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Comparative Structural Analysis of Meca Encoded Beta-Lactam Resistance in MRSA

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ABSTRACT

As the level of antibiotic resistance in bacteria rises, the threat of patients facing fatal consequences from a simple cut or infection grows dramatically. One example of an emerging antibiotic-resistant threat is MRSA, methicillin-resistant staphylococcus aureus, which is a growing cause of hospital-acquired pneumonia, skin infections, and even sepsis through cross-contamination in health care settings. However, methicillin resistance like that exploited by MRSA is also highly conserved with that of another species of staphylococcus bacteria, *Staphylococcus sciuri* which is the ancestral bacterium of *S. aureus*. *S. sciuri* is a typically animal-associated bacterium with increasing clinical relevance as the amount of human infections from it has grown. Both these bacteria share the commonality of having the mecA gene that determines methicillin resistance transported through the mobile *Staphylococcal* cassette chromosome. This study focuses on the relationship between the mecA mobile element in *S. sciuri* compared to *S. aureus* and explores the specific role of *S. sciuri* in the creation of the mecA cassette. Understanding the cassette evolution and similarities and differences between *S. sciuri* and *S. aureus* will enable the continued tracking of *S. sciuri* as an emerging clinical threat. To answer all these questions, BLAST analysis, InterProScan, and additional computational analysis were used to characterize differences in homology, structure, function, and effect on infectivity, robustness of antibiotic resistance, and morbidity. The overall goal of this analysis is to characterize the differences between mecA and methicillin-resistant genes in clinically relevant MRSA as well as environmental samples such as *S. sciuri*.

Introduction

As the World Health Organization reported in 2014, antibiotic resistance is a serious healthcare concern throughout the world. Standard antibiotics to treat bacterial infections may no longer work, and this can very quickly lead to epidemics and higher mortality rates. This resistance often arises when bacteria treated with a specific antibiotic develop mutations or new chromosomal components allowing them to evade antibiotics and continue reproducing.

Staphylococcus aureus is one such bacteria that developed resistance to methicillin, a beta-lactam family antibiotic, giving rise to methicillin-resistant S. aureus (MRSA). MRSA strains are now emerging in the community with a mortality rate of 29% in hospitals and the death of over 100,000 people in 2019 alone (1). S. aureus is the predominant cause of skin and soft tissue infections like abscesses, furuncles, and cellulitis (2). It can even lead to more serious issues such as pneumonia or bone infections (2). It is most often spread by contaminated hands and is especially dangerous to people who are immunocompromised, have open wounds, or have invasive medical devices (2). It is found in the environment and also in regular human flora on the skin and mucous membranes of most healthy individuals but it becomes dangerous if it enters the bloodstream or internal tissues (2).

S. aureus was first treated by penicillin, another beta-lactam family antibiotic (3). Penicillin kills bacteria by binding its four-membered beta-lactam ring to S. aureus' transpeptidase, effectively inhibiting its activity. Transpeptidase is an essential enzyme in bacterial cells that cross-links peptidoglycan chains to form a net-like, rigid composition of the outer cell wall (3). Being a gram-positive bacteria, S. aureus has thick cell walls and high levels of peptidoglycan which were essential to resisting environmental and molecular pressures (3). Therefore, penicillin was extremely effective until the 1940's when S. aureus began producing beta-lactamase, an enzyme that destroys the beta-lactam ring of penicillin making it ineffective (3). Subsequently scientists moved on to using second-generation penicillins which included methicillin (3). By the 1960's, MRSA had emerged with a new resistance method. MRSA was able to upregulate a low-affinity penicillin-binding protein that would not bind to the second-generation penicillins, effectively ensuring bacterial cell survival and providing a dangerous blanket resistance to all beta-lactam antibiotics (3).

Since its emergence, MRSA infections have sky-rocketed since 2000. As shown in Figure 1 it seems to be on the rise again in 2022 after a brief dip during the covid-19 pandemic. There are two main types of MRSA: healthcare-acquired MRSA(found mainly in hospital patients and long-term care facility residents) and community-associated MRSA (3).

