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Prevalence and types of methicillin-resistant *Staphylococcus aureus* (MRSA) in meat and meat products from retail outlets and in samples of animal origin collected in farms, slaughterhouses and meat processing facilities. A review

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## MRSA in meat and meat products. A review



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The great variety of methods used for the determination of MRSA highlights the **need to develop a standardized protocol** for the study of this microorganism in foods

Strains of MRSA were detected in **84.3%** (156 out of 185) of retail establishments and **86.5%** (64 out of 74) of farms, slaughterhouses and meat processing facilities

MRSA was detected in **under 20% of samples** from retail establishments, and **under 10% in samples** from farms, slaughterhouses and meat processing facilities

The meat and meat products most often contaminated with MRSA were **pork and chicken**

**TITLE**

Prevalence and types of methicillin-resistant *Staphylococcus aureus* (MRSA) in meat and meat products from retail outlets and in samples of animal origin collected in farms, slaughterhouses and meat processing facilities. A review

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**ABSTRACT**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a frequent cause of nosocomial and community infections, in some cases severe and difficult to treat. In addition, there are strains of MRSA that are specifically associated with food-producing animals. For this reason, in recent years special attention has been paid to the role played by foodstuffs of animal origin in infections by this microorganism. With the aim of gaining knowledge on the prevalence and types of MRSA in meat and meat products, a review was undertaken of work published on this topic since 2001, a total of 259 publications, 185 relating to meat samples from retail outlets and 74 to samples of animal origin collected in farms, slaughterhouses and meat processing facilities. Strains of MRSA were detected in 84.3% reports (156 out of 185) from retail outlets and 86.5% reports (64 out of 74) from farms, slaughterhouses and meat processing facilities, although in most of the research this microorganism was detected in under 20% of samples from retail outlets, and under 10% in those from farms, slaughterhouses and meat processing facilities. The meat and meat products most often contaminated with MRSA were pork and chicken. In addition to the *mecA* gene, it is crucial to take into consideration the *mecB* and *mecC* genes, so as to avoid misidentification of strains as MSSA (methicillin-susceptible *Staphylococcus aureus*). The great variety of methods used for the determination of MRSA highlights the need to develop a standardized protocol for the study of this microorganism in foods.

**KEYWORDS.** MRSA, meat, prevalence, types

## 1. INTRODUCTION

*Staphylococcus aureus* is a Gram-positive, catalase-positive bacterium that can infect different animal species and humans. This microorganism usually colonizes the host asymptotically, lodging in the skin and nasal cavities, but it can also cause a wide variety of infections, for example, pneumonia, wound infections and bacteraemia. It is one of the bacteria that has most often been associated with nosocomial infections in recent years, belonging to the group of bacteria called “ESKAPE”, comprising *Enterococcus* spp., *S. aureus*, *Klebsiella* spp., *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. (Thwala et al., 2021). Furthermore, *S. aureus* is an important cause of food poisoning (De Jonge et al., 2010).

Infections caused by antimicrobial-resistant bacteria are a problem of increasing size worldwide (Buyukcangaz et al., 2013). In recent decades, strains of *S. aureus* that are resistant to a wide range of antibiotics have emerged, in both hospitals and community settings (Thwala et al., 2021). The majority of nosocomial *S. aureus* infections are caused by methicillin-resistant strains (MRSA), which have become a widely recognized cause of morbidity and mortality, being associated with prolonged hospital stays and heavy costs for healthcare systems (Ripari et al., 2023; Ardic et al., 2006; Ho et al., 2008; Pesavento et al., 2007; De Boer et al., 2009; Kwon et al., 2006). In addition, several studies from different geographical areas have reported the presence of enterotoxin genes in several MRSA food isolates. Molecular typing studies have revealed genetic relatedness of these enterotoxigenic isolates with isolates incriminated in human infections (Sergelidis and Angelidis, 2017).

Penicillin and its derivatives were very effective when they were first used against staphylococcal infections. However, penicillin-resistant *S. aureus* strains quickly emerged and spread rapidly throughout the world (Idrees et al., 2023). Methicillin thus became the antibiotic of choice for the treatment of infections caused by penicillin-resistant *S. aureus* strains (Nikolic et al., 2023). MRSA was first described in 1961 in the United Kingdom, shortly after the

marketing of methicillin for clinical use (Normanno et al., 2007). In 1995, Kluytmans et al. (1995) described the first food-borne MRSA outbreak, which caused the death of five out of the twenty-one patients affected in a hospital in the Netherlands. This microorganism is resistant to practically all available beta-lactam antibiotics, making it the most frequently isolated antibiotic-resistant pathogen in many parts of the world, and especially in Europe, America and the Middle East.

In the European Union, MRSA is responsible for approximately 150,000 hospital-acquired infections per year, resulting in more than 7,000 deaths and hospital costs of 380 million euros per year (Cassini et al., 2019). Moreover, in recent years, multi-resistant MRSA strains have been emerging, greatly limiting the options available for controlling infections (Pereira et al., 2009). Hence, MRSA is considered a critically important human pathogen and its transmission routes are currently being investigated, particular attention being paid to the potential role of animals used for food and products obtained from them in infections by this microorganism (Weese et al., 2010a).

Initially MRSA was recognized as a hospital-acquired pathogen (HA-MRSA), with human infection usually occurring through direct contact with infected people or contaminated healthcare products and equipment. In addition, many patients admitted to hospitals have weakened immune systems, making them more vulnerable to infections and favouring the spread of the microorganism. However, since 1990, community-associated MRSA (CA-MRSA) began to cause infections outside healthcare settings. In recent years, the incidence of MRSA infections has increased in livestock and a third epidemiological type, livestock-associated MRSA (LA-MRSA) has been recognized (Papadopoulos et al., 2018). Infections that are associated with otherwise healthy people in the community having no history of hospitalization usually affect the skin and certain soft tissues (Dyzenhaus et al., 2023). On the other hand, there are more invasive infections that can cause death, such as sepsis, endocarditis,

osteomyelitis or pneumonia, and these are most often linked to a hospital environment (Tchamba et al., 2023). Because the divergence between HA-MRSA and CA-MRSA strains took place decades ago, any analysis of the genetic changes associated with the transition from hospitals to the general community is complex (Dyzenhaus et al., 2023). Furthermore, strains isolated from pets and from livestock such as pigs, goats, horses, sheep, buffalo, cows, rabbits and poultry are also genetically distinct from human isolates (Silva et al., 2023). Unlike the longer-standing MRSA strains found in hospitals, the new versions are able to invade community environments and affect people without any risk factors predisposing them to infection. This evolution has continued with a burgeoning reservoir of MRSA in pets and livestock (Lakhundi and Zhang, 2018). Consequently, foods of animal origin represent a potential source of MRSA, and their handling and consumption has the potential to constitute a vehicle for transmitting the infection to humans (Papadopoulos et al., 2018; Ektik et al., 2017). In this context, the presence of a specific strain of MRSA (CC398) has been reported in animals reared for food, especially intensively farmed pigs, calves and chickens (European Food Safety Authority, 2022).

