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Pig as a source of Methicillin-resistant *Staphylococcus aureus* in
Ireland

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1. Introduction

Staphylococcus aureus, a gram-positive, non-spore forming bacterium was discovered by a surgeon in 1880, Sir Alexander Ogston, in pus from surgical abscesses. Friedrich Rosenbach, a German physician and microbiologist, in 1884, named two characteristically different colored colonies, one white and one golden-yellow, *Staphylococcus albus* and *Staphylococcus aureus* respectively.

It has been since commonly noted that *Staphylococcus aureus* occurs as a commensal organism, one existing without causing apparent harmful effects. Under stressful conditions or after a skin injury though, *Staphylococcus aureus* could cause infection, making it an opportunistic pathogen. It is a versatile pathogen that commonly colonizes areas of nasal cavity or the skin, but since it does not cause an infection, it rarely requires treatment. Staphylococcal infections are of major importance in both veterinary and human medicine. In humans, clinical signs may vary from minor skin infections like impetigo (highly contagious skin infection producing blisters), boils and pimples, to even more serious conditions such as post-operative wound infections and cellulitis. Last but not least *S.aureus* is capable of causing pneumonia, bacteremia, meningitis and sepsis¹. Not only can humans get colonized by the organism, but also other wild and domesticated animals such as horses, dogs, cats, chicken, cattle and pigs can contract the bacterium. As far as treatment is concerned, antibiotics have always been the first line of defense in treatment of the condition in both animals and humans but unfortunately due to excessive usage of antibiotics some bacteria, among them *Staphylococcus aureus*, developed resistance mechanisms and these bacteria are to be considered further for public health. Resistance to methicillin, indicating resistance to all β -lactams, was reported in 1961, date which marks the appearance of Methicillin-resistant *Staphylococcus aureus*.

The aim of this essay is to report the prevalence of MRSA in Irish pig sector from pig farms to pig slaughterhouses and retail pork meat, as well as highlight the necessary alterations in perception, of anyone associating with swine, for the disease and acknowledgment of its significance.

2. Antibiotic Resistance

In order to treat and prevent bacterial infections we use medicines such as antibiotics. Antibiotic resistance occurs, when microorganism such as bacteria, fungi, parasites and viruses undergo a series of changes upon their exposure to antimicrobial drugs, including antibiotics, antifungals, anthelmintics and antivirals. Meaning that, the microorganism, through a series of changes and alterations, gains the ability to resist the effect of a medicine that was previously used to successfully treat them. Hence, it refers to a partial sensitivity or complete insensitivity of a microorganism to a number of antibacterial agents. Bacteria are capable of becoming resistant to more than one antibiotic, thus these bacteria are termed as multi-resistant. *S.aureus* is considered as such. As an outcome, since medicines tend to be ineffective, there is persistence of an infection and at the same time an increasing risk of transmission and spreading (fig 1). This unavoidably leads to an increase in medical costs, prolonged hospitalization and increased mortality. In EU drug-resistant bacteria are estimated to cause 25.000 deaths and cost above 1.4 billion euro annually in productivity losses and medical expenses². Antimicrobial resistance was encountered almost simultaneously with the discovery of antimicrobials themselves (fig 2 & fig 3). What is alarming though is the rate at which antibiotic resistance develops and how fast it spreads among different bacteria and around the earth. During his 1945 Nobel Prize lecture, Alexander Fleming reminded the world of the importance of using antibiotics cautiously to ensure their long term effectiveness.

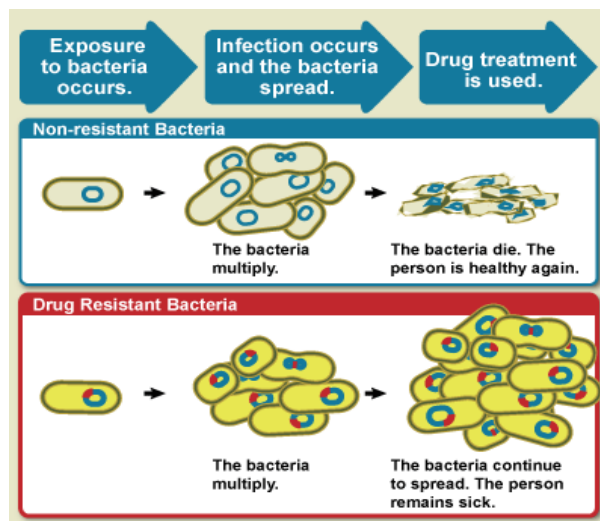


Figure 1. Difference between non-resistant and drug resistant bacteria³.

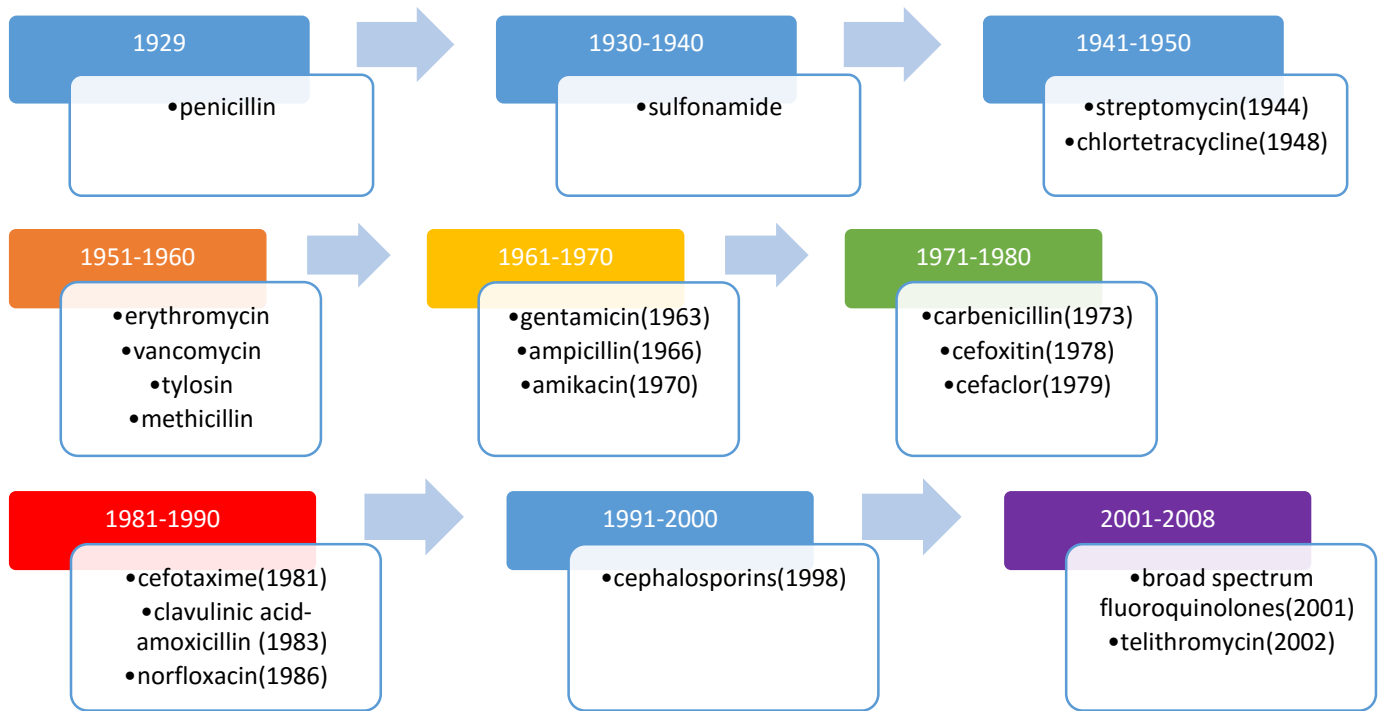


Figure 2. The Antibiotic Timeline⁴

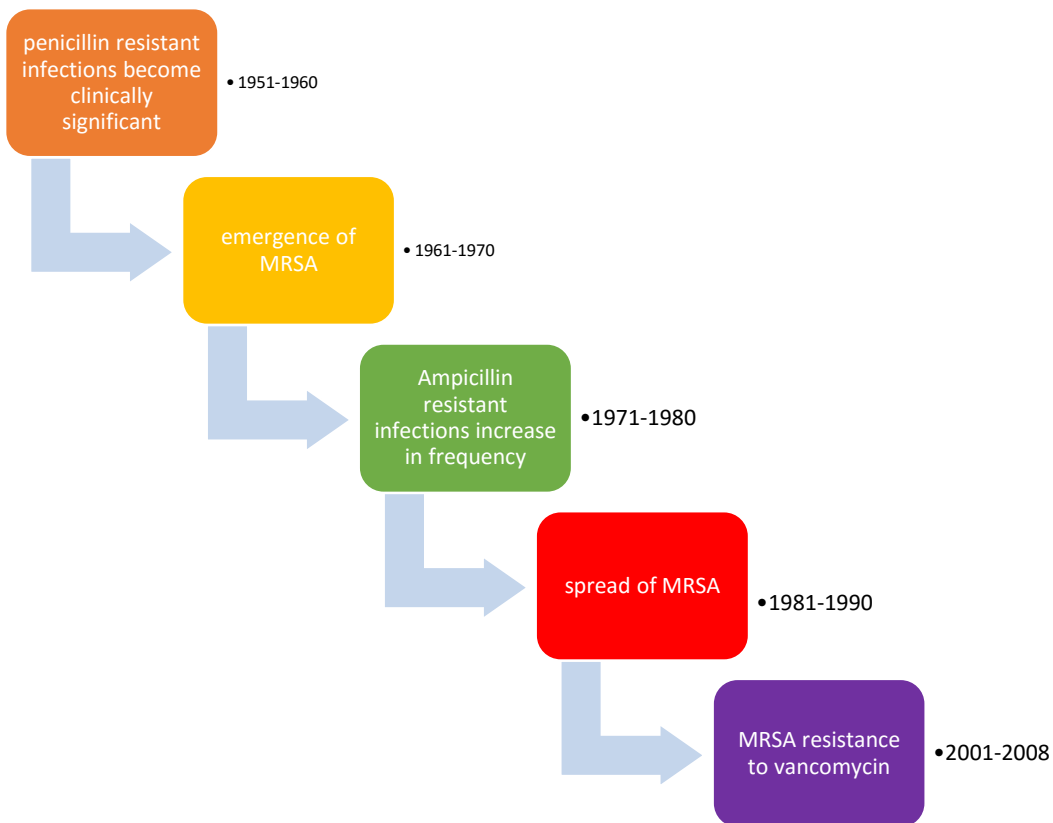


Figure 3. The antibiotic resistance timeline⁵

2.1. Molecular mechanisms/Strategies of bacterial resistance

2.1.1. Intrinsic resistance to antibiotics

Intrinsic resistance can be explained by 4 mechanisms:

- I. Inactivation or degradation of the antibiotic,
With the enzyme beta-lactamase, bacteria are able to hydrolytically deactivate the beta-lactam ring in penicillins and cephalosporins. Since penicilloic acid is inactive, it will not be able to bind to PBP's, and consequently protecting cell wall synthesis. The most usual way of bacterial resistance, is by enzymatic inactivation of the antibiotic agent.
- II. Prevent the antibiotic from reaching its target, with reducing its ability to penetrate into the cell,
Porin channels are the passage through which antibiotics cross the outer membrane of the bacterium to reach their target site. Some bacteria have developed the ability to modify the cell membrane porin channel selectivity, size and frequency for antibiotics such as beta-lactams and aminoglycosides. Through this process beta-lactams are unable to reach PBP's and aminoglycosides can't reach ribosomes, hence their action is diminished.
- III. Modify the antibiotic target within the bacteria,
By camouflaging or reprogramming certain target sites, bacteria make it impossible for the antimicrobial agent to recognize them and thus inhibiting any further binding or inhibition.
- IV. Reduced intracellular antibiotic accumulation by reducing the permeability and/or increasing active efflux of the antibiotic⁶,
Some bacteria have membrane proteins acting as efflux pumps for specific antibiotics. The antibiotics can be excreted at the same rate as they enter. This causes an insufficient intracellular concentration of the antibiotic making it incapable of eliciting a reaction.