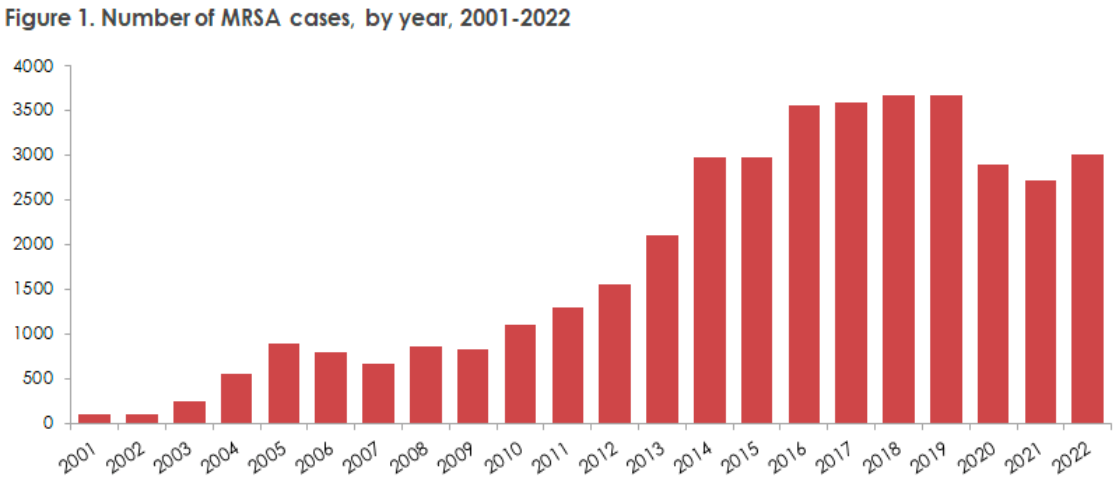

Figure 1. Number of MRSA cases by year from 2001-2022 and showing an incline at 2022 again. Adapted from No 34a - 2023 Methicillin-resistant Staphylococcus aureus (MRSA) 2022. (n.d.). No 34a—2023. Retrieved from https://en.ssi.dk/news/epi-news/2023/no-34a---2023

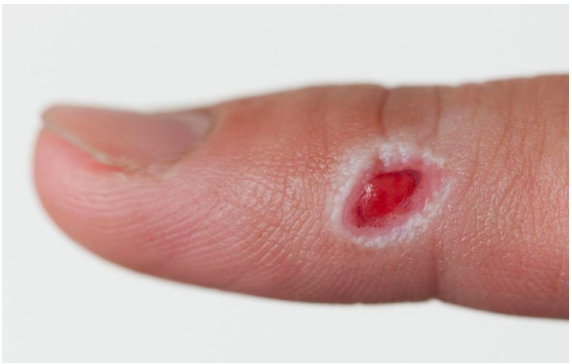

Figure 2. Image of MRSA infection on skin surface. Adapted from What are the Best MRSA Precautions? (With pictures). (2023, November 3). http://www.wise-geek.com/what-are-the-best-mrsa-precautions.htm

All MRSA contain an exogenous mec gene called mecA that has been indicated to develop from a harmless core gene called mecA1 that encodes penicillin-binding protein D (PBP D) in a different Staphylococcal species called S. sciuri. S. sciuri is an animal-associated bacterial species that is commonly found in environmental soil, sand, marsh, and water samples (4). However, S. sciuri has also begun showing signs of antibiotic resistance according to a 3-month study in Serbia where 73% of hospital-isolated S. sciuri strains demonstrated resistance to one or more antibiotics (4). With S. Sciuri demonstrating similar patterns to MRSA, the potential health risks only grow and it becomes more and more important to understand the mecA gene's role in resistance. One way to do so is by comparing S. Sciuri and S. aureus. The mecA gene in MRSA produces a modified PBP with low beta-lactam affinity called PBP2a. This protein has a C-terminus known to have transpeptidation functions and an N-terminus which is the non-binding domain (5). PBP2a has a lower affinity for beta-lactam antibiotics because of a slower rate of acylation with the beta-lactam ring due to a disordered active site that provides higher flexibility of the N-terminal (5). Residue 403 on the mecA gene is serine that produces an effective nucleophilic attack (bond formation from an electron-rich species meeting an electron-deficient species) of the beta-lactam ring and subsequent acylation (5). Pinpointing similarities like this in the domains of S. aureus and S. sciuri could tell a lot about how to combat antibiotic resistance in the future and how to eventually address it in strains like MRSA as well.