### **1.1. Resistance mechanisms in MRSA**

#### **1.1.1. PBP2a**

The main mechanism of resistance to methicillin in *S. aureus* is based on the synthesis of an altered penicillin-binding protein (PBP), PBP2a, an enzyme involved in the formation of the bacterial cell wall (peptidoglycan synthesis) that presents a low binding affinity to most beta-lactam antibiotics, including those having a broad spectrum (Ali et al., 2021; Idrees et al., 2023; Lakhundi and Zhang, 2018). This acts by blocking the antibiotic from reaching its target location (Silva et al., 2023; Ripari et al., 2023). The PBP2a protein is encoded by an acquired gene, *mecA*, which is carried on a mobile genetic element (MGE), termed staphylococcal chromosome cassette *mec*, or SCC*mec* for short (Lakhundi and Zhang, 2018).

PBP2a is an elongated protein composed of a transmembrane domain, a non-penicillin-binding domain, and a transpeptidase domain (Liu et al., 2021). This penicillin-binding protein has a transpeptidase activity the same as that of the intrinsic pool of PBPs (PBP 1 to 4) of *S. aureus*, but differs in having a low affinity for many beta-lactam antibiotics. In comparison with the active sites of native PBPs, the active site of PBP2a is less accessible to beta-lactams (Lakhundi and Zhang, 2018; Zhang et al., 2021). Hence, cell wall synthesis continues despite the presence of inhibitory concentrations of beta-lactam antibiotics, this preventing cell lysis and bacterial death (Shalaby et al., 2020).

### 1.1.2. *mecA*

So far, at least three different *mec* genes have been identified: *mecA*, with three allotypes (*mecA*, *mecA1* and *mecA2*), *mecB* and *mecC*, with four allotypes (*mecC*, *mecC1*, *mecC2* and *mecC3*). The *mecA* and *mecC* genes can be located on the SCC*mec*, while the *mecB* gene has been detected on a plasmid (Tchamba et al., 2023).

The *mecA* gene is not a native gene of *S. aureus*, but rather has been acquired from some extraspecific source through an unknown mechanism (Lakhundi and Zhang, 2018). Although the *mecA* gene is the most common PBP2a-encoding gene, *mecC* has also been detected as part of SCC*mec* type XI (Silva et al., 2023).

### 1.1.3. *mecC*

This gene was initially named *mecA*<sub>LGA251</sub>, and the protein produced showed approximately 63% homology at the amino acid level with the original PBP2a protein, which explains the negative results in tests for *mecA* by polymerase chain reaction (PCR) and for PBP2a by slide agglutination that were observed after resistance to oxacillin and cephoxitin was detected (Dierikx et al., 2023). This *mecA* homologue was renamed *mecC* in 2012 by IWG-SCC, the International Working Group on the Classification of Staphylococcal Cassette Chromosome elements (Paterson et al., 2014). Like *mecA*, the *mecC* gene is also located in a



SCC*mec* element in the 3' region of *orfX*. Recombinant *mecC* PBP2a is associated with increased resistance to oxacillin as compared to cephoxitin. In contrast, PBP2a of *mecA* showed greater resistance to cephoxitin, with a minimum inhibitory concentration (MIC) = 400 µg/ml, than to oxacillin (MIC = 200 µg/ml), as noted by Lakhundi and Zhang (2018).

Strains with *mecC* are sometimes confused with methicillin-susceptible *S. aureus* (MSSA), this posing major implications in tracking MRSA (Paterson et al., 2014). Although the proteins encoded by *mecA* and *mecC* possess different biochemical properties, *mecC* confers resistance to methicillin. Laboratories using antimicrobial susceptibility testing are likely to identify these strains correctly as MRSA. However, there are difficulties when only molecular methods are used for the identification and confirmation of MRSA (Bali et al., 2021). To avoid these problems, laboratories should incorporate universal *mec* gene primers for PCR detection or add *mecC*-specific primers to differentiate between *mecA* and *mecC* MRSA. It should also be noted that commercial slide agglutination assays using *mecA*-encoded PBP2a will erroneously identify *mecC* MRSA as MSSA (Lakhundi and Zhang, 2018).

#### 1.1.4. *mecB* and *mecD*

In addition to the *mecA* and *mecC* genes, other *mec* genes (*mecB* and *mecD*) have been identified as responsible for methicillin resistance in the *Staphylococcaceae* family. The *mecB* and *mecD* genes were initially described on the chromosome, a plasmid, or both, of *Micrococcus caseolyticus*. The *mecB* gene has also been detected in a plasmid of an MRSA isolated from a human patient. In contrast, *mecD* has not hitherto been detected in staphylococci (Tchamba et al., 2021).

Like *mecA* and *mecC*, *mecB* in *S. aureus* also confers methicillin resistance. Laboratories using antibiotic susceptibility testing can correctly identify MRSA carrying *mecB* as MRSA, and not MSSA. However, in the case of PCR, *mecB*-specific primers must be incorporated so as to identify these strains correctly as MRSA (Lakhundi and Zhang, 2018). As for the *mecD*

gene, it has been suggested that it may confer resistance to all classes of beta-lactam antibiotics, including the anti-MRSA cephalosporins, cephtobiprole, and cephtaroline (Lakhundi and Zhang, 2018).

#### 1.1.5. Staphylococcal Chromosome Cassette *mec* (SCC*mec*)

It has been discovered that the emergence of methicillin-resistant Staphylococcaceae lineages was due to the acquisition of the SCC*mec* by susceptible strains. There are three basic structural-genetic elements in SCC*mec*. These are: firstly, the *mec* gene complex, which contains the *mec* gene (*mecA*, *mecB*, *mecC* or *mecD* and combinations thereof) and the regulatory elements that control its expression (inducer-*mecR1*, which encodes transducer protein signals, and repressor-*mecI* which encodes a repressor protein); secondly, the *ccr* gene complex, which includes three *ccr* genes (*ccrA*, *ccrB* and *ccrC*, with different variants encoding the chromosomal cassette recombinase); and, thirdly, junction regions (J regions), and surrounding open reading frames (ORFs), responsible for SCC*mec* integration and excision from the chromosome (Lakhundi and Zhang, 2018; Tchamba et al., 2023). SCC*mec* includes three junction (J) regions. The J1 region is located above the *ccr* gene complex (L-C region) and may include several ORFs and regulatory genes (*pls* and *kdp*). The J2 region is located between the *ccr* gene complex and the *mec* gene complex (C-M region) and may include the Tn554 transposon that encodes erythromycin resistance. The J3 region is located below the *mec* gene complex (M-R region) and can include different inserted genetic elements such as plasmid pT181, plasmid pUB110, transposon Tn4001 or combinations of these (Tchamba et al., 2023) (Figure 1).