Most but not all, mechanisms of resistance are encoded by plasmids. Plasmid is a DNA molecule located inside the cell that is physically separated from a chromosomal DNA, with the ability to replicate on its own. Plasmids can be transmitted to other bacteria. A study by University College Dublin (UCD), while performing plasmid profiling of a small number of MRSA strains, suggested that the most resistant strains possess the greatest number of plasmids, indicating that a certain amount of resistance elements are carried on them.

Figure 4, shows an overview of intrinsic resistance mechanisms. The example shown is of β -lactam antibiotics, targeting a penicillin-binding protein (PBP). Antibiotic A can enter the cell via a membrane-spanning porin protein, reach its target and inhibit peptidoglycan synthesis. Antibiotic B can also enter the cell via a porin, but unlike Antibiotic A, it is efficiently removed by efflux. Antibiotic C cannot cross the outer membrane and so is unable to access the target PBP⁷.

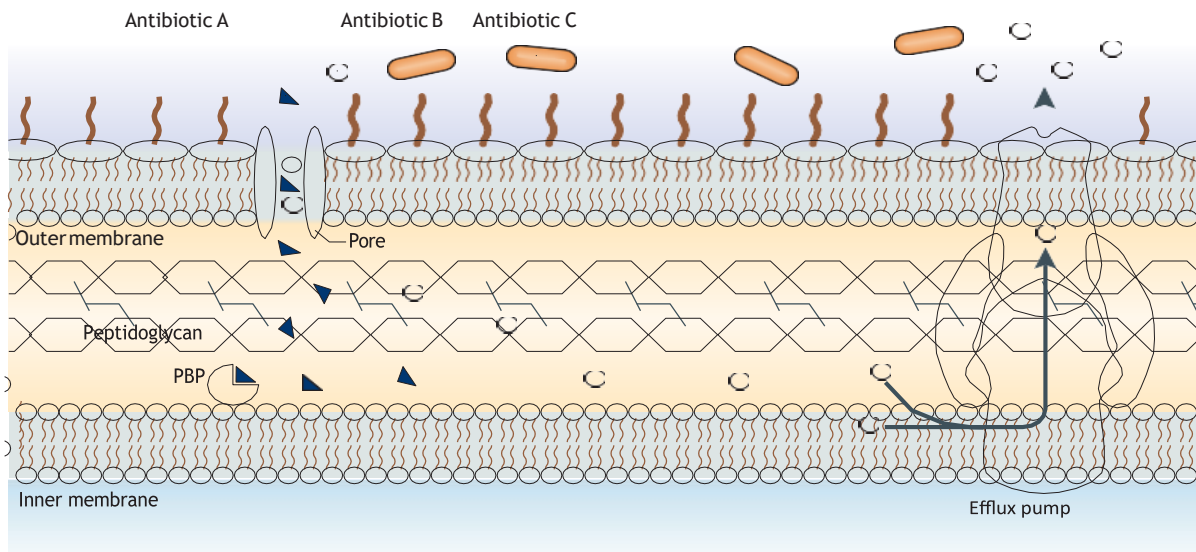


Figure 4. Intrinsic mechanisms of resistance.

The ability of bacteria to use the above mentioned strategies in order to resist antimicrobial compounds is genetically encoded. Intrinsic resistance is the natural ability of bacterial species to resist action of particular antimicrobial agents through its innate functional or structural characteristics. Intrinsic resistance is expressed by almost all strains belonging to the bacterium and is coded naturally.

2.1.2. Acquired resistance to antibiotics

Acquired resistance, can be the outcome of a successfully exchanged gene and/or an exchange involving: mutation or horizontal gene transfer via conjugation, transformation or transduction. Acquired resistance refers to alterations in the bacterial genome, which may lead to structural and functional fixtures alteration and consequently result in resistance against particular antimicrobial agents. *Staphylococcus* acquired *mecA* genes. *MecA* genes are found on a mobile genetic element which is called ‘staphylococcal cassette chromosome’⁸ which is responsible for coding penicillin

binding proteins (PBP). Penicillin binding proteins are protein involved in the final steps of synthesis of peptidoglycan, considered the major component of bacterial cell wall⁹. β -lactam antibiotics bind to PBP's and inactivate it and thus they inhibit the bacterial cell wall synthesis, which makes staphylococcus resistant to methicillin. Unlike intrinsic resistance, the traits that correlate with the acquired resistance are only encountered in some strains of each particular species of bacteria.

In table 1 a comparative overview of different bacterial mechanisms of resistance, alongside the mode of action of antibiotics is offered.

Table 1. Overview of the antibiotic mode of action and the bacterial mechanism of resistance¹⁰

Antibiotics	Antibiotic mode of action	Bacterial mechanism of resistance
Beta-lactams (penicillin, ampicillin)	Target and bind to penicillin-binding proteins, inhibiting bacterial cell wall synthesis, causing it to rupture	- Enzymatic destruction of beta-lactam rings - Target(PBP)modification - Reduced IC accum.
Glycopeptides (vancomycin)	Inhibit the last stage of cell wall synthesis by preventing cross-linking reactions	- Target modification - Produce false targets
Quinolones (ciprofloxacin)	Inhibit DNA gyrase, thus inhibiting bacterial DNA synthesis (topoisomerase inhibitor)	- Target modification - Reduced IC accum.
Aminoglycosides (gentamicin, amikacin, streptomycin)	Target and bind at 30s ribosomal subunit, resulting in inhibition of protein synthesis	- AB modification - Target modification - Reduce uptake
Macrolides (Erythromycin, Tylosin)	Target and bind to 50s ribosomal subunit, inhibiting protein synthesis	- Reduce IC uptake - Target modification

Tetracyclines (oxytetracycline, doxycycline)	Target and bind to 30s ribosomal subunit, inhibiting protein synthesis	- Reduced IC accum - Target modification
Rifamycins (Rifampin, Rifabutin)	Interact with bacterial RNA polymerase to block RNA synthesis	- Target modification
Sulfonamides (Sulfectamide, Sulfadiazine)	Targets DHPS and prevents addition of PABA, inhibiting folic acid synthesis	- Target modification

2.2. Resistance to penicillin

Before the introduction of antibiotics the mortality of patients with *S.aureus* bacteremia, reached 80%¹¹. With the introduction of penicillin in the early 1940s, this percentage drastically dropped and the prognosis was improved. However, in the 1942 penicillin-resistant staphylococci were discovered, firstly in hospitals and subsequently in the community¹². 20 years later, in the late 1960's 80% of staphylococci, that were isolated from hospitals and communities, were resistant to penicillin. It was Kirby who first demonstrated penicillin being inactivated by penicillin resistant strains of *S.aureus*¹³.

2.3. Resistance to methicillin

The first semisynthetic penicillinase-resistant penicillin was introduced in the 1959. It was called, Methicillin. Not long after its introduction it was accompanied by reports of isolates being methicillin resistant¹⁴. The result of infection as an outcome of methicillin resistant, is far worse than the result of an infection from methicillin-sensitive strains¹⁵. Same as penicillin resistant strains, MRSA isolates commonly carried resistance genes to different antimicrobial agents¹⁶. Due to the excessive use of penicillin, it is assumed that, *Staphylococcus aureus* acquired resistance by expressing *mecA* gene, which results in alteration of penicillin binding protein to penicillin binding protein 2 (PBP's2), which has a low affinity for most β -lactam antibiotics, and consequently allowing *S.aureus* to replicate under the presence of methicillin and related antimicrobial agents¹⁷.

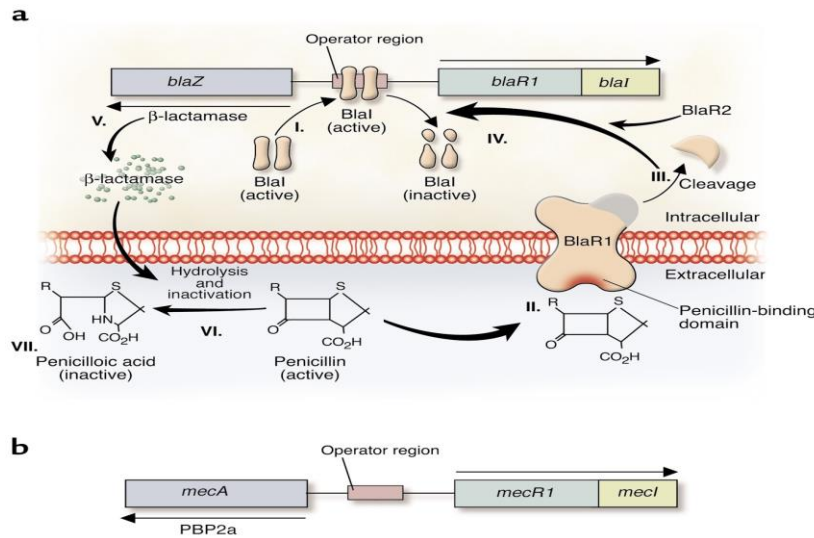


Figure 5. Description of staphylococcal β -lactamase synthesis, in the presence of penicillin ¹⁸

blaZ: β -lactamase gene

β -lactamase: an EC enzyme synthesized when Staphylococci are exposed to β -lactam antibiotics, it hydrolyzes the β -lactam ring, inactivating it¹⁹

blaR1: gene that encodes a signal transducing membrane protein

blaI: gene that encodes a repressor protein

- a. Description of staphylococcal β -lactamase synthesis, in the presence of penicillin (fig 5).
 - i. BlaI binds to the operator region, repressing RNA transcription from both *blaZ* and *blaR1-blaI*. With penicillin not present, β -lactamase is decreased
 - ii. Binding of penicillin to the glycoprotein BlaR1 stimulating BlaR1 autocatalytic activation
 - iii. Active BlaR1 breaks down BlaI into inactive fragments, enabling transcription of both *blaZ* and *blaR1-blaI* to start
 - iv. β -Lactamase, the extracellular enzyme encoded by *blaZ*,
 - v. hydrolyzes the β -lactam ring of penicillin
 - vi. renders it inactive (vii)
- b. Describes the mechanism of *S. aureus* resistance to methicillin. Synthesis of PBP2a is similar to the production of β -lactamase, and *mecA* gene is responsible for its production. Exposure of MecR1 to a β -lactam antibiotic, induces the synthesis of MecR1. In turn MecR1, inactivates MecI, allowing the synthesis of PBP2a. MecI and BlaI regulate the expression of PBP2a and β -lactamase²⁰.