A mobile genetic element called Staphylococcal cassette chromosome mec (SCCmec) carries mecA and is composed of a mecA complex and a cassette chromosome recombinase (ccr) complex that aids in movement (5). SCCmec has also been known to carry diverse genes relevant for Staphylococcal survival such as heavy metal resistant scenes, modification functions, and immune protections (5). This cassette is transferred through the ccr's use of recognition sites, but the path of extrachromosomal SCC elements after they are formed is still elusive (5). The MecA homolog's native location in S. sciuri is 200 Kb from the orfX operon and research on the Staphylococcal species has indicated that S. sciuri is the most distant in homology of the genes flanking SCCmec (psm-mec and ugpQ) while species such as S. fleurettii are most similar to MRSA as indicated by Figure 3 (5).

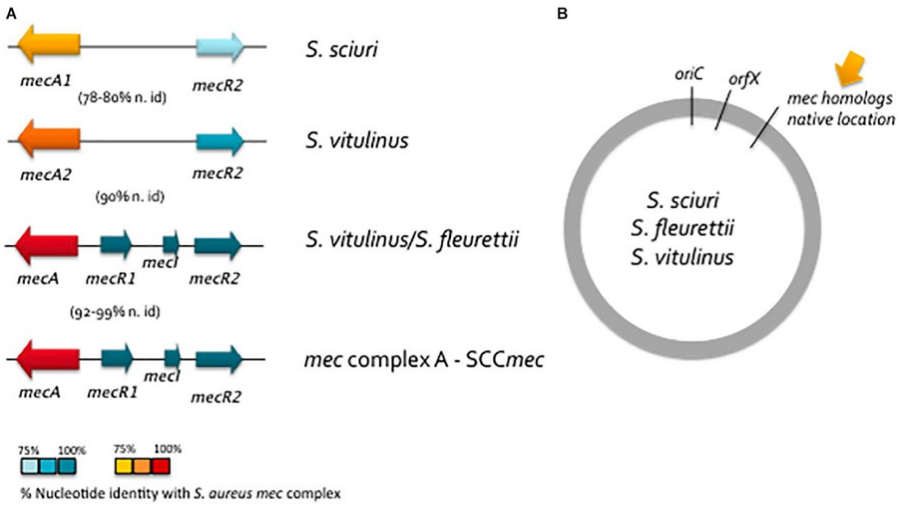

Figure 3. Representation of the structure of mec native region in S. sciuri, S. fleuretti, S vitilinus, and S. aureus indicating varying levels of homology. (5)

As demonstrated by Figure 3, S. sciuri is the furthest ancestry of mecA and has a 85% nucleotide sequence homology while S. fleuretti and some S. vitulinus have around 94% homology to S. aureus (5).MecA precursors were probably related to cell wall synthesis and not antimicrobial resistance but the stepwise process

between them led to a modified PBP with resistance capabilities (5). Seeing antimicrobial resistance in S. sciuri the furthest ancestor of MRSA is cause for worry and the differences between the mecA homolog's nucleotide sequences must be observed to understand the underlying differences that allowed for antimicrobial resistance. Using FASTA sequences from Uniprot, S. sciuri and S. aureus' sequences were analyzed in this study to increase our understanding of the structural similarities and differences (6).

Methods

Clustal Omega

Clustal was used to generate a Multiple Sequence Alignment (MSA) of the protein sequences of S. aureus against S. sciuri (7). Clustal Omega uses seeded guide trees and HMM profile-profile techniques to generate medium-large alignments. The resulting alignment of S. aureus against S. sciuri was color-coded and analyzed in sections to note particular amino acid differences between the two species and consequent differences in structure and function.