SCC*mec* are classified as varying types and subtypes, with an increasing level of SCC*mec* I to V in MRSA isolates (Youssef et al., 2022). Up to the present day, a number of differing types of SCC*mec* have been identified in MRSA, on the basis of varying combinations of the *ccr* and *mec* gene complexes, with various subtypes distinguished because of differences in J

regions of the *SCCmec* (Lakhundi and Zhang, 2018). As indicated previously, J regions contain characteristic genes, pseudo-genes, non-coding regions and mobile genetic elements, such as insertion sequences, and plasmids or transposons, which are utilized to define the subtypes of *SCCmec* (Uehara, 2022).

Furthermore, pseudo, composite, and hybrid *SCCmec* versions have also been described. A pseudo *SCCmec* lacks the *ccr* gene complex (Tchamba et al., 2023). A composite *SCCmec* contains different genetic elements, including two or more SCCs in tandem, and carries the *ccr* gene complex, which catalyzes the integration and cleavage of SCCs (Urushibara et al., 2020). A hybrid *SCCmec* carries genes that encode resistance to other antibiotics or to antiseptics, factors associated with virulence, or combinations of these (Tchamba et al., 2023).

Currently, eleven main types of *SCCmec* are recognized, numbered I to XI. *SCCmec* types I, IV, V, VI, and VII generally confer resistance only to beta-lactam antibiotics, but *SCCmec* types II and III harbour resistance to multiple classes of antibiotics, this being due to additional genetic elements carrying drug resistance genes integrated into the *SCCmec*, such as plasmids and transposons. HA-MRSA includes *SCCmec* types I, II, III, VI, and VIII; CA-MRSA includes *SCCmec* types IV, V, and VII; and LA-MRSA includes types IX, X, and XI. MSSA strains can become MRSA strains by acquiring *SCCmec* elements (Liu et al., 2021).

#### 1.1.6. Other resistance mechanisms

There are two other resistance mechanisms that result in weak resistance to methicillin and oxacillin in which the role of the *mecA* gene is unclear. Strains with modifications in the affinity of PBPs 1, 3 and 4 (low-affinity PBP) show weak resistance to methicillin, and strains that hyper-produce beta-lactamases have limited resistance to oxacillin (Silva et al., 2023). Unlike penicillin resistance, methicillin resistance in *S. aureus* is not mediated by plasmid-borne beta-lactamases (Lakhundi and Zhang, 2018). Methicillin resistance not mediated by *mecA* in *S. aureus* may be due to excess beta-lactamase production, resulting in low-level

oxacillin-resistance. In such cases, the strains are termed BORSA, that is, borderline oxacillin-resistant *S. aureus* (Sawhney et al., 2022). BORSA isolates are susceptible to cephoxitin and do not carry the *mecA* or *mecC* genes, but have an oxacillin MIC between 1 and 8 µg/ml (Krupa et al., 2014). They have mechanisms not dependent on PBP2a, such as the presence of other low-affinity PBPs, the hyper-production of beta-lactamase or the production of some other methicillinase (Heo et al., 2008).

## 1.2. Detection and identification of MRSA

MRSA strains can be identified by phenotypic assays, for example by the cephoxitin disc-diffusion method or by PBP2a latex-agglutination, as well as by the presence of the *mecA* gene, which encodes the alternative penicillin binding protein 2a (PBP2a). Furthermore, MIC determinations, as described by the Clinical and Laboratory Standards Institute (CLSI), are used to confirm resistance to standard beta-lactam antibiotics, in this case oxacillin. The CLSI considers *S. aureus* isolates whose MICs for oxacillin are equal to, or greater than 4 µg/ml in Mueller-Hinton broth to be resistant to this antibiotic, and also resistant to first-generation cephalosporins (Ersoy et al., 2021).

It should be noted that prevalence data can vary considerably, depending on the isolation methods, sample types, and sample collection schemes used. There is thus a need for a harmonized protocol for the detection of MRSA from food samples (Murugadas et al., 2016).

### 1.2.1. Phenotypic methods

Several phenotypic methods have been developed for the detection of MRSA isolates, including the oxacillin-agar screening test and the cephoxitin test. There are also commercial automated assays, such as the MRSA latex-agglutination test, the Vitek 2 system (card GPS-SA) and Microscan. However, as these methods are often not sensitive or specific enough, the *mec* gene is usually detected by performing a PCR (Normanno et al., 2007).

Different culturing methods have been used to detect MRSA. Conventional microbiological procedures are generally laborious, requiring the isolation of *S. aureus* before testing for methicillin resistance. Long-standing techniques for the detection of MRSA include inoculation on blood agar plates and selective agar media, followed by confirmatory testing of suspicious colonies. In the case of foods, since the population density of MRSA is usually small and there is often a wide variety of background microbiota, direct isolation on such seeding media will rarely be successful. Methods for isolating MRSA from foodstuffs should thus include preferential sample enrichment followed by plating on selective solid media. This increases the detection frequency for MRSA. As staphylococci are relatively tolerant to high concentrations of salt, the addition of 6.5% NaCl may favour the growth of these organisms relative to that of contaminating microbiota. Although a broth with 6.5% or 7.5% NaCl is a commonly used enrichment medium in MRSA isolation protocols from different samples, it has been shown that the growth of certain strains may be inhibited by NaCl concentrations higher than 2.5% (Pang et al., 2015). Phenol red mannitol broth (PHMB), supplemented with cephtizoxime and aztreonam, has been shown to be an effective and sensitive means of distinguishing MRSA.

New chromogenic media for the detection of MRSA have recently been marketed, which are much more specific and sensitive than those supplemented with oxacillin used previously. According to the ISO 11133 standard, specificity of a culture medium is defined as the demonstration, under defined conditions, that non-target microorganisms do not show the same visual characteristics as the target microorganisms. Productivity (sensitivity) is the level of recovery of a target microorganism from the culture medium under defined conditions. Selectivity is defined as the degree of inhibition of a non-target microorganism on or in a selective culture medium under defined conditions. The use of chromogenic media allows differentiation between MRSA and MSSA, reduces the number of confirmatory tests required,

and achieves isolation and presumptive identification in a single step. For example, CHROMagar MRSA medium has been found to have 100% sensitivity and specificity for MRSA (Fadel and Ismail, 2015). Brilliance MRSA Agar also showed high specificity, but low sensitivity (Traversa et al., 2015). However, both CHROMagar MRSA and Brilliance MRSA have low selectivity in isolating MRSA. There is hence a need to achieve further confirmation of colonies showing typical appearances on both media using other methods, such as PBP2a or PCR, so as to avoid false positive results, overdiagnosis, and overtreatment for MRSA (Stewart-Johnson et al., 2019b).