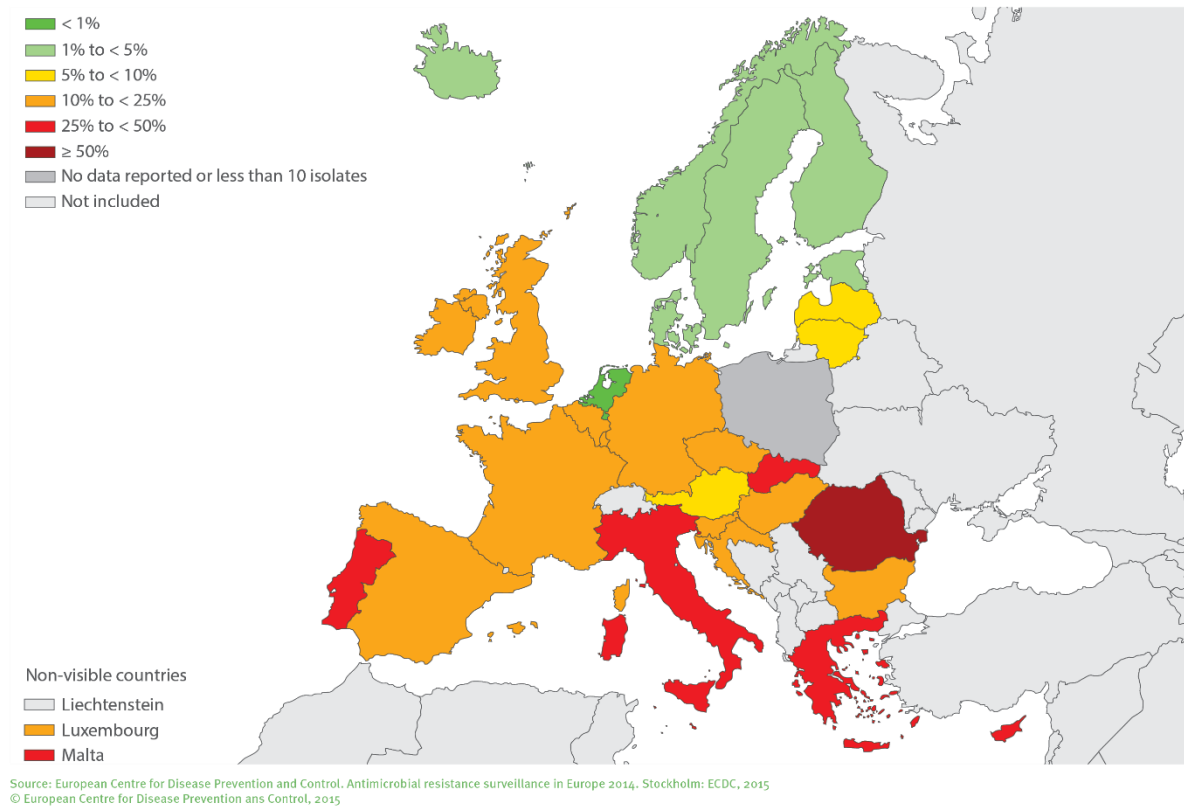


Figure 6. *Staphylococcus aureus* invasive isolates with resistance to methicillin in 2014

Figure 6 demonstrates the percentage of *Staphylococcus aureus* invasive isolates with resistance to methicillin, as detected by European Centre for Disease prevention and Control (ECDC) on 2014. A great variation in MRSA percentages is observed among different countries with a range between <1% to >60%. However according to ECDC the population weight mean percentage of MRSA infections has decreased since 2010 when the last survey was conducted²¹.

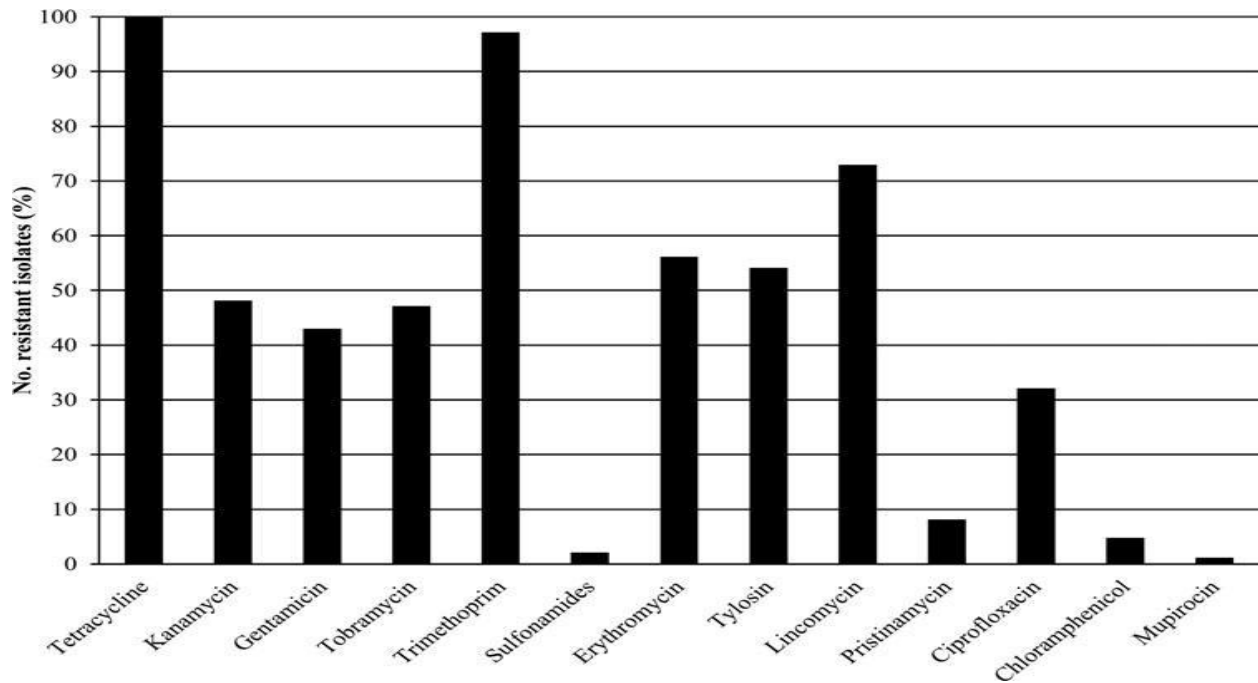


Figure 7. Resistance of MRSA strains

Figure 7, indicates the resistance of MRSA strains, isolated from pigs, in different antimicrobial agents. More specifically among 643 MRSA isolates, 32% were resistant to Fluoroquinolones, 73% were resistant to Lincosamides and 97% to trimethoprim²².

3. Molecular MRSA-typing

It is an important and effective way of monitoring and describing epidemiological trends. There are a lot of techniques available for typing MRSA strains. The most notable though are:

- Pulsed Field Gel Electrophoresis (PFGE), a variation of agarose gel electrophoresis, used for production of DNA fingerprint from the bacterium
- Multilocus Sequence Typing(MLST), through analysis of Staphylococcal Cassette Chromosome it defines the clonal complex(CC) type of MRSA strains
- Staphylococcal Cassette Chromosome mec (SCCmec) typing, classifies SCCmec elements (they are used in order to capture foreign DNA segments, to produce a defense against a dangerous environment for the bacteria) based on their difference in structure
- Single Locus Sequence Typing (SLST), compares the variation in the region X of protein A gene(*spa*) and coagulase (*coa*)²³

4. Methicillin-resistant *Staphylococcus aureus* (MRSA)

Before the introduction of antibiotics for the treatment of *S.aureus* infections, the mortality rate of the disease was about 80%²⁴. With the introduction of antibiotics in the 1940's it was soon after discovered that some organisms, including *S.aureus*, a Gram-positive bacterium, were developing a resistance to antibiotics such as penicillin. It was later found that the resistant bacteria were producing an enzyme which breaks down penicillin making it ineffective, an enzyme called penicillinase. In 1959 an antibiotic called methicillin was the first semi-synthetic penicillin derivative to be developed that resisted hydrolysis by staphylococcal β -lactamase²⁵(β -lactamases are enzymes produced by bacteria providing multi resistance to β -lactam antibiotics). At the beginning, isolates of *S.aureus* appeared susceptible to penicillin but it was only one year later that the first case of MRSA infection was recorded in UK²⁶. Since then MRSA has reached to become a worldwide concern of both veterinary and human medicine. Although methicillin is no longer used in practice and is replaced by isoxazolyl penicillins, like flucloxacillin, MRSA has continued to be used as an acronym. The annual epidemiological report of 2014 from the European Centre for Disease Prevention and Control (ECDC) stated that the percentage of *S.aureus* isolates that were reported as MRSA is stabilizing or decreasing significantly over the past 4 years. Although this observation may seem optimistic, the percentage of MRSA is still above 25% in almost 1/3 of reporting countries, mainly located in southern and Eastern Europe²⁷. MRSA in animals is a spillover of the prevalence of MRSA in people, arising from use of methicillin in people. In pigs infections are rarely caused by *S.aureus*, with the most common staphylococcal pathogen being *S.hyicus*.

4.1. Types of MRSA

4.1.1. Hospital-associated/acquired MRSA (HA-MRSA)

They are nosocomial infections, which are acquired by hospitalized patients while receiving healthcare. Many different risk factors have been connected with hospital-associated (HA) MRSA and include surgery, enteral feeding, fluoroquinolone²⁸ and macrolide administration, intravenous (IV) catheterization²⁹ and previous hospitalization^{30,31}. The majority of this particular MRSA strain in Europe has emerged with the introduction of SCCmec, the staphylococcal cassette chromosome mec, harboring *mecA*, the methicillin-resistance gene, into five clonal complexes

CC5, CC8, CC22, CC30, CC45, as they are defined by Multi-Locus Sequence Typing (MLST)³². The European Antimicrobial Resistance Surveillance System is employed by most European countries to record the occurrence of cerebrospinal and bloodstream infections of MRSA, mostly representing HA-MRSA morbidity (fig 8). “The prevalence of HA-MRSA has decreased in recent years in certain countries of Europe, e.g France, Ireland, UK, and Greece. In different European countries the prevalence has remained unaltered”³³.

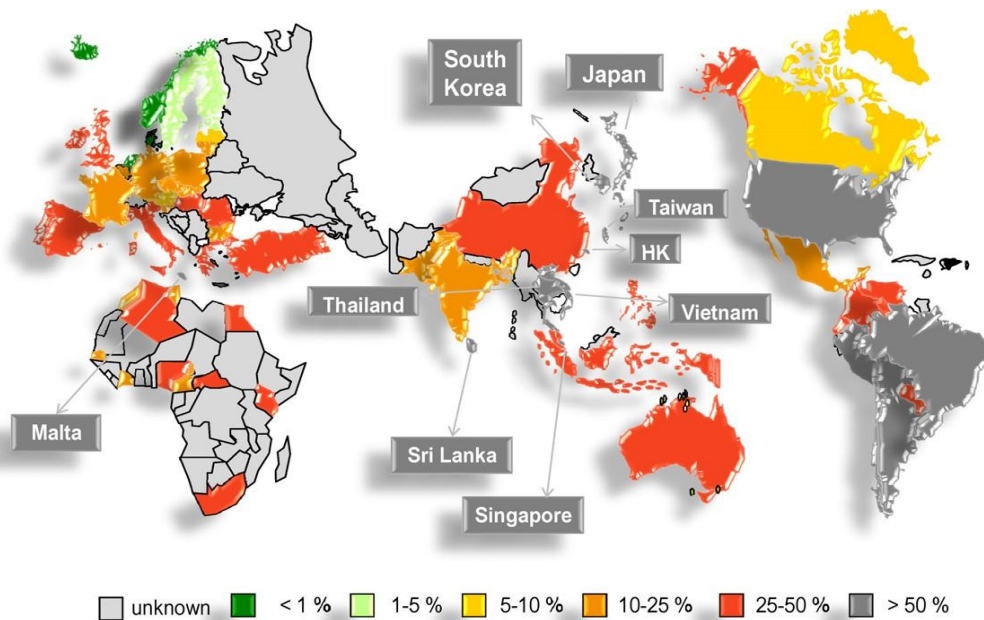


Figure 8. Worldwide prevalence of hospital acquired methicillin resistant *Staphylococcus aureus*³⁴.