Galaxy

Galaxy is an open-source platform that allows users to process large datasets through various plug-ins and programs (8). Galaxy's NCBI BLAST + blastp-short (optimized for queries under 30 residues) was used to compare S. aureus, S. sciuri, and MRSA individually to the MadLandDB protein sequence database and identify alignments listed in order of highest similarity to the protein query's pattern. An expectation value cutoff of 0.15 was used and the top 5 similar alignments were analyzed for evolutionary patterns or connections to the query sequence.

InterProScan

InterPro integrates predictive information about protein's function to give a visual and overall overview of a protein's families, domains, and sites (9). Interpro was used to compare S. aureus and S. sciuri domain length and site information by feeding the program FASTA sequences. It was also used to generate result images that visually indicated varied domain lengths in key areas.

Uniprot

Uniprot is a comprehensive resource of protein sequence and functional details, images, and information (6). The FASTA protein sequences for S. aureus and S. sciuri that were used in InterPro, Galaxy, and Clustal were taken from Uniprot. Additional properties were also analyzed through Uniprot's data collection.

Results

The mecA orthologous complex in both S. aureus and S. sciuri was compared in terms of domain lengths and differences in amino acid construction of domain areas to better understand the different penicillin-binding proteins (PBP) produced by each species and the subsequent effect on antibacterial resistance and environmental interactions.

S. aureus vs. S. Sciuri: InterPro

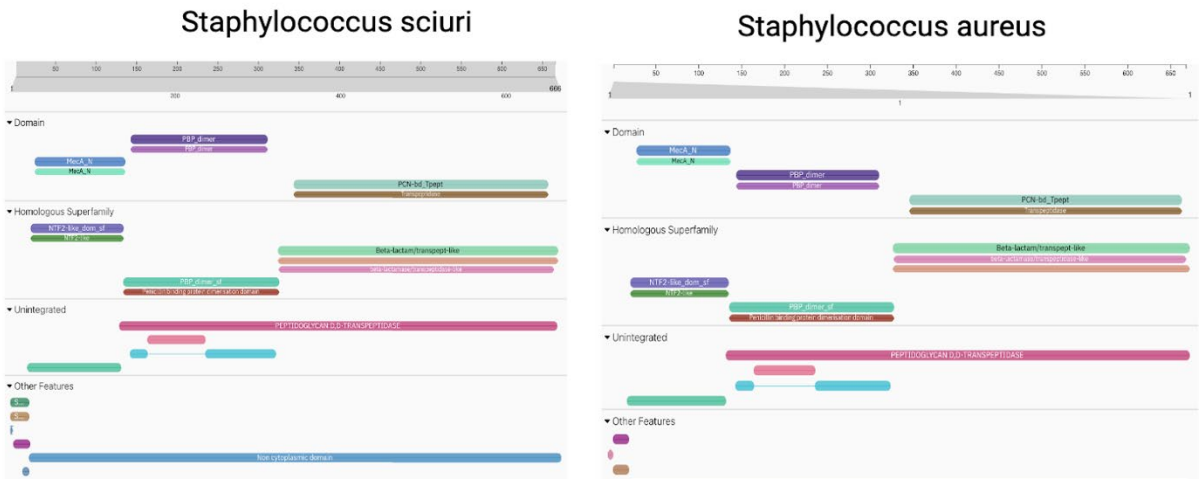

Figure 4. Interpro Scan results of Protein Domain Differences between S. aureus and S. sciuri

InterPro Scan analysis of the protein domains of the gene encoded by MecA between S. aureus (right) and S. scuri (left). The results are broken down into three sections: domain, superfamily, unintegrated, and other features. The MecA-N terminal Transpeptidase Domain is residues 34-140 in S. aureus and residues 31-139 in S. sciuri. Penicillin Binding Protein(PBP) Dimerisation Domain is residues 148-310 in S. aureus and residues 147-311 in S. sciuri. Penicillin Binding Protein Transpeptidase Domian is residues 346-656 in S. aureus and residues 344-650 in S. sciuri (9).