The Kirby-Bauer, or disc-diffusion, test is a standard method for determining the susceptibility of isolates to antimicrobial agents, while the test on ORSAB medium, an oxacillin-resistance screening agar-base supplemented with oxacillin, at two milligrams per litre (2 mg/l) is a presumptive test that helps identify possible methicillin-resistant isolates (Ndahi et al., 2014). A cephoxitin disc-diffusion assay has been found to be superior to methicillin and oxacillin disc-diffusion assays for the detection of MRSA (Ruban et al., 2017a). Since cephoxitin has been shown to be better than oxacillin as an indicator of methicillin resistance (Bulajić et al., 2017), in the absence of molecular techniques, cephoxitin disc-diffusion testing is recommended in conjunction with any other phenotypic method to improve MRSA detection (Fri et al., 2018).

The identification of MRSA strains by the disc-diffusion method offers high sensitivity, and is the method of choice in many laboratories for detecting MRSA, because it is economical and easy to perform. The accuracy of the MRSA-Screen latex-agglutination method for the detection of PBP2a comes close to that of PCR, and it is more precise than any susceptibility test used on its own in confirming the presence of MRSA. A diagnostic strategy using the disc-diffusion method followed by confirmation of MRSA-positive strains with the PBP2a test constitutes an accurate, cost-effective and affordable option (Stewart-Johnson et al., 2019a).

Lee et al. (2004) compared the MRSA latex-agglutination test with an oxacillin-agar detection test, MIC determination, and the detection of *mecA* by PCR. The latex-agglutination test outperformed the widely used oxacillin-agar test, with a sensitivity and specificity of 100%. With PCR taken as the reference method, the MRSA-Screen latex-agglutination test demonstrated 100% sensitivity and 100% specificity.

### 1.2.2. Genetic methods

In the case of PCR, it is of interest to combine the detection of the *mecA* gene with the detection of PBP2a, *mecA* homologues, such as *mecC*, mobile elements, transposons and phages that can harbour other genes responsible for resistance (Usman et al., 2016). It is essential to look for the *mecC* gene in all *mecA*-negative *S. aureus* isolates that present resistance to oxacillin, cephalosporins or both (Giacinti et al., 2017).

Isolation and identification of MRSA, including differential enrichment and plating on selective agar, followed by confirmation by biochemical tests, PCR assays, or both, requires approximately three to seven days. Real-time polymerase chain reaction (RT-PCR) technology has been used as an alternative to such culturing methods for the rapid detection of *S. aureus* and MRSA. Detection by this form of PCR can reduce analysis time to just eighteen hours after enrichment. In some research work (Anderson and Weese, 2007; Kim et al., 2021; Velasco et al., 2014), a RT-PCR assay is reported to have allowed the detection of the *mecA* gene in samples that tested negative for *S. aureus* when conventional PCR and identification methods were used. Recently a RT-PCR technique was developed that simultaneously detects two key components of the MRSA genome: *mecA* and *orfX*. This RT-PCR test (IDI-MRSA, GeneOhm Sciences, San Diego, CA) has a high level of agreement with standard culture methods ( $\kappa = 0.82$ ) when used directly on human nasal swabs (Anderson and Weese, 2007; Warren et al., 2004). The detection limit in pure cultures and artificially contaminated food samples was  $10^2$  cfu/ml for *S. aureus*, *S. capitis*, *S. caprae*, and *S. epidermidis*. Moreover, RT-PCR successfully

detected strains isolated from various food matrices (Kim et al., 2021). Multiplex RT-PCR could detect more *S. aureus*-positive samples than the conventional culture/PCR method alone. Possible reasons for these discrepant results include: amplification of DNA by the RT-PCR from very low levels of *S. aureus* that were not detectable by the bacteriological methods due to competition or non-viable *S. aureus* in the samples, or false-positive RT-PCR results as a result of cross-reaction rather than false-negative culture results (Velasco et al., 2014). Reducing the detection time for *S. aureus* and MRSA in food is important, since this permits control measures to be adopted quickly, and thus reduces the risk of spread of these strains into the food chain. If two-step selective enrichment is used together with the RT-PCR method, the total analysis time is under two days, which is a significant time saving compared to the six to seven days needed for culture methods including selective enrichments, plating, biochemical tests and standard multiplex PCR for confirmation. However, the presence of MRSA must still be confirmed by culturing if isolates are required for follow-up studies (Velasco et al., 2014).

MRSA isolates that carry the *mecA* gene but are oxacillin-susceptible are called OS-MRSA, and have an oxacillin MIC  $\leq 2 \mu\text{g/ml}$  (Luo et al., 2020). Particular care needs to be taken so as not to misidentify these isolates as MSSA. These strains have been associated with food, animals, and clinical samples and their importance derives from the fact they can easily acquire resistance to beta-lactams (Thwala et al., 2021). Given the difficulty of correctly distinguishing between OS-MRSA and MSSA, it is highly advisable to compare different phenotypic methods for detecting methicillin resistance (cephoxitin disc-diffusion, plating on agar with oxacillin, plating on chromogenic MRSA agar ID, or latex-agglutination test for penicillin-binding protein antigen 2a), along with PCR for the *mecA* gene. The sensitivity of the cephalosporin disc-diffusion method may be lower in areas with a high prevalence of OS-MRSA, and here a combination of cephalosporin disc-diffusion testing with plating on MRSA ID agar or latex-agglutination is recommended (Nair et al., 2021).



### 1.3. MRSA Typing

Different molecular techniques have been used to identify and to type MRSA strains, including pulsed-field gel electrophoresis (PFGE), based on macro-restriction patterns of genomic DNA, multilocus sequence typing (MLST), which determines the allelic profile of seven housekeeping genes, and Staphylococcal protein A (*spa*) typing based on sequencing of the polymorphic X region of the protein A gene, this being a useful method for the differentiation of strains, particularly those that cannot be distinguished by PFGE (De Boer et al., 2009). The discriminatory power of PFGE has been shown to be greater than that of MLST and *spa* typing (Farahmand et al., 2020). Despite the difficulties present in reproducibility, interlaboratory reliability, and hard work, it is agreed that PFGE remains the gold standard, particularly for short-term surveillance. MLST is a good typing method for long-term and global epidemiological investigations, but it is not suitable for outbreak investigations; *spa* typing is the most widely used method today for first-line typing in the study of molecular evolution, and outbreak investigation (Chadi et al., 2022). A combination of two methods can increase precision in epidemiological studies (Buyukcangaz et al., 2013; Murugadas et al., 2017). Feßler et al. (2011) observed that direct repeat unit (*dru*) typing had the highest discriminatory power, followed by *spa* typing, SCC*mec* typing, and lastly MLST in the typing of MRSA.