4.1.2. Community associated/acquired MRSA (CA-MRSA)

In the 1980’s and early 90’s individuals in the community were found to be infected with MRSA³⁵. The infection was encountered among schoolchildren, prison inmates, soldiers and athletes³⁶. The difference between hospital associated and community associated MRSA is that in the latter, individuals acquired the infection without having any contact with health care facilities or any known then predisposing risk factors for MRSA, like surgery, previous hospitalization etc. Upon further investigation, it was discovered that factors such as overcrowding, or any activity that would inflict skin injury like contact sports or even personal equipment and belongings like razors, could increase the probability of people within these population to get infected with MRSA³⁷. Consequently this new MRSA classification was termed community-associated (CA) MRSA.

Further research was carried through and it was discovered that these strains carried genes encoding the exotoxin, Panton-Valentine leucocidin (PLV) toxin³⁸, which increases the virulence of certain Staphylococcal strains. In contrast to HA-associated MRSA, CA-associated was found to be susceptible to a number of antimicrobial agents ³⁹(table 2).

Table 2. Comparison of HA-MRSA and CA-MRSA

Characteristics	Hospital-associated MRSA	Community-associated MRSA
Risk group	Hospitalized, IV catheterization	Athletes, children
Antimicrobial resistance	Multidrug	Beta-lactams
Clinical signs	Surgical site, catheter-related infections	Skin and soft tissue infections ⁴⁰
PLV toxin	Rare	Frequent
SCCmec ^{41,42,43}	1, 2 ,3	4, 5, 6

Due to identification of HA-MRSA strains circulating within the community and the opposite⁴⁴, researchers are now focusing on molecular and microbiological characteristics rather than epidemiological factors for the classification of MRSA as HA or CA⁴⁵.

4.1.3. Livestock associated/acquired MRSA (LA-MRSA)

In 1972, MRSA was isolated from cows with mastitis⁴⁶. In the following 25 years, this sporadic case was followed by only a few similar cases. From 2000 however, reports have become more frequent, and in 2007 transmission of MRSA between cows and humans was reported⁴⁷. This was not the first occurrence of the disease in humans. In 2005 many decades after the discovery of HA-MRSA and CA-MRSA, the first LA-MRSA was encountered preoperatively in a 6-month-old girl and in pig farmers in the Netherlands⁴⁸. The MRSA isolate discovered, was non-typeable by Pulsed-Field Gel Electrophoresis (PFGE). Further research revealed, that these ‘new’ strains were resistant to digestion from the enzyme SmaI, commonly used for this test, and thus, they acquired the name Non-Typeable MRSA (NT-MRSA). An investigation was carried out concerning the association of NT-MRSA (‘non-typeable’ MRSA) in humans, with a reservoir in animals. From 2002 and 0%, NT-MRSA exceeded 21% by 2006⁴⁹. Through substitution of the SmaI enzyme with an alternative, it was discovered through molecular typing with Multilocus Sequence Typing

(MLST), that these strains belonged to clonal complex 398 (CC398), with the highest proportion of the strains belonging to the sequence type 398 (ST398)⁵⁰. The strains carry SCCmec cassette types 4 or 5⁵¹. They are resistant to aminoglycosides, tetracycline, macrolides and lincosamides.

4.1.3.1. LA-MRSA CC398

It is documented to be the most widely disseminated LA-MRSA strain. The first demonstration of CC398 in animals was conducted in France in 2005⁵². Afterwards it was discovered by Voss in Netherlands, and subsequently this strain has been identified in many more European countries, Asia as well as North America⁵³ and has become pandemic. However, CC398 can be absent from certain countries or at least not detected. According to comparative genome analysis, CC398 lineage is believed to have evolved from humans MSSA and then jumped to livestock, where it acquired the SCCmec cassette and developed resistance to methicillin⁵⁴. Despite being associated primarily with animal colonization, serious human infections and spreading within the health care system have been documented⁵⁵. This clone is generally susceptible to all antibiotics other than β -lactams and more specifically tetracyclines, which is a fact implying that the heavy use of tetracyclines in pig farming, favored this clone's emergence. Studies in Netherlands have showed that 39% of pigs at slaughter and >20% of pig farmers tested positive for MRSA⁵⁶. The pig-associated MRSA strain (ST398) is considered to be responsible for >33% of all human MRSA cases in Netherlands.

4.2. Humanosis or zoonosis

From 1970 until 2000, it was not common to isolate MRSA from animals; in the rare case that it would, the strains isolated were thought to be of human origin according to bio-typing and due to the fact that the majority of MRSA infections that were detected in companion animals was resembling human strains. Hence, it was assumed that the primary route of transmission between humans and animals, could only be achieved from the former to the latter-a 'humanosis'⁵⁷. To the contrary, the strains found in food producing animals, cattle⁵⁸ chicken⁵⁹ and pigs⁶⁰, tended to originate from farm animals. A recent study shown light into this dispute when it demonstrated, that MRSA can be transmitted in both directions and not only from humans to animals as it was previously believed⁶¹. Factors causing stress, such as overcrowding, skin injury, contaminated surfaces and fomites alongside poor hygiene, can contribute to the transmission of MRSA. These

factors are applicable to both animals and humans. Shared contaminated surroundings can facilitate the transmission of the disease⁶².

4.3. LA-MRSA in pigs

LA-MRSA is believed to be the predominant MRSA strain found in pigs. An extensive spreading of CC398 has been reported among pigs. Multiple studies from the Netherlands report the prevalence of farms being positive, between 23%-81%, with the occurrence of the disease among individual pigs varying from 11% to 39%⁶³. When studies were carried out to investigate the possible contamination of the environment of pigs, which were already tested positive, researchers suggested a strong correlation between the positive results from the environment and the pigs, hinting that the disease can be transmitted through dust/air within the farm. Furthermore the presence of finishing pigs in the farm, the farm size, the farming system (conventional/organic, open/closed), the usage of disinfectants and the amount of zinc added in the feed, influence the prevalence. The number of ST398 bacterial cells were increased in pigs' nostril's when zinc was added in the feed⁶⁴. Due to colonization in the nares and skin of clinically normal pigs in an infected farm, it is shed with skin scales and also nasal secretions. It can be transmitted from the sow's skin to the piglets at parturition and also by coming in contact with colonized pigs, contaminated pens, workers, as well as vehicles. A study showed that upon vaginal inoculation of the sow shortly prior to farrowing results in stable colonization, indicating that vertical transmission can be an effective mean of spread⁶⁵. In fact it is 1-4 times more likely for piglets to be colonized when they are farrowed by a MRSA positive sow. Last but not least, trade of pigs' further implicates transmission. The majority of LA-MRSA reports in pigs originate from Netherlands⁶⁶, but in Europe it has also been found and recorded in pigs located in Germany⁶⁷, Denmark⁶⁸ and Ireland⁶⁹. In a research done by European Food Safety Authority (EFSA) in 2009 MRSA was identified in pig farms in 17EU member states⁷⁰. The disease is an occasional cause of arthritis, mastitis, osteomyelitis, skin conditions and abscesses in pigs. As a matter of fact the only clone in Europe that has been connected with swine is ST398. Although it is more common in pigs, LA-MRSA CC398 has been reported in dairy cattle as well as poultry. In a research done in Belgian farms at which pigs were colonized with LA-MRSA CC398, it was discovered that other species including goats, dogs, cats and humans residing in this farm were colonized⁷¹.

4.4. LA-MRSA in humans

Humans can get infected with MRSA by coming in contact with contaminated fomites, skin to skin contact with already infected person, by pus from an infected wound, direct contact with contaminated animals and eating or handling contaminated meat. Additionally potential sources of the disease could be visitation to a farm, having a house member employed in farming or contaminated environment. Transmission between hosts is achieved primarily through contact. MRSA was primarily thought to be a human pathogen, but the detection of the disease in Belgian dairy cattle in 1972 with mastitis changed that perception⁷². MRSA transmission was reported between Hungarian cows with subclinical mastitis and a worker who was found to be throat swab positive⁷³. Once an animal gets exposed to MRSA it can become colonized, and serve as a reservoir. Through this way the disease can be transmitted to other animals as well as their human handlers. Many studies have shown that veterinarians and animal caretakers that come in contact with colonized animals may become colonized by MRSA themselves^{74,75,76,77}. Nasal colonization has also been found in slaughterhouse workers⁷⁸. Furthermore a lot of studies have proven transmittance of the MRSA CC398 from animals to their human handlers. Risk factors for the acquisition of LA-MRSA was working or encountering with pigs and cattle⁷⁹. However 15% of all LA-MRSA CC398 human cases in the Netherlands had no physical contact with pigs⁸⁰. Moreover close association between density of farming and occurrence of LA-MRSA has been described before by van Loo⁸¹ indicating its significance in livestock-dense areas. Hence it is suggested that spread of MRSA-CC398 occurs independently from possible contact with livestock in the areas heavily populated by pigs⁸². A threefold increase in MRSA occurrence in a Dutch hospital⁸³ and also 22% of people admitted in a German hospital⁸⁴ both located in areas with high density of MRSA-CC398 positive pigs carried the bacterium. Human to human transmission of LA-MRSA CC398 was believed to be a very rare incident. In contrast with pigs, CC398 in humans can cause similar infections with *S.aureus*. MRSA-CC398 can effectively cause severe diseases in humans including endocarditis, pneumonia, bacteremia, skin and soft tissue infections. The strain has been isolated from hospitals causing septicemia, joint infections or post-operative infections of the surgical site. The majority of LA-MRSA infections are resistant to most antimicrobial agents, although there are still sufficient options for treatment. Currently LA-MRSA is resistant

to macrolides, lincosamides, tetracyclines and β -lactams, and partly to fluoroquinolones. Instead it is susceptible to glycopeptides, rifampicin, daptomycin.

4.4.1. MRSA and swine farm workers

In the summer of 2004 in Netherlands, a 6-month-old girl was preoperatively diagnosed with MRSA. No member of the family had a history of travelling or being admitted to a foreign hospital. Attempts for decolonization took place, but they were unsuccessful and subsequently the girl's parents were diagnosed positive for MRSA. The family lived on a farm and raised pigs. In the autumn of the same year further investigations were carried out, to the pigs of the MRSA positive father/farmer as well as to other regional pigs. During the process of the study four (4) more incidents of MRSA arose, the first was from a pig farmer in a different region, the second in the son of a veterinarian who worked with pigs, the veterinarian himself and also from the nurse in the hospital into which the kid was admitted. The investigators found an unknown type of MRSA in the pigs of both farmers, with the molecular typing methods allowing researchers, to determine that the pig isolates and the isolates from the pig owner are identical. This unique MRSA clone was found to belong to ST398 and also to a group or related spa types. Through a broader survey that was conducted in the area, they estimated that pig owners had a 760 times higher chance of carrying MRSA than the general population⁸⁵, who were not exposed to swine. In pig farmers the colonization rate was found to be 23%, a number which is 100 times higher than that of the normal colonization rate in Netherlands. Since this initial study was performed many more studies that were conducted in France⁸⁶, Netherlands⁸⁷ and Canada⁸⁸, came to prove the assumption, that there is a higher risk of contamination when working closely with pigs. Dust in rooms with heavily colonized pigs can also be a source of human contamination. Two different papers, documented the high incidence (77-86%)⁸⁹ of nasal colonization of humans working in rooms with MRSA-positive pigs, but the degree of colonization seems to be dependent upon intensity of interaction alongside the duration of exposure⁹⁰.