The mecA gene segment in S. aureus was also determined to have a region of the membrane-bound protein predicted to be embedded in the membrane from residues 7-24 and the residues 1-6 to be outside of the membrane in the cytoplasm (Fig 4). S. sciuri's mecA domain InterPro analysis revealed that residues 5-24 of the membrane-bound protein were predicted to be embedded in the membrane (Fig 4). Additionally, residue 4-15 of S. sciuri was a hydrophobic region of the signal peptide (spanning 1-23) which had an N-terminal from residues 1-3 and C-terminal from residues 16-23 (Fig 4). The N-terminal has a free amino group and often contains targeting signals while the C-terminal has a free carboxyl group and often contains retention signals for protein sorting. The Interpro Scan reveals that there is a hydrophobic region (4-15) between the C and N-terminals indicating the region of the membrane-bound protein predicted to be embedded in the membrane is 4-15 (Fig 4). This is another difference between the protein domain ranges of S. aureus and S. sciuri. mecA in both S. aureus and S. sciuri were predicted to have the same molecular function of penicillin-binding and peptidoglycan L,D-transpeptidase activity.

For further analysis of specific differences among these domains, Clustal Omega was used to perform a multiple sequence alignment on S. aureus and S. sciuri.

S. aureus vs. S. sciuri: Clustal Omega Comparison

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tr|O54286|O54286_STAAU      MKKIKIVPLILIVVVVGFGIYFYASKDKEINNTIDAIEDKNFKQVYKDSSYISKSDNGEV  60
tr|O54277|O54277_MAMSC      MK-KLIIAIVIVIIAVGSGVFFYASKDKKINETIDAIEDKNVKQVFKNSTYQSKNDNGEV  59
                            **   *: ::::::.** *::*******:**:**.******.***:*:*:* **.*****
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Figure 5. Clustal Omega Protein Alignment of MecA N-terminal Transpeptidase domain in residues 30-34 The boxed section indicates a significant residue change at the ":".

For analyzing the given color of residues: red indicates small, hydrophobic properties; blue indicates acidic; magenta indicates basic residues; green indicates hydroxyl, sulfhydryl, and amine groups; and gray indicates unusual amino acids. For analyzing consensus symbols in the alignment: "*" indicates fully conserved residues, ":" indicates conservation between groups of strongly similar properties; and "." indicates conservation between groups of weakly similar properties (9).

Clustal sequence alignment, residues 30-34 are boxed to indicate the differences in starting amino acids of the MecA N-terminal Transpeptidase domains for S. aureus against S. sciuri that was found through InterPro Scan comparative analysis (Fig 5). S. sciuri starts at residue 31 and has a blue (acidic) element at the 33rd residue which S. aureus does not have at the 33rd residue (Fig 5). The ":" at this location indicates a conservation between groups of strongly similar properties referencing S. aureus having a green residue at that placement, indicating hydroxyl, sulfhydryl, and amine groups which are all hydrophilic (Fig 5). Similarly, at the 33rd residue, S. sciuri has glutamine while S. aureus has asparagine, which are both polar, neutral, and hydrophilic (10). Changes very similar to this can be seen along the entire segment, indicating that many parts of the gene stay the same but there must be other larger changes elsewhere to generate such a significant change in mecA function in S. sciuri vs. S. aureus.

Figure 6. Clustal Omega Protein Alignment of MecA N-terminal Transpeptidase domain in residues 217-231

Residues 217-231 in Figure 6 highlight amino acid differences in the PBP Dimerization domain which aids protein folding and synthesis. For analyzing the given color of residues: red indicates small, hydrophobic properties; blue indicates acidic; magenta indicates basic residues; green indicates hydroxyl, sulfhydryl, and amine groups; and gray indicates unusual amino acids. For analyzing consensus symbols in the alignment: "*" indicates fully conserved residues, ":" indicates conservation between groups of strongly similar properties; and "." indicates conservation between groups of weakly similar properties (9).