## 2. METHODOLOGY

The objective of this work was to compile details of the literature covering the prevalence of MRSA in meat and meat products from retail outlets, and in samples of animal origin (mainly meat and meat products) collected in farms, slaughterhouses and meat processing facilities, the typing of strains and the description of the methods used in each case. The intention was to

broaden the knowledge of this microorganism in foodstuffs and identify the methods commonly used for detection, identification and typing in MRSA-positive samples.

Various databases were consulted, including Web of Science, Scopus, Pubmed and ScienceDirect, so as to compile a list of all studies on MRSA in meat and meat products published between 2001 and 2024. The key words used to search for articles were: “prevalence or incidence”, “MRSA”, “methicillin-resistant *Staphylococcus aureus*”, followed by the terms for each of the food groups evaluated. No date, language, article type or text availability restrictions were applied. A total of 185 articles were selected for meat products from retail outlets and 74 for samples of animal origin collected in farms, slaughterhouses and meat processing facilities, these having been published between January 2001 and February 2024. These were tabulated by year of publication, and within each year by alphabetical order of the authors of the articles. The dates and place of the study, the prevalence of MRSA and the typing of the MRSA strains found in the study were analysed (Suppl Table 1; Suppl Table 2). In the absence of further clarification in Suppl Table 1 and Suppl Table 2, detection of the *mecA* gene was taken as MRSA positive. A numerical code explained in the footnotes to the Suppl Table 1 and Suppl Table 2 was created to identify the protocol followed in each piece of research in the articles among the various MRSA identification methodologies.

### 3. RESULTS AND DISCUSSION

A total of 185 articles covering meat and meat products from retail outlets were reviewed. In 59.5% of the research works (110 out of 185), no prior enrichment was performed. Double enrichment of the samples was described in only 22.2% (41 publications), this step usually being associated with a higher MRSA frequency of recovery. Just two investigations referred to triple enrichment of the samples, firstly in buffered peptone water, secondly in Mueller-Hinton broth (MHB) supplemented with 6.5% NaCl, and thirdly in tryptone soy broth (TSB)

supplemented with 7.5 mg/l of aztreonam and 5 mg/l of cephoxitin. In 40 of the publications consulted, TSB was used as the culture medium. Among these, the majority supplemented this with 10% NaCl (nine cases), with 10% NaCl and 1% sodium pyruvate and TSB at double concentration (six instances), or with 7.5% NaCl (six cases). In a total of 29 studies, MHB supplemented with 6.5% NaCl was used. Few articles, just 18 out of the total (9.7%), referred to any use of a selective chromogenic medium for MRSA, and only 10 articles mentioned oxacillin-resistance screening agar-base (ORSAB) supplemented with oxacillin at 2 mg/l. With respect to the method of confirming the presence of MRSA, it was noted that four main techniques were in use. In the vast majority of research works (69.7%, 129 out of 185), the amplification of the *mecA* gene was carried out by PCR. In 32 investigations there was amplification of the *mecA* and *mecC* genes by PCR, in 17 studies a test of susceptibility to cephoxitin (30 µg) and oxacillin (1 µg) by the disc-diffusion method was applied, and in 10 articles reference was made to the MRSA latex-agglutination test (MRSA latex-agglutination of penicillin-binding protein 2a). MRSA was not detected in 15.7% (29 out of 185) of the reports consulted. Most publications described a prevalence of positive samples of below 20%, although percentages of as high as 90% were obtained within *S. aureus* isolates. The meats most often found to be contaminated with MRSA were pork and chicken. Regarding the location of the research works (Figure 2), it was observed that the three most frequent locations were: United States of America (USA, 22 articles), Egypt (22 articles) and China (19 articles). Most detected SCC*mec* and ST types in the different research works were: SCC*mec* V (24 articles), IVa (19), IV (14) and III (8); ST398 (36), ST5 (20), ST9 (17) and ST8 (5) (Figures 3 and 4).

A total of 74 articles relating to samples of animal origin collected in farms, slaughterhouses and meat processing facilities were reviewed (Suppl Table 2). No prior enrichment was performed in 37.8% of the research recorded (28 studies out of 74). Double

enrichment of the samples was performed in 24.3% of them (18 publications), this normally being associated with a higher MRSA frequency of recovery. Only one of the articles described triple enrichment of the samples, firstly in buffered peptone water, secondly in MHB supplemented with 6.5% NaCl, and thirdly in TSB supplemented with 7.5 mg/l aztreonam and 5 mg/l of cephoxitin. TSB was the culture medium used in a substantial part of the research consulted (23 instances out of 74), in most cases supplemented with salt, 10% NaCl being mentioned in four publications, 6.5% NaCl in 20 investigations. In 23.0% of the items (17 out of the 74), which is a higher percentage than that observed in the case of meat and meat products from retail sources (Suppl Table 1), a selective chromogenic medium for MRSA was used, and only four articles recorded the use of ORSAB supplemented with oxacillin (2 mg/l). With regard to the techniques used to confirm the presence of MRSA, four principal methods were used. In by far the majority of the research (75.7%, 56 articles out of 74), amplification of the *mecA* gene was achieved by using PCR. In 14 studies amplification of the *mecA* and *mecC* genes was carried out by PCR, in seven investigations a susceptibility test to cephoxitin (30 µg) and oxacillin (1 µg) using the disc-diffusion technique was applied, and nine articles recorded use of an MRSA latex-agglutination test (latex-agglutination test of penicillin-binding protein 2a). Ten of the papers reviewed recorded no finding of MRSA, and most of the publications noted a prevalence of under 10%. Regarding the origin of the publications (Figure 5), it was observed that the four most frequent locations were: Korea (9 articles), Nigeria (6 articles), Switzerland (5 articles) and The Netherlands (5 articles). Most frequently detected SCC*mec* and ST types are shown in Figures 6 and 7. These were: SCC*mec* V (23 articles), IV (12), IVa (10) and III (7); ST398 (26), ST9 (8), ST1 (8) and ST5 (6).