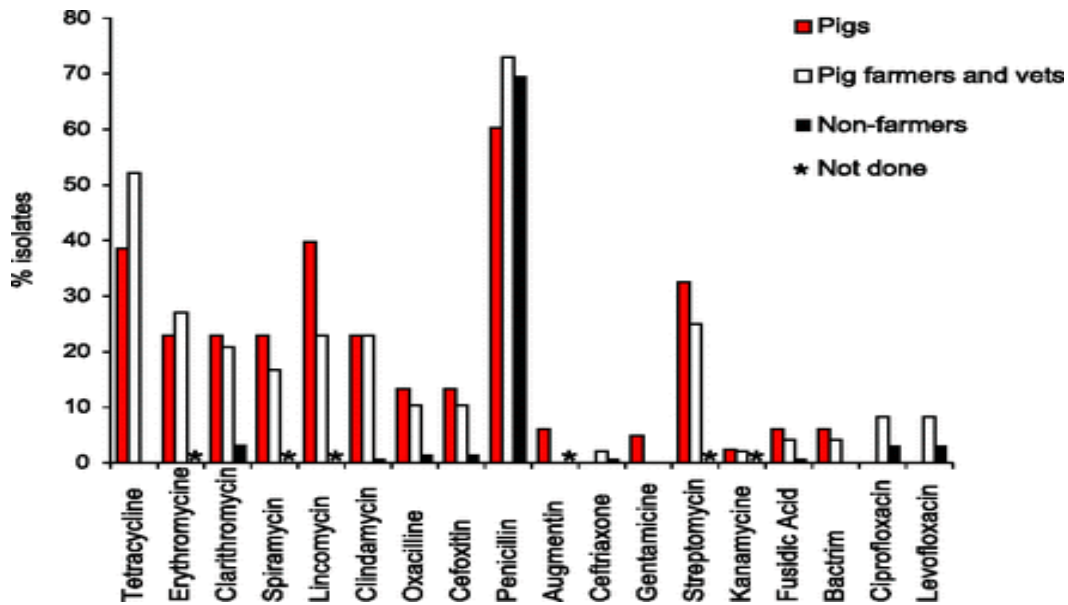


Figure 9. Nasal origin isolates resistant to antimicrobials⁹¹

According to Figure 9 one can draw the conclusion, that people closely associated with pig farming, have a greater resistance to antimicrobial agents compared to people having no contact with pigs. The level of antimicrobial resistance between farmers, veterinarians and pigs is essentially the same. It was also noted on this survey, that the strains of MRSA detected were identical in both humans and pigs. Due to comparative surveys being conducted, there is a suspicion that resistance might be altered with decreased usage of antimicrobial agents in pig farming. A recent study in Germany taking into consideration a declining usage of antibiotics in animal farming, documented a decrease in MRSA prevalence in pigs and LA-MRSA occurrence in humans, regardless of physical contact with the animals⁹². In the Netherlands, in order to reduce the usage of antibiotics a national program has been implemented since 2010. However, different patterns concerning antimicrobial resistance might be related to different farming techniques as well as management, for example difference in the number of animals hosted in a farm. Particularly in the Netherlands the usage of antibiotics is closely related with the farm size. Farm containing less than 250 sows use an average of 15 daily dosages whereas a farm with more than 600 sows uses 50 daily dosages of antibiotics⁹³. Table 3 shows the prevalence of ST398 and carriage of MRSA in healthy population, a summary from different studies indicating that working or living in a swine farm is a risk for MRSA acquisition⁹⁴.

Table 3. Prevalence of ST398 and carriage of MRSA in healthy population

Population	Country	No. of subjects	MRSA	ST398
Pig farmers ⁹⁵	Netherlands	26	23%	23%
Veterinarians ⁹⁶	International	345	7%	0%
Pig veterinarians ⁹⁷	International	235	14%	13.1%
Veterinarians and veterinary personnel ⁹⁸	Czech Republic	280	0.7%	0%
Veterinarians ⁹⁹	Belgium	146	9.5%	7.5%
Pig farmers ¹⁰⁰	Belgium	127	37.8%	37.8%
Pig farmers(exposed to MRSA positive pigs) ¹⁰¹	Germany	113		86%
Pig workers ¹⁰²	USA	20	45%	45%
Pig farmers ¹⁰³	IRELAND	100	2%	0%
Slaughter house workers ¹⁰⁴	Netherlands	249	5.6%	

4.4.2. MRSA and slaughterhouse workers

With the use of human and environmental samples three big slaughterhouses were studied in the Netherlands in 2008. The study proved that working with live pigs was the most important factor for abattoir workers getting colonized with MRSA (table 4).

Table 4: prevalence of, slaughterhouses' workers, nasal MRSA carriage¹⁰⁵

Contact with swine	Function	Total samples	MRSA positive	Percentage
Live pigs	Livestock transport worker	41	9	22%
	Official veterinarian +auxiliary	13	2	15.4%
	Lairage worker	32	2	6.3%
	Dirty area worker	7	1	14.3%
Dead pigs	Clean area worker	127	0	0%
Other	Administrative personnel	29	0	0%
Total		249	14	5.6%

The prevalence of MRSA found on the employees of the slaughterhouse is low (5.6%). Taking into consideration that the prevalence of the disease in retail meat is considerable, one can assume that the employees do not seem to be a significant source of meat contamination with MRSA. People in this study that did not work with live pigs all turned out to test negative for MRSA carriage. Hence, it is more probable that meat contamination with MRSA occurs through the equipment and the slaughterhouse environment. This cross-contamination has also been demonstrated for *Salmonella* spp. in slaughterhouses¹⁰⁶.

4.5. LA-MRSA in pork

Staphylococcus aureus is considered to be one of the most common pathogens causing food poisoning. Food poisoning from *S.aureus* is the result of staphylococcal enterotoxin production. Many type of enterotoxins, have been found and reported in connection with *S.aureus*¹⁰⁷. These enterotoxins have an immunosuppressive action; triggering nonspecific proliferation of T-cells. As an outcome, symptoms like abdominal pain, vomiting, high fever and diarrhea 2-6 hours after the consumption of contaminated food occur. In general though, clinical signs of food poisoning from *S.aureus* are mild. Hence, we can safely assume that the actual number of incidents of food poisoning derived from *S.aureus* is much higher than reported.

The isolation of MRSA strains from several food producing animals has been noted. During slaughtering MRSA-positive animals, there is a possibility of contaminating the carcasses with MRSA and eventually contamination of the meat of these animals may occur. Meat can frequently be contaminated with MRSA and can potentially be a vector for transmission of the disease after handling unprocessed meat or after ingestion. Ham has been described as the most frequently associated with food poisoning cause by staphylococci. There is a great variation in *S.aureus* isolates in pork. Recovery from 5%¹⁰⁸ of the samples has been reported, but also recovery from more than half fresh pork samples has been reported to be found positive as well¹⁰⁹. In Germany according to national surveillance programs in pig meat at retail it was found to be contaminated at a percentage of 16%¹¹⁰. In Netherlands out of 309 pork meat samples collected 33 (10.7%)¹¹¹ of them were positive for MRSA contamination. However food intoxication involving MRSA CC398 has not been reported yet and additionally it is found to be rare for this clonal complex to carry enterotoxin genes¹¹². Van Loo detected 2(2.5%) MRSA strains out of 79 samples of raw beef and pork¹¹³, although in that study a different detection method was applied which could explain

the low positive percentage. Raw unprocessed meat may contain MRSA as a result of contamination during the slaughtering process. Although nasal areas are believed to be the primary location of the organism it has been reported that it also resides in the intestines¹¹⁴. Hence, during slaughtering, carcasses can get contaminated through intestinal tract contents, from the infected personnel or from the slaughtering environment. Food is not considered an important route of transmission of the disease although one study discovered one patient colonized with MRSA-CC398 reporting; buying from a farm shop, could be a potential risk factor for acquiring the disease. Undoubtedly, food could be a possible route of transmission, but factors like compliance with kitchen hygiene as well as the frequency of consumption need to be taken into consideration when committing further future researches. It is important to state that this far there are no signs that LA-MRSA in pork has significantly contributed to the dissemination of the disease among humans.

4.6. The swine's industry point of view

Before the research and investigation done in Netherlands very few MRSA infections in swine workers had been reported and documented. *S.aureus* is not considered to have a considerable economic impact on swine production and therefore an insignificant impact on the industry. Additionally only a small number of human infections caused by this particular clone has been severe in Europe, and only one case was fatal. For all the above mentioned reasons, the industry has little motivation to study *S.aureus* or MRSA in particular. In contrast to the swine industry, some researchers think that ST398 has significant implications for the swine industry. P. van der Wolf concluded in a paper presented at the Iowa State University Swine Disease Conference in 2007 that, "a new type of MRSA has established itself in livestock throughout Europe and other parts of the world forming a reservoir of infection for humans who are in close contact to these animals. This establishment has large consequences for the livestock industry and the people working in that industry. Interventions are largely unknown and research is ongoing."¹¹⁵ Thomas Blaha concluded that, "Although there is no acute threat to human health due to MRSA ST398 (no steep increase of human cases reported) it is advisable to watch the occurrence and epidemiology of the pig-associated MRSA clonal line ST398 closely. Humans occupationally exposed to pigs should be educated about the potential risk for themselves and about their potential of carrying MRSA into a hospital."¹¹⁶

5. Methicillin-resistant *Staphylococcus aureus* in Ireland

5.1. MRSA in Irish human population

During 2002, it was found that out of 998 *S.aureus* isolates from human patients with bacteremia 416 (42%) were resistant to methicillin. This statistic placed Ireland fourth highest out of 27 European countries who submitted data to the European Antimicrobial Resistance Surveillance System (EARS). On 2008, 100 individuals were screened through nasal swabs, for nasal carriage of MRSA during Tullamore Pig Health Symposium. The individuals were farmers as well as personnel employed in the pig industry in Ireland. Only two individuals were identified to be nasal carriers of MRSA. The first ST398 isolation occurred at 2012 on an elderly patient resident of a rural nursing home. On 2009 he was admitted to a hospital and screened for MRSA. After nasal swabbing he tested positive. Decolonization followed after which the patient tested negative in all subsequent screenings until 2011. On October of that year, upon another admission of the same patient to the regional hospital when screened for MRSA, he tested positive on a swab taken from his throat while results came back negative from nasal and perineal samples. MRSA isolates that were recovered were submitted to the Irish National MRSA Reference Laboratory (NMRSARL) for molecular typing. Out of the two MRSA isolates that were recovered, typing suggested that they were distinctively different. One was found to be ST22-MRSA-IV and the other one ST398 as confirmed by DNA microarray profiling. ST22-MRSA-IV belong to clonal complex (CC) CC22, while ST398 belongs to CC398. It was not possible at the time, for investigators to trace the source of the infection. “This is the first reported case of ST398 MRSA in Ireland and although it was not possible to trace its source, it is important to note the similarity of this isolate with other spa type t011 ST398-MRSA strains of swine, bovine, and chicken origin,” the report states¹¹⁷. In Figure 10, the total number of invasive isolates tested (N) and percentage with resistance to methicillin (MRSA) including 95% confidence intervals (95% CI), in Ireland 2011-2014¹¹⁸, is described.

