	0.489
2019-01-3465	0.471	0.419	0.285	0.392
2019-01-3472-1	0.851	0.687	0.514	0.684
2019-01-3472-4	0.592	0.412	0.455	0.486
2019-01-3481	0.922	0.516	0.472	0.637
2019-01-3483	0.651	0.577	0.346	0.525
2019-01-3560	0.395	0.355	0.128	0.293
2019-01-3588	0.288	0.201	0.312	0.267
2019-01-3618	0.707	0.627	0.469	0.601
2019-01-3619	0.474	0.345	0.284	0.368
2019-01-3793	0.699	0.435	0.592	0.575
2019-01-3794	0.675	0.401	0.413	0.496
2019-01-3796	0.646	0.388	0.348	0.461
2019-01-3895	0.789	0.518	0.447	0.585
2019-01-3912	0.819	0.65	0.563	0.677
2019-01-3922	0.794	0.663	0.349	0.602
2019-01-3928	0.684	0.44	0.27	0.465
2019-01-3929	0.407	0.244	0.247	0.299
2019-01-3930	0.546	0.272	0.325	0.381
2019-01-3932	0.534	0.419	0.436	0.463

2019-01-3989	0.613	0.402	0.522	0.512
2019-01-3971	0.654	0.43	0.384	0.489
2019-01-3992	0.723	0.55	0.505	0.593
2019-01-4118	0.71	0.494	0.488	0.564
2019-01-4119	0.498	0.327	0.331	0.385
2019-01-4121	0.714	0.537	0.544	0.598
2019-01-4122	0.019	0.013	0.007	0.013
2019-01-4123	0.622	0.429	0.407	0.486
2019-01-4124	0.523	0.434	0.396	0.451
2019-01-4125	0.369	0.26	0.254	0.294
2019-01-4126	0.79	0.496	0.422	0.569
2019-01-4127	0.551	0.3	0.328	0.393

Biofilm results - Thailand

Sample ID	Round 1	Round 2	Round 3	Average
1PK	0.73	0.662	0.506	0.633
5PK	0.535	0.497	0.379	0.470
9PK	0.596	0.648	0.451	0.565
10PK	0.509	0.538	0.379	0.475
13PK	0.364	0.482	0.244	0.363
15PK	0.567	0.687	0.444	0.566
16PK	0.549	0.752	0.394	0.565
17PK	0.03	0.013	0.005	0.016
19PK	0.368	0.756	0.278	0.467
20PK	0.02	0.008	0.006	0.011
21PK	0.702	0.656	0.537	0.632
23PK	0.687	0.652	0.449	0.596
25PK	0.007	0.011	0.021	0.013
26PK	0.005	0.008	0.001	0.005
27PK	0.627	0.624	0.459	0.570
31PK	0.504	0.498	0.347	0.450
38PK	0.009	0.004	0.006	0.006
39PK	0.714	0.698	0.543	0.652
40PK	0.47	0.394	0.255	0.373
41PK	0.231	0.101	0.182	0.171
43PK	0.001	0.012	0.008	0.007
49PK	0.828	0.755	0.559	0.714
50PK	0.932	0.02	0.006	0.319
51PK	0.476	0.421	0.279	0.392
55PK	0.577	0.482	0.419	0.493
56PK	0.295	0.219	0.164	0.226
57PK	0.687	0.635	0.523	0.615

60PK	0.762	0.63	0.664	0.685
70PK	0.717	0.767	0.456	0.647
76PK	0.787	0.639	0.496	0.641
77PK	0.134	0.016	0.056	0.069
78PK	0.634	0.522	0.45	0.535
79PK	0.665	0.514	0.529	0.569
80PK	0.016	0.031	0.008	0.018
83PK	0.59	0.542	0.423	0.518
85PK	0.005	0.023	0.003	0.010
86PK	0.674	0.53	0.501	0.568
87PK	0.002	0.002	0.007	0.004
88PK	0.87	0.332	0.389	0.530
89PK	0.732	0.642	0.524	0.633
90PK	0.547	0.003	0.004	0.185
95PK	0.136	0.553	0.567	0.419
96PK	0.524	0.585	0.531	0.547
99PK	0.569	0.547	0.413	0.510
101PK	0.721	0.715	0.612	0.683
104PK	0.588	0.134	0.261	0.328
105PK	0.617	0.492	0.536	0.548
127PK	0.429	0.655	0.792	0.625
137PK	0.518	0.015	0.018	0.184
139PK	0.83	0.622	0.737	0.730
140PK	0.981	0.662	0.91	0.851
143PK	0.727	0.592	0.671	0.663

Specification of media and equipment

Media/ equipment	**Producer**
Blood agar	Oxoid
Mueller Hinton (MH) agar	Thermo Fisher Scientific
Simmons citrate 1% inositol agar (SCAI)	Oxoid, Sigma
Luria Bertani (LB) broth	Merck KGaA, Darmstadt
LB broth - MIC	Thermo Fisher Scientific, Inc.
Nunc™ Nunclon™ microtiter	Nunc A/S, Roskilde, Denmark
EUVSEC	Thermo Fisher Scientific, Inc.
1% crystal violet	Sigma-Aldrich, St. Louis, MO, USA
LB broth without NaCl	Bacto-tryptone 10 g/liter, yeast extract 5 g/liter
Aceton:etanol	Prolab, VWR
Peptone water	Difco
Sterilized distilled water	Merc KGaA, Darmstadt
Amoxicillin	Sigma
MALDI-TOF	Bruker MALDI Biotyper. Version 4.1.90 (PYTH)
Matrix solution	α-cyano-4-hydoxycinnamic acid (HCCA), Acetonitrile, Trifluoroacetic acid and sterilized distilled water
Spectrophotometer	Multiscan MS; Thermo Fisher Scientific, Inc.
MagNa Pure 96 machine	Roche diagnostics
Hamilton NGS Star	Roche diagnostics
Quant-iT™ 1x dsDNA HS assay	Roche diagnostics
Illumina Miseq	Illumina

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