Different genes related to methicillin-resistance have been observed. In most of the articles consulted, the *mecA* and *mecB* genes were evaluated, but further analysis of the *mecB* and *mecD* genes is recommended. Primers for both of these genes were designed using primer-

blast (NCBI) and included primers MecB2-r 5'-ACTACACAGAAACGGGATTGAT-3', 5'-TCGTCGGAAATGCCGAACAT-3', Macro-MecD-r 5'-AGGAGAGGAAACGCCTTCTG-3', and Macro-MecD-f 5'-ACCCACAAACCATCCAATTTGT-3'. Reference strains used as positive controls were *Micrococcus canis* DSM 101690 (*mecB*) and *M. caseolyticus* IDM0819 (*mecD*) (Klempt et al., 2022). When *S. aureus* isolates are negative for *mecA* and *mecC* in MRSA screening, but show methicillin resistance, the presence of the plasmid carrying the *mecB* gene should be investigated. The *mecB* homologue of *S. aureus* shows a 60% nucleotide sequence similarity to the originally identified *mecA* gene of *S. aureus*. As with the *mecA* and *mecC* genes, *mecB* in *S. aureus* results in methicillin resistance and therefore strains carrying this gene should be accurately identified as MRSA, rather than MSSA. This can be achieved by antibiotic susceptibility testing. However, for accurate identification of MRSA strains, the PCR method with *mecB*-specific primers should also be used (Cikman et al., 2019).

Traditional detection of MRSA by culture method is time-consuming, laborious and difficult to carry out *in situ*. Zhao et al. (2022) developed a device for rapid detection (within 30-40 minutes) of MRSA, which can detect the *nuc* gene in SA and the *mecA* gene in MRSA simultaneously. After simple sample processing, the mixture can be loaded directly onto the chip device and the detection results can be directly determined by a color change. This isothermal amplification chip device can be widely applied in many fields with simple operation (Zhao et al., 2022).

#### 4. CONCLUSIONS

From the review of MRSA in meat, it is clear that the products widely reported to be contaminated with this microorganism are pork and chicken. In addition to the *mecA* gene, it is essential to study the *mecB* and *mecC* genes, so as to avoid misidentification of the strains as methicillin-susceptible *Staphylococcus aureus* (MSSA). Pre-enrichment of the samples allows

a higher detection of positive samples. Double and triple enrichment with media such as Mueller-Hinton broth (MHB) supplemented with 6.5% NaCl, and tryptone soy broth (TSB) supplemented with 7.5 mg/l of aztreonam and 5 mg/l of cephoxitin increases the detection frequency for MRSA. The great variety of methods used to investigate MRSA highlights a need to develop a harmonized protocol for the study of this microorganism in foods.

### **CRedit AUTHORSHIP CONTRIBUTION STATEMENT**

**Camino González-Machado:** Conceptualization, Formal Analysis, Investigation, Writing - Original Draft, Writing - Review & Editing. **Carlos Alonso-Calleja:** Conceptualization, Resources, Writing - Review & Editing, Supervision, Funding acquisition. **Rosa Capita:** Conceptualization, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

### **DECLARATION OF COMPETING INTEREST**

The authors declare no conflict of interest.

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### **Appendix A. Supplementary data**

The following is the Supplementary data to this article.

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**FIGURE CAPTIONS**

Figure 1. Explanatory diagram of *SCC<sub>mec</sub>* structure (McClure-Warnier et al., 2013)

Figure 2. Research works on MRSA in meat and meat products grouped by location.

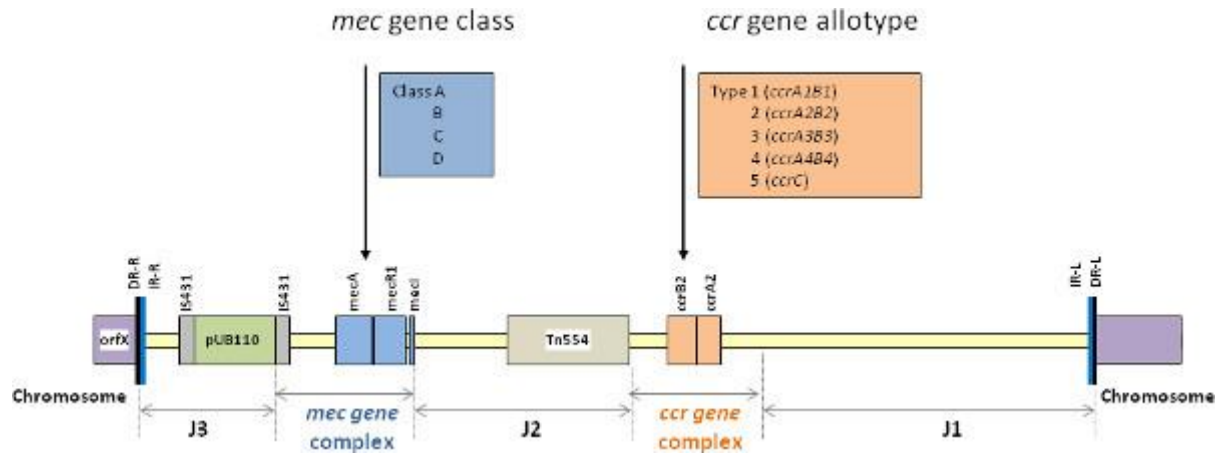
Figure 3. Most frequently detected staphylococcal cassette chromosome *mec* (*SCC<sub>mec</sub>*) types in the reviewed research works on MRSA in meat and meat products (number of articles are indicated in parentheses).

Figure 4. Most frequently detected sequence types (ST) in the reviewed research works on MRSA in meat and meat products (number of articles are indicated in parentheses).