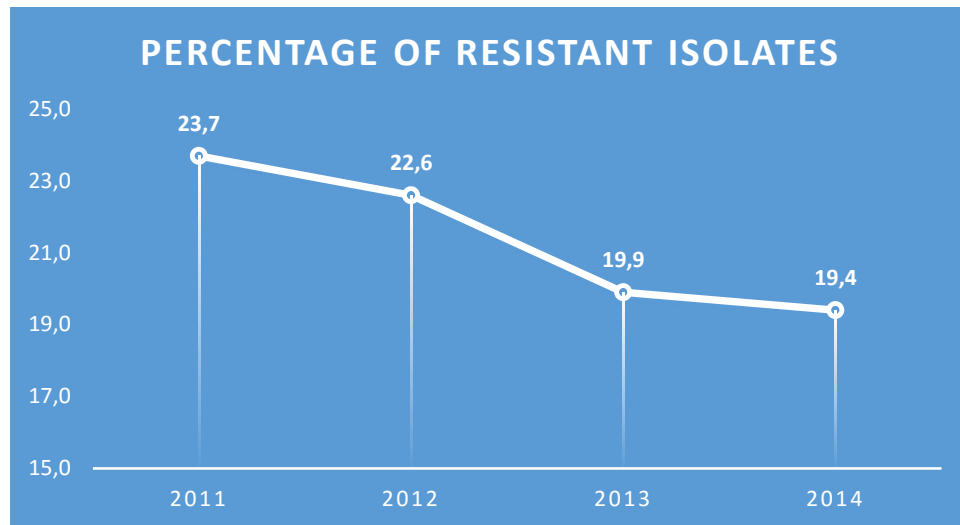


Figure 10. Percentage (R%) with resistance to methicillin (MRSA) from 2011-2014¹¹⁹.

As the prevalence of human infection of MRSA in Ireland is among the highest in Europe, it is possible that both pig and human strains exist in a percentage of Irish farms with pigs. Additionally the presence of foreign workers who constitute an accountable percentage of the labor on Irish pig farms, could potentially be a source of introduction of MRSA strains in Ireland.

5.1.1. National MRSA Reference Laboratory

Investigates MSSA and MRSA isolates upon the request of microbiological laboratories throughout Ireland. It has the ability to include isolates that were recovered from different patients and environmental sites from both community and hospital sources. It also contributes to the analysis of all MRSA bloodstream infection isolates, from Irish patients, hospitalized in institutions that participate in The European Antimicrobial Resistance Surveillance Network (EARS-Net) project.

5.2. MRSA in Irish pig farms

5.2.1. Early reports (2007-2011)

The National Reference Laboratory (NRL) had conducted two surveys in the look for evidence of MRSA in pigs. The first one was conducted in 2007-2008, investigating the presence of MRSA in the nasal cavity of pigs present in a slaughterhouse. 960 samples were taken from 96 herds, in 8 abattoirs over a course of 6 weeks. No positive samples were detected. Upon the second survey

which was conducted in 2008, as a part of a European baseline study for the presence of Salmonella and MRSA in breeding pigs, 190 dust samples were collected and for once more, none of the samples was found to contain MRSA (fig 11).

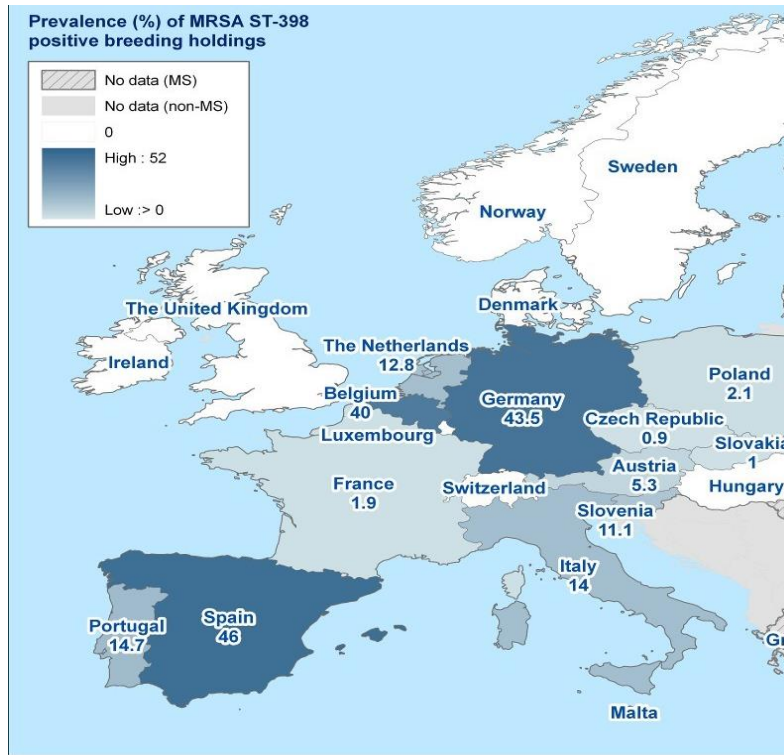


Figure 11. Prevalence of MRSA ST398 positive breeding holdings, MRSA EU baseline survey in breeding pigs, 2008¹²⁰.

Out of 440 pigs that were screened for MRSA on 2008, from 41 geographically distributed farms, no swine was found to carry it. In addition 15 dust samples from 3 abattoirs (5 in each) were collected which were again found to be negative for MRSA. During this study, the pig farms that were selected were located within high density pig farming locations. The size of the herds within the farms tested was varying from 246 pigs to 15,050 pigs. In 2008, the southern part of Ireland represented 68% of the pig population on the island according to the Central Statistics Office. This trend is present until today (fig 12).

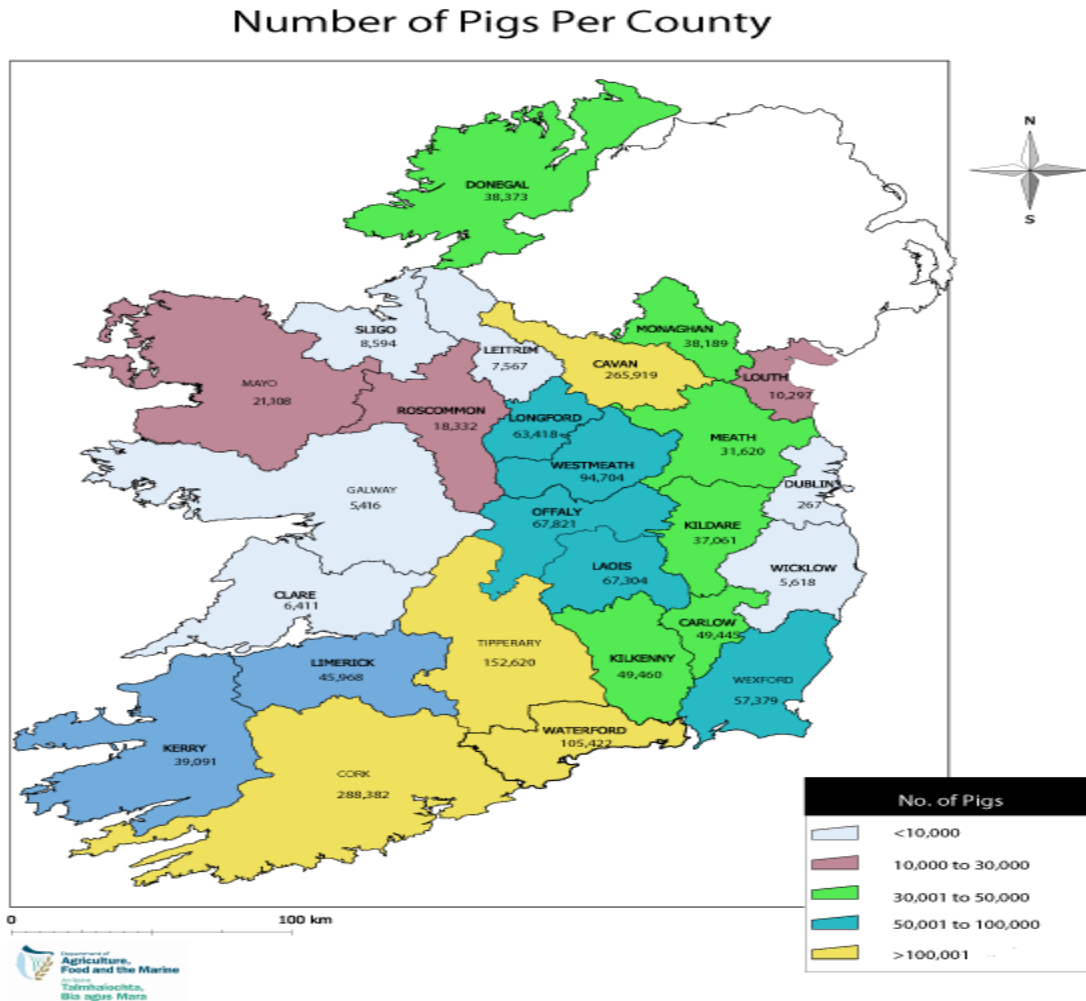


Figure 12. Pig population in Ireland ¹²¹

On a European survey of MRSA-ST398 isolates in 2009, Ireland was not included in the countries with the highest proportions of the incidence, ranging from 1.6% to 11.9%¹²². The possible explanation for the low prevalence in Ireland until that time was the low importation of pigs, which led to limited opportunities for the importation and spread of MRSA-ST398. Until 2011 Irish pigs were considered free of MRSA. Until 2012 no report on Irish animals indicated the presence of MRSA ST398 among them, despite the prevalence of ST398 in Europe among pigs, associated workers and their families.

5.2.2. Most recent reports (2012-present)

On 2012/2013 a MRSA-CC398 isolate was identified in a joint abscess of a pig, during a post mortem examination at the University College Dublin Veterinary Hospital (UVH). The farm,

FARM A, to which the MRSA positive pig was identified was visited. Nasal swabs from 100 pigs and 5 farm workers who were in contact with the pigs were collected. 8/100 pigs and 4/5 farmers tested positive for MRSA-CC398. This farm had been repopulated prior to the isolation of MRSA with gilts originating from Ireland and Germany. As a continuation of this investigation, another farm was sampled, FARM B, which was the finishing unit of FARM A, meaning that all weaned pigs from FARM A were delivered to FARM B at around 12 weeks of age. 10 farm workers were tested from FARM B. MRSA isolates were recovered from 5/10 nasal swabs from workers of FARM B. All isolates were found to belong to CC398¹²³. According to NMRSARL CC398 *S.aureus* represented 0.19% of *S.aureus* genotyped between 2010 and 2014¹²⁴. At this point it needs to be mentioned that MRSA infection in animals is not considered notifiable in Ireland. Thus no requirement for screening of imported animals exists. This policy may need to be reconsidered since importation of pigs from Germany, a region which has been documented to have a high prevalence of CC398 in both animals and humans, led to spreading of the disease in Irish pigs and farmers. On another occasion University College of Dublin (UCD) investigated two farms. The setting was the same. Farm A was providing second stage weaners to farm B. Farm A was composed by 2000 sows and was found MRSA positive. Farm B, introduced pigs from farm A, into its holding on June and July 2012 prior to MRSA detection in farm A. Depopulation, cleaning and disinfection was applied in farm B. Farmer repopulated the unit by introducing pigs into his holdings from a MRSA free farm. Within two hours after their introduction, pigs tested positive for MRSA. Further investigations were carried through in order to identify the source of infection. 42 nasal swab samples were collected randomly throughout the farm. 23.8% of swine sampled was found to be MRSA positive. Additionally, environmental swabs were collected from both an occupied and an unoccupied room. Environmental samples from occupied finisher room were found to be 42% positive on MRSA¹²⁵. Unoccupied room was found to be MRSA free. After molecular typing the spa type was determined to be t105 which belongs to CC398. It was also determined that the source of infection and recolonization was the loading ramp¹²⁶.