At residue 217 S. aureus contains a lysine (magenta - basic element) compared to S. sciuri's glutamine (green - hydroxyl+sulfhydryl+amine group) at the same placement (Fig 6). While both lysine and glutamine are polar and hydrophilic, glutamine is neutral compared to lysine's positive charge (10). This indicates that both tend to be outside of the membrane due to their hydrophilic side chains but glutamine has more potential in forming multiple hydrogen bonds as a neutral amino acid, preferring to be projected into the aqueous phase. Glutamine has also been known to remove excess ammonia and is a building block to make essential proteins such as glucose (11). In comparison lysine has a positive charge indicating that it carries more amine groups than carboxyl groups. Lysine, like many other positive amino acids, has great potential as a topogenic determinant in membrane proteins, meaning that they aid formulation of peptide sequences to orient and organize the cell membrane (12). Lysine is also known to degrade to form glucose, providing an energy source for cells as well (12). While glutamine in S. sciuri and lysine in S. aureus aid protein synthesis and glucose production, lysine in S. aureus has the specific capability to reorient membrane surface organization which could lend itself to new structural capabilities going in to resist antibiotics. The ":" also indicates roughly equivalent residues and a level of conservation (Fig 6).

At residue 220, S. sciuri has asparagine compared to S. aureus' aspartic acid. This distinction is interesting because asparagine is the amide of aspartic acid, meaning it doesn't carry a formal charge. The amide is easily hydrolyzed, allowing it to convert from asparagine to aspartic acid (13). This process has been shown to have ties with the molecular basis of aging and damage to DNA (13). Another difference is that asparagine has a higher attraction to form hydrogen bonds and is more likely to be found on the membrane surface as a common

attachment site for carbohydrates (13). For this process to occur cellular glutamine is used to hydrolyze asparagine. In the boxed residues being analyzed we have already identified the nearest glutamine source(residue 217) in S. Sciuri paired with S. sciuri's asparagine at residue 220 creates the perfect conditions for hydrolysis to aspartic acid and subsequent impacts to bonding (Fig 6). This is a potential area of further exploration to identify the specifics of this hydrolyzed interaction of asparagine that can impact molecular aging in connection to S. sciuri's sensitivity to antibiotics such as penicillin, and methicillin or other molecular functions (Fig 7).

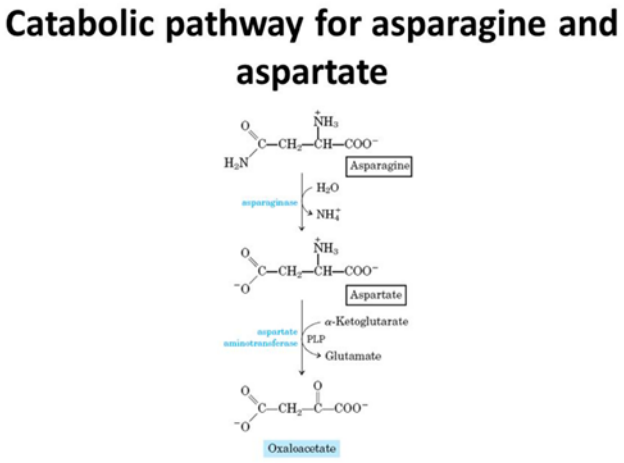

Figure 7. Catabolic pathway for Asparagine and Aspartate(aspartic acid)- potential links to S. sciuri function (14)

Residue 228 is marked with a ":" indicating equivalent residues and indicating another point of interest on the PBP Dimerization domain (Fig 6). S. aureus has an alanine at this placement while S. sciuri has a valine. They are both red indicating small hydrophobic properties, suggesting that they are found within the inner, hydrophobic section of the plasma membrane in the protein complex. However, alanine is known to be an ambivalent molecule, meaning that it can be inside or outside of the protein molecule, possibly changing the local membrane architecture because in S. sciuri valine can only be inside the membrane (hydrophobic) (15). Alanine is also a much better helix-forming residue than valine (16). Meanwhile, valine is a precursor in the penicillin biosynthesis pathway and is important for the correct formation of protein structure. Playing an essential role in building protein structures for recognition and binding due to its hydrophobic properties, valine in S. sciuri may demonstrate how it ensures PBP protein synthesis with fewer mutations. Perhaps the lack of valine in this domain in S. aureus, allows mutations to take place evolving into the modified PBP2a protein that enables methicillin resistance in the species. Dimerization domains enhance and enable protein folding and synthesis; in the case of PBP2a synthesis with mecA, these differences in the dimerization domain could create differences in protein synthesis and folding leading to different functional capabilities in mecA's ability to synthesize protein resistant to methicillin such as in MRSA.