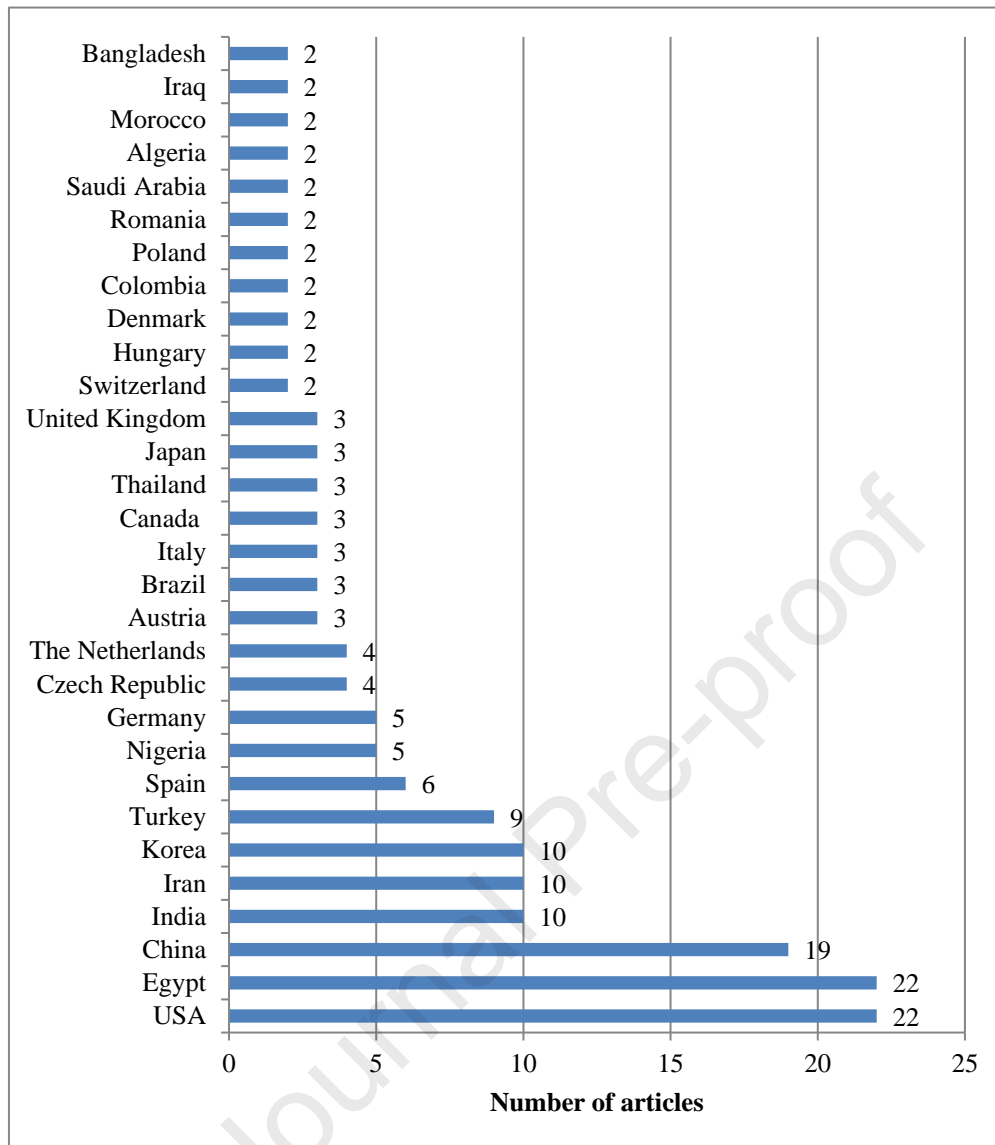
Figure 5. Research work on MRSA in meat and meat products obtained directly from slaughterhouses and grouped by location.

Figure 6. Most frequently detected staphylococcal cassette chromosome *mec* (*SCC<sub>mec</sub>*) types in the reviewed research works on MRSA in meat and meat products obtained directly from slaughterhouses (number of articles are indicated in parentheses).

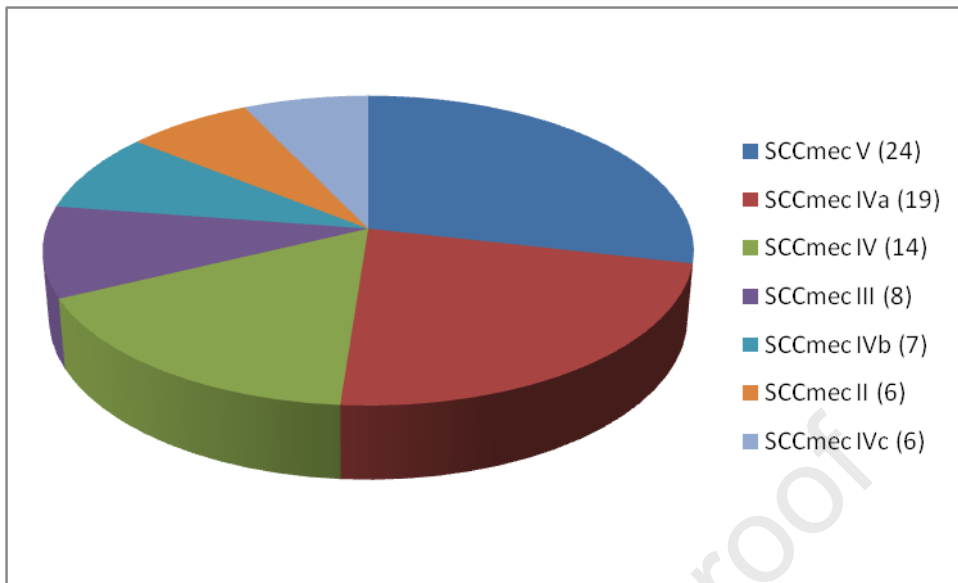
Figure 7. Most frequently detected sequence types (ST) in the reviewed research works on MRSA in meat and meat products obtained directly from slaughterhouses (number of articles are indicated in parentheses).