Antimicrobial susceptibility testing was carried out, the results of which showed that the majority of strains were resistant to β -lactams, lincomycin and erythromycin. Only one isolate showed resistance to tobramycin (Fig 13).

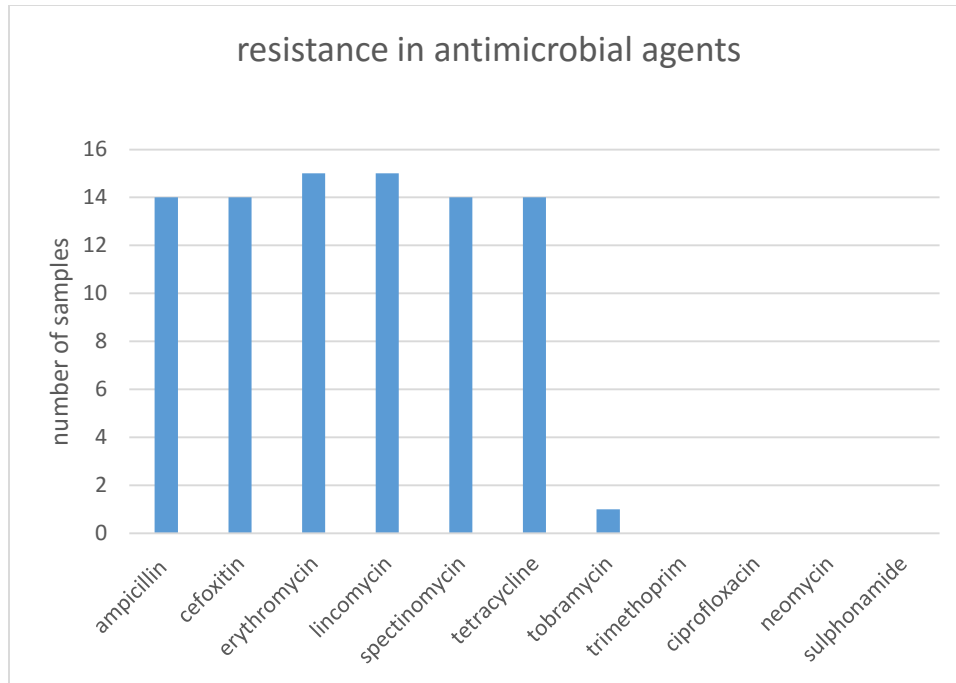


Figure 13. Resistance in antimicrobial agents¹²⁷.

An additional research was put on motion by UCD to further investigate MRSA in pigs, as an emerging pathogen of public health significance. Unfortunately due to the detection of MRSA ST398 during the late stages of the field study the researchers decided to use their discovery in order to perform a longitudinal study investigating the dissemination of the organism within the different production stages. Investigation was carried through on 4 farms, of which their colonization rate varied between 15%-70%¹²⁸. It was found that colonization level tended to peak during first stages of weaning, which was attributed to the increased level of stress as well as with the mixing of animals, taking place during that period. The sows were found to be a significant source of colonization for the piglets. Those born to nasal-positive sows had 12 times higher chances to become positive themselves. While being born to nasal and vaginal-positive sows increased those chances threefold. Through molecular typing two different clonal complexes were discovered. CC9, with the majority of MSSA isolates on Irish pig farms belonging to this clonal complex, and also MSSA ST398 being multidrug resistant but not to methicillin¹²⁹. This project deduced that, if control measures for the prevention of the introduction of the bacterium inside the farm are followed then they will be useful. Such are, strict disinfection, only buy and introduce pigs from MRSA-free herd and reduction of antibiotics usage as much as possible. Finally it was suggested that nasal swab sampling is not the most sensitive technique, due to the multiple

organism colonization in the tonsils. Probably this finding is of greater relevance for individual animals which may be brought into a herd and need a MRSA-free certification.

6. Control and eradication mechanisms

6.1. Antimicrobial agents consumption

Usage of antibiotics in animals potentially increases the risk factor for dissemination and selection of resistant microorganisms. A lot of antibiotics used in animals is the same as the components used in human medicine. Every time an antibiotic is used resistance-a natural process- develops. Risk of resistance increases, when the antibiotics are used too often, for too short duration, for too long, at low dosages and for treating a disease not susceptible to the specific antibiotic agent. Antibiotics are a useful tool once used appropriately. The European Surveillance of Veterinary Antimicrobial Consumption programme (ESVAC) was introduced. Since 2009 Health Products Regulatory Authority (HPRA) has published data for antimicrobial sales. These data are collated by HPA for ESVAC. Data on antimicrobial use in animals are limited, but some indicative information can be obtained through that initiative. As Dr. Micheál O' Mahony mentioned “these data (fig 14) have many limitations, yet are the best available, and much more useful than were available before this initiative. Notably they come from central records of drug companies, not prescribing data, hence they indicate what is sold and not what is prescribed. As an outcome drugs marketed for pigs and prescribed to fish under cascade will appear here as going to pigs. Furthermore these data come in crude tones, which don't take into account greater potency of some molecules (lower tones might have more effect). Also it does not take into account differing populations, Ireland has more cattle than sheep, or different dosages, cattle are bigger than sheep. The various DANMAP reports pertaining to food production in Denmark are much more useful set of data but just for that country”. DANMAP is the Danish program for surveillance of antimicrobial consumption and resistance bacteria from animals, food and humans.

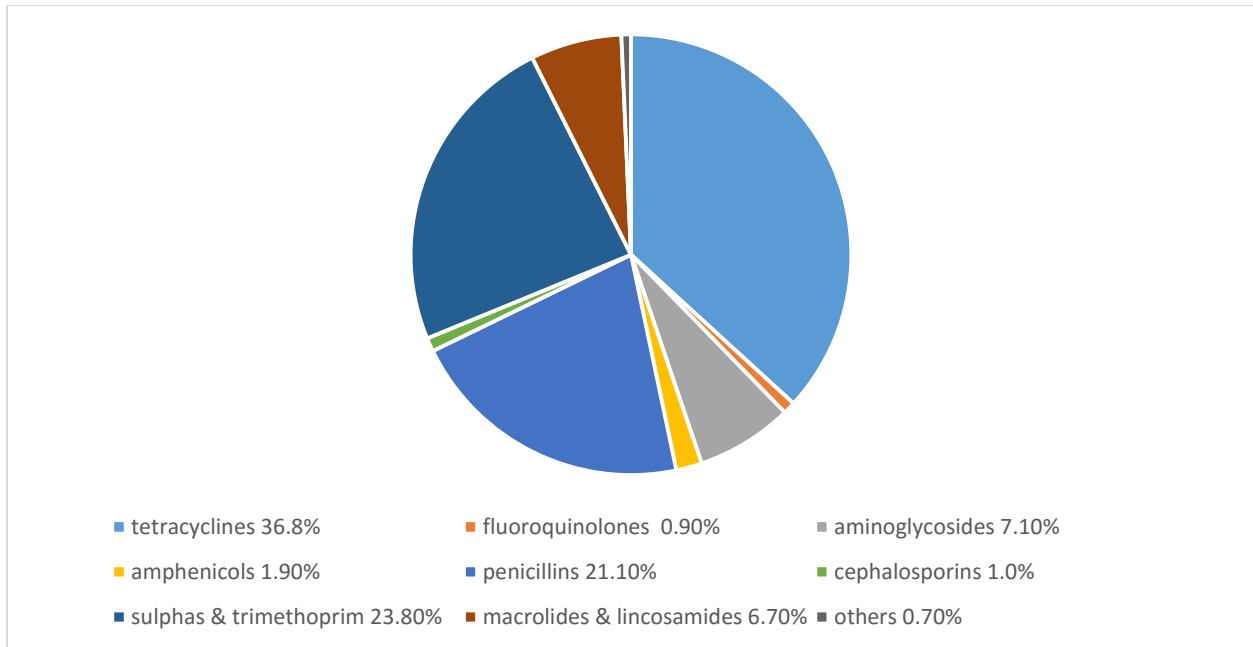


Figure 14. Distribution of sales (tons) of antibiotics supplied for veterinary use in 2013 in Ireland¹³⁰

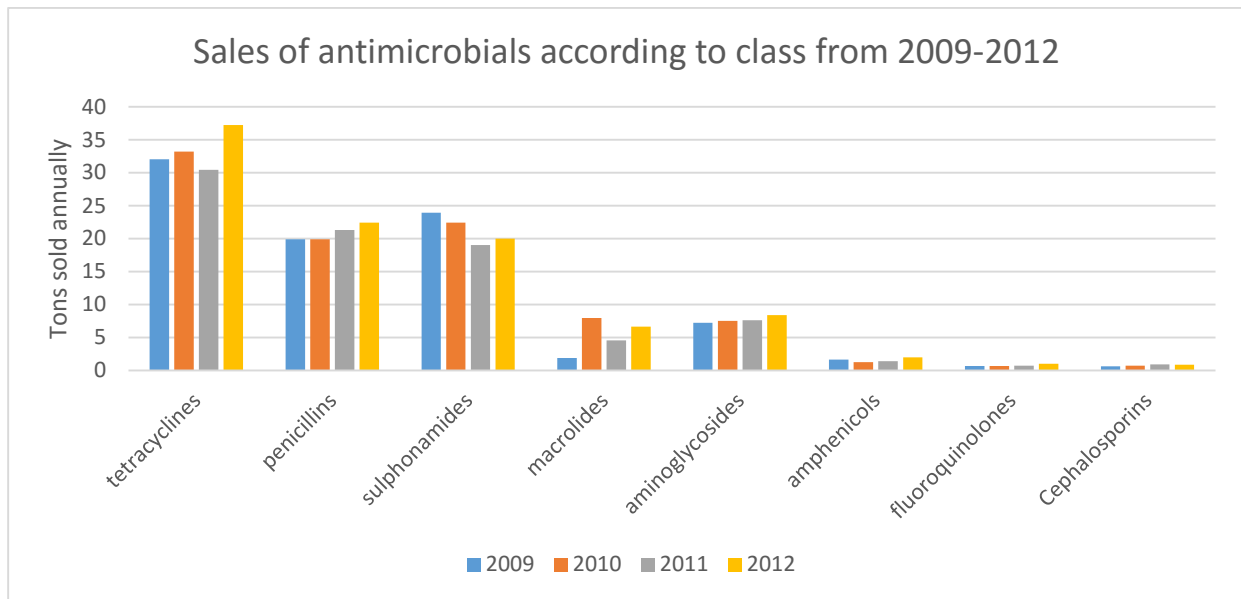


Figure 15. Antimicrobial sales in Ireland 2009-2012¹³¹.

From figure 15 one can safely estimate the usage of β -lactam antibiotics over the course of the given years. Furthermore, data currently available allowed researchers to estimate the use of antimicrobial agents in pig production in Ireland during 2011, estimated to be between 96.6 to 152.5mg/kg pig meat while in Denmark, Danish data indicate a 40mg/kg pig meat of antimicrobial

agent during the same year¹³². MRSA in animals is a spillover of the prevalence of MRSA in people, arising from use of methicillin in people and therefore the primary method of tackling the issue would be to reduce the selection pressure i.e. prescribing of methicillin in human medicine. Secondary approaches would probably target spread to animals, human clinical waste or normal organic waste management, to prevent transmission to animals. Tertiary methods would not aim at controlling prevalence in animals, but try to minimize spread from animals to humans.

6.2. International Trade

Imported food needs to be taken into consideration while developing systems for surveillance of antimicrobial resistant bacteria in food. The following data (table 5) collected by Central Statistics Office indicate that in Ireland more meat is imported than is produced. A great proportion will be re-exported following processing. Hence, the importance of international trade also needs to be considered, as far as introduction and spreading of antimicrobial resistant organisms is concerned.

Table 5. Pig-producing sector in Ireland 2011¹³³

Total animal numbers	Meat category	'000 tons produced	% exported	'000 tons home-produced sold in Ireland	'000 tons imported
1.5 million pigs	Pig meat	235	77	54	77

6.3. Cleaning and disinfection

On a research published on 2015 which was conducted in Germany, strict disinfection and decontamination was employed in order to investigate the possibility of complete MRSA eradication from the pig holdings. The rate of MRSA prevalence prior to decontamination was 32.7%. All pigs in the farm prior to decontamination were culled. An extra new unit was built during this period to host the new pig arrivals. Upon introduction of the new pigs, no mixing occurred, and all of them settled in the new stable (stable B). However though, pigs would go to the old unit (stable A) for insemination. During the cleaning, all equipment of the farm was dismantled and disposed. New technical equipment and pipes were reinstalled after the disinfection process. After decontamination and during the course of investigation, showering and a complete

change in clothing was obligatory before entrance to the farm. Moreover to any person that had contact with pigs in the previous 48 hours, access was denied. Two days after the arrival of the new pigs, when 30 of them were screened for MRSA, 25% in the old stable and 4.5% in the new stable tested positive for MRSA. After resampling 2 months later the percentage of MRSA carriage reached 37.5% (fig 16) which is close to the initial levels, prior to decontamination. Resampling took place again 12 months later. 38 pigs were screened in total from both new and old units. MRSA was recovered out of 12, hence a colonization rate of 31.6%.

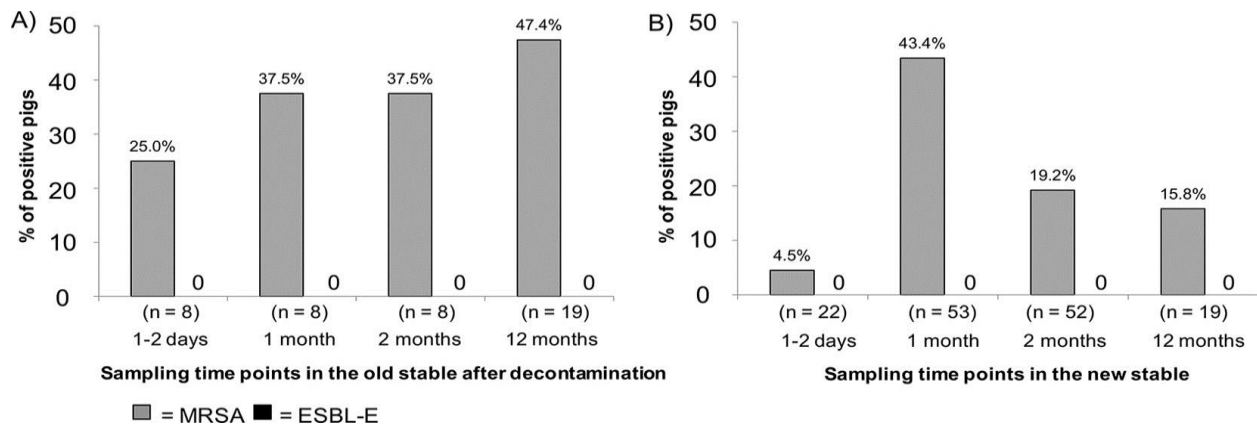


Figure 16. Positive samples of pigs after culling and decontamination¹³⁴.

MRSA isolates were also detected from environmental samples, dust, water and air samples 24 to 48 hours after introduction of the new sows in the unit, MRSA isolates were present in both new and old units. According to this study, with the process of decontamination followed, the initial strains of MRSA were eliminated and recolonization occurred by different strains. The origin and source of these strains were not investigated. They concluded that control of MRSA can be achieved through aggressive control measures, total decontamination and new construction of stables. Pigs already present in the farm need to be culled, workers should be decolonized and infection control measures should be applied. Due to costly measures taken during this research the question arising is whether active screening and strict application of protocols for infection control would have been sufficient for complete elimination of LA-MRSA. It has been concluded that effective cleaning and disinfection, including ventilation systems, prior to the arrival of the new pigs is necessary in order to avoid transmission of MRSA between animals¹³⁵. It is possible to eradicate resistant bacteria from pig farms but the process however is costly. On the other hand, it should convey benefits to the farmer.

6.4. Food processing

Animal origin foodstuffs are a considerable source of antibiotic-resistant bacteria within the food chain. Undercooked or raw food is much more likely to contain bacteria, as well as antibiotic-resistant bacteria, that come from primary production^{136,137}. However, foods that receive microbiocidal treatment, like high pressure cooking, are less likely to be a source of antimicrobial-resistant bacteria. Instead, if intact DNA coding for resistance is present, then it may persist¹³⁸. It is currently uncertain whether bacteria in the human gut uptake the resistance genes in food. Furthermore, not only the bacteria deriving from primary production can pose a threat. Processed food can get contaminated during storage, preparation and serving. Currently in Ireland, MRSA in food is not of primary concern and there is a larger focus on *Campylobacter* and *Salmonella* presence in feedstuff.

6.5. Education

It is of major importance for pig farmers, pig transporters, pig slaughtering facilities and veterinarians to be made aware that CC398 is spreading within Irish pig industry. People working closely with live pigs ought to understand that they are more likely to get contaminated by LA-MRSA and need to consider the possible health implications in case they require surgical intervention or if they develop immunosuppressive conditions. Currently farmers, handlers and veterinarians are aware of the bacterium and the consequences, in particular when the immune system is compromised. However, MRSA is not a notifiable disease. Therefore, people cannot be forced to test for it and they are unable to stop imports from infected herds making the situation worse. Veterinarians need to be aware of the possibility of MRSA infection in animals as well as their possible participation in transmission. Veterinary practices, should employ infection control procedures for the bacterium in order to reduce its transmission. Appropriate advice to owners of MRSA infected livestock should also be provided. Moreover, pig farmers should strictly apply standard hygiene i.e. wash hands after contact with pigs, use specific clothing for the piggery. These measures should be reinforced by the use of protective gloves and the use of respirator mask while carrying out activities generating dust.

6.6. Medicine

6.6.1. Phages

Phages are the most ubiquitous microbes on Earth. Our dental plaque, gastrointestinal tract, skin, and other organs, as well as our drinking water and food, are loaded with these microorganisms, and we live harmoniously with them¹³⁹. Currently research teams and companies around the world are actively researching in order to develop phage products for the treatment of antibacterial-resistant infections. Phages firstly attach on the bacterium and puncture its membrane with the use of 2 enzymes, holins and lysins. Phages' DNA is injected, inhibiting bacterial DNA transcription. New phages are produced. Eventually the bacterial wall will burst to release phages. Researchers as an alternative are trying to isolate the phage enzyme instead. No phage therapies for MRSA are currently in human use. Phages could have use in livestock for the prevention and treatment of diseases. They could potentially increase the effectiveness of antibiotics and assist in overcoming antibiotic resistance.

6.6.2. Oritavancin

In 2014 United States approved Oritavancin for treatment of skin infections. It is a glycopeptide antibiotic used for treatment of serious Gram+ bacterial infections. Its chemical structure is similar to vancomycin.

6.6.3. Vaccination

Staphylococcus aureus is currently the leading cause of soft tissue and skin infection and one of the most important health-care associated infections, yet no vaccine has been commercialized until today. A lot of candidate vaccines have failed. However, substantial development has taken place and efforts will resume in order to tackle an infection which is becoming even harder and more expensive to treat.

7. Conclusion

After its introduction in Netherlands, LA-MRSA has caused significant threat to humans. It has spread across Europe by both humans and animals. CC398 was not discovered until recently in Ireland and studies have determined that strains of MRSA can be found in both pigs and swine workers and they can be of same origin. Factors like, failure to routinely use protective equipment, daily exposure to big numbers of swine and working with swine carrying MRSA increase the risk for farm workers to get infected. Government guidelines or regulations should be considered in order to minimize each of these factors. Controlled use of antibiotics is essential in order to minimize the occurrence of resistance in bacteria such as *Staphylococcus aureus*, and all Europe is having the same aim with Netherlands having already decreased the usage of antibiotics by almost 70%. Further strategies and legislations should be developed concerning this issue. Confirmation of LA-MRSA in pig slaughterhouses and the slaughterhouses' workers, as well as retail meat, hints that further caution should be applied and screening for MRSA on such occasions may be required routinely. Without a doubt elimination of the organism from the farm currently poses a great cost for the farmer, and includes replacement of the stock, cost of interrupting the production, as well as the cost of depopulation. Screening of the workers should take place as well in order to eliminate the possibility of human-to-pig transmission. Human decolonization would also add to the overall costs. Until medical breakthroughs occur related to this issue, the best someone could do, is avoid introduction of this organism into the unit, either this is a farm or a slaughterhouse.

8. Summary

It was 1942 when the first penicillin resistant *S. aureus* was detected and only 20 years later that the first MRSA strains were isolated. Since then MRSA became a major concern for both human and veterinary medicine. *S. aureus* rarely causes clinical signs and the problem is only exacerbated when the immune system of the host is compromised. MRSA is categorized into HA-MRSA, CA-MRSA and LA-MRSA. Different molecular typing techniques have been developed in order to detect and differentiate different types. LA-MRSA is spreading via contact with contaminated fomites, pus from infected wound, eating/handling contaminated meat, visitation or employment in a swine farm as well as presence in contaminated environment with dust being an important source of human contamination. Moreover factors like overcrowding, skin injury, contaminated surfaces as well as poor hygiene contribute to the transmittance of the diseases. Pig owners, have 760 times higher chance of carrying MRSA than that of general population. Prevalence of MRSA within a farm is influenced by the size of the farm, the farming system, the level of antibiotic usage and the density of livestock around the area that the farm is located. Clonal complex 398 is documented to be the most widely disseminated LA-MRSA strain and is believed to have evolved from human MSSA. Although in pigs MRSA rarely causes significant clinical signs, in humans, especially when the immune system is compromised, MRSA CC398 can cause severe diseases like, endocarditis, septicemia, pneumonia as well as skin and soft tissue infection. The prevalence of human infection of MRSA in Ireland is among the highest in Europe with the first CC398 isolate being detected in 2011 with many more isolations having followed the primary detection. Clinical signs of food poisoning with MRSA are mild. Food intoxication involving MRSA CC398 has not been reported yet and it is rare for this clonal complex to carry enterotoxin genes. Food is not considered to be an important route of transmission of the disease. It is important though to state, that this far, there are no signs that LA-MRSA in pork has contributed significantly to the dissemination of the disease among humans. Furthermore, since *S. aureus* has no considerable economic impact on swine production and therefore an insignificant impact on the industry little or no motivation to further study *S. aureus* or MRSA in particular exists.

9. Bibliography

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