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tr|O54286|O54286_STAAU    IVDDNSNTIAHTLIEKKKKDGKDIQLTIDAKVQKSIYNNMKNDYGSGTAIHPQTGELLAL  360
tr|O54277|O54277_MAMSC    IIDDNNK-VIDTLIKKKKKDGKDIKLTIDARVQKSIYNNMKDDYGSGTAIHPQTGELLAL  358
                          *:***.: : .***:*********:*****:**********:******************
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Figure 8. Clustal Omega Comparison Residues 360-374 from mecA PBP Transpeptidase Domain.

The boxed section highlights nucleotide differences in the transpeptidase domain for both Staphylococcal species which are critical to function.

For analyzing the given color of residues: red indicates small, hydrophobic properties; blue indicates acidic; magenta indicates basic residues; green indicates hydroxyl, sulfhydryl, and amine groups; and gray indicates unusual amino acids.

For analyzing consensus symbols in the alignment: "*" indicates fully conserved residues, ":" indicates conservation between groups of strongly similar properties; and "." indicates conservation between groups of weakly similar properties (9).

Transpeptidase is an enzyme that catalyzes the carbonyl substitution process required for peptidoglycan cross-linking in bacterial cell walls (3). This enzyme is the primary target for antibiotics such as penicillin and methicillin, therefore this is the main segment of interest for understanding mutations that may have led to MRSA. Antibiotics bind to the active site of transpeptidase and block the enzyme's function, effectively hindering bacterial cell wall growth (3). Methicillin and other antibiotics of the beta-lactam family utilize a four-membered beta-lactam ring to bind and inhibit enzyme function (3). The subsequent thinning of the bacterial cell wall causes the vulnerable cell to die quickly from molecular pressures, yet human cells aren't impacted because they don't have a bacterial cell wall (3). Health risk examples of antibiotic resistance such as MRSA, which evolved from S. aureus, upregulate a low-affinity form of penicillin binding protein that can avoid inhibition by antibiotics and allow MRSA to continue growing even in the face of beta-lactam antibiotics such as methicillin. Majority of S. aureus strains (83.1%) have been resistant to penicillin by producing beta-lactamase, which destroys penicillin's beta lactam rings (17). Then second-generation penicillins such as methicillin were used on S. aureus, however MRSA evolved to circumvent these new antibiotics as well. This continued evolutionary growth of MRSA makes it an evolving health risk and therefore understanding the transpeptidase domain could bring to light some of the mechanisms that played a role in antibiotic resistance.

Residues 360-374 of the Transpeptidase domain show interesting differences between the residues of S. aureus and S. sciuri that may reveal how S. aureus evolved to maintain antibiotic resistance against second-generation penicillins through PBP2a synthesis done by MRSA (Fig 8). Residue 361 is isoleucine for S. sciuri and valine for S. aureus (Fig 8). Valine plays a pivotal role in protein structures for recognition and binding and it is in red indicating hydrophobic properties (18, Fig 8). Valine would be found in the hydrophobic inner section of the plasma membrane where it could form the internal basics for protein surface recognition that is needed for the low-affinity penicillin-binding proteins that form the basis of MRSA antibiotic resistance (18). Meanwhile the isoleucine in S. sciuri is also hydrophobic but is known for strong helical-forming powers for the evolution of larger protein molecules. This may indicate that the replacement of isoleucine with valine from S. sciuri to S. aureus causes a shift in protein structure and formation which allows the production of lower affinity binding proteins.

Residue 365 is marked by a "." indicating weak similarity at this amino acid (Fig 8). S. aureus has serine at this residue compared to S. sciuri's asparagine. Both are green indicating they are both hydrophilic and are on the outer and inner surface of the membrane, rather than the inside, therefore this part of the function remains relatively the same. Serine aids in the production of natural antibodies which could lend itself to antibiotic resistance in MRSA, while asparagine aids protein modification more than any other function (19).

Residue 370 is also marked by "." indicating weak similarity and has aspartic acid for S. sciuri and histidine for S. aureus (Fig 8). The aspartic acid is blue and acidic while histidine is green indicating the presence of hydroxyl, sulfhydryl, and amine groups which allows for more bonds to form creating complex protein structure. This difference could affect the protein structure exposed out of the membrane. These differences in protein structure could be the key difference in the upregulation of low-affinity PBP2a compared to the use of other beta-lactamases in response to antibiotics. This makes MRSA more dangerous because even second-generation penicillins like methicillin can no longer stop bacterial growth if the structure of PBP2a proteins evolves differently over time at its current rate.

Galaxy Analysis of Species Similar to S. aureus: S. pseudintermedius

Table 1. Top 5 similar species to S. aureus produced by BLAST-p analysis done on Galaxy (8)
Blast-p analysis done on the second most similar species to understand key parts of S. aureus function in antibiotic resistance.

NCBI Identifier	Similarity	Description
tr|O54286|O54286_STAAU	100	MULTISPECIES: PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA
tr|O54286|O54286_STAAU	100	PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA [Staphylococcus pseudintermedius]
tr|O54286|O54286_STAAU	99.85	PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA [Staphylococcus aureus]
tr|O54286|O54286_STAAU	100	PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA [Staphylococcus aureus]<>penicillin binding protein 2a
tr|O54286|O54286_STAAU	99.85	PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA [Staphylococcus aureus]

Highlighted in red, the species S. pseudintermedius retains 100% similarity with S. aureus (Table 1). This species has also developed methicillin resistance known as Methicillin Resistant S. pseudintermedius (MRSP) which often causes canine pyoderma and affects open wounds with infection (20). Since 2006 there has been significant emergence of MRSP and the growth of this infection has even led to humans becoming transient carriers of S. pseudintermedius (20). The first clinical case in humans was in 2020 in Argentina and caused severe infection, leading to more emergent studies aiming to further understand the evolution of MRSP as well (20).

Studying the mechanisms of MRSP infection could also shed light on S. aureus' evolution to methicillin resistance. SCCmec variants III, IV, V, and VII were found in US isolates of S. pseudintermedius (20). Comparing MRSA and MRSP, healthcare-associated MRSA harbors SCCmec types I, II, and III, while community-acquired MRSA has SCCmec types IV, V, VI, VII, and VIII (21). The types represent various site-specific recombinases called cassette chromosome recombinases (ccr) that are responsible for integration of SCCmec and the beta-lactam resistance phenotype (22). This significant overlap between MRSA and MRSP could indicate similar SCCmec integration processes.

Additionally, ccr gene complexes have three distinct allotypes: ccrA, ccrB, and ccrC that have all been identified in MRSA with nucleotide sequence similarities below 50% (22). The distinction is made because ccr genes with more than 85% nucleotide similarity are assigned to the same allotype while similarities between 60% and 82% belong to different allotypes. MRSA isolates have been classified as ccrA, ccrB, or ccrC while S. pseudintermedius has been classified as ccrA (22). More specifically a few extra ccr forms have been identified one being ccrA5 which is the ccrA gene of S. psuedintermedius. Meanwhile ccrA7 has been found in S. sciuri (5). The overlap of some ccr gene complex allotypes may also highlight key parts of SCCmec integration that play the largest role in beta lactam resistance observed in Staphylococcal species.

Another interesting similarity between S. aureus and S. pseudintermedius are that both are coagulase positive while all other species of staphylococci are coagulase negative (23). This test references the bacteria's ability to produce secretory proteins that cause blood clotting through prothrombin activation and the subsequent conversion of fibrinogen to fibrin (23). S. aureus strains have bound coagulase, known as clumping factor, which directly acts on the fibrinogen of organisms which then causes clumping of the Staphylococcal strains themselves. This can be detected by a coagulase slide test (23, Fig 9). S. aureus also exhibits free coagulase properties where the same result is achieved by globulin-like plasma factor to produce a thrombin-like enzyme. This factor catalyzes fibrinogen to insoluble fibrin and can be detected by a coagulase tube test (23). S. pseudintermedius only expresses free coagulase which may provide an explanation behind its similarities to S. aureus through this overlap. Overall the