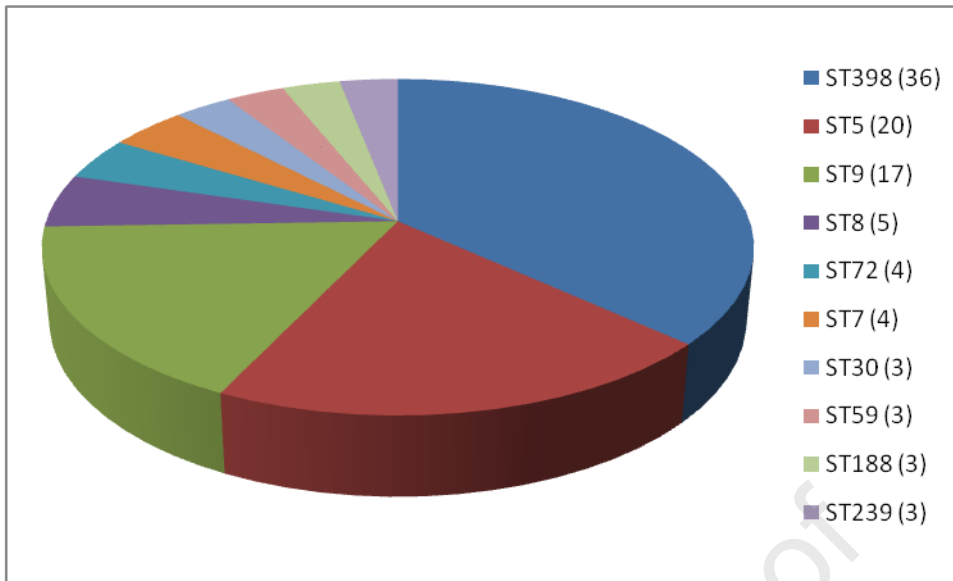


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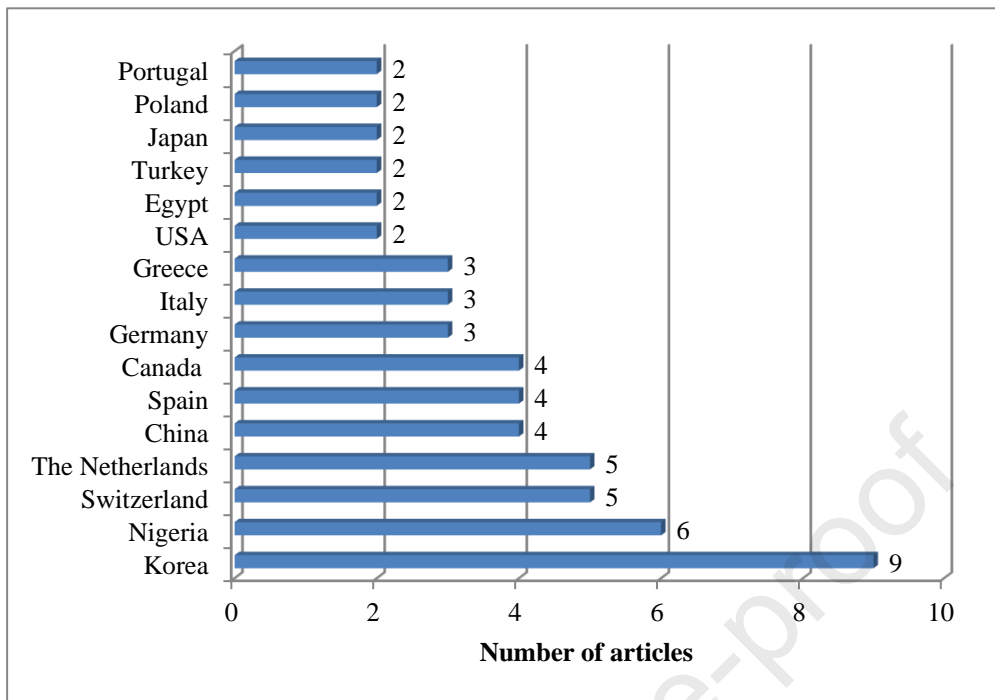


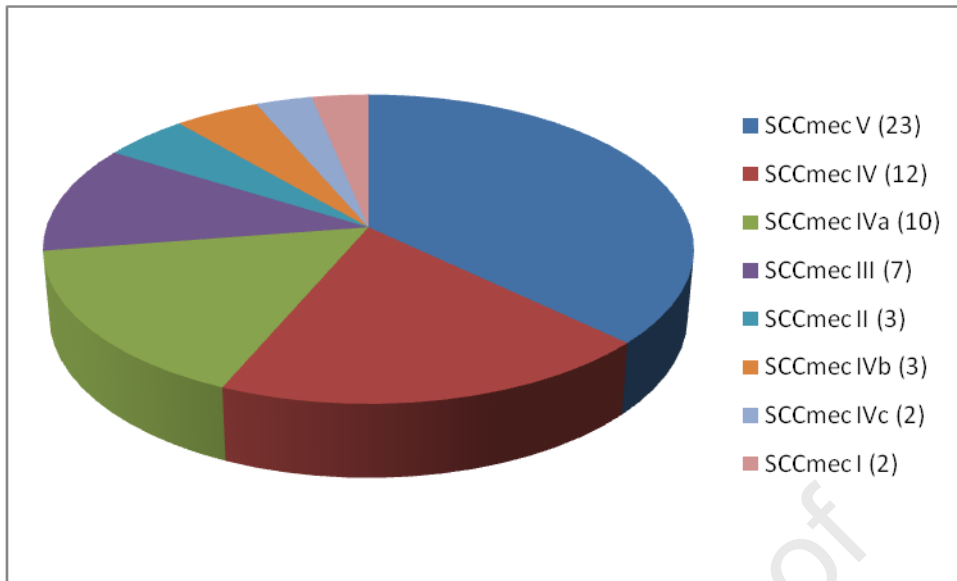




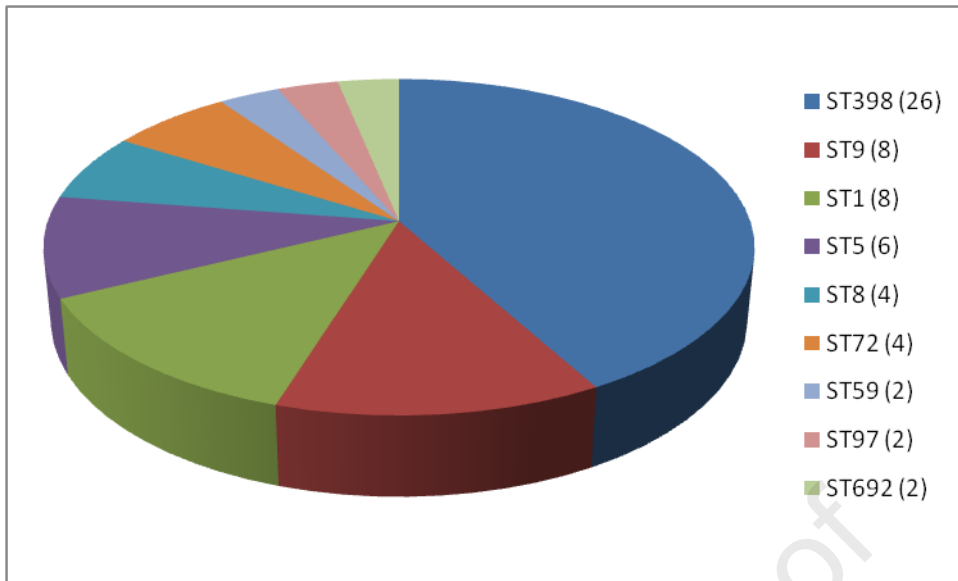


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- > A total of 259 research reports into MRSA in meat and meat products were reviewed
- > MRSA was detected in 84.3% (retail outlets) and 86.5% (abattoirs) of the researches
- > Prevalence was < 20% in foods from retail outlets and < 10% in those from abattoirs
- > The highest prevalence of MRSA was observed in pork and chicken samples
- > It is essential to study the *mecB* and *mecC* genes in addition to the *mecA* gene

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**AUTHOR DECLARATION**

We wish to draw the attention of the Editor to the following facts which may be considered as potential conflicts of interest and to significant financial contributions to this work.

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.

We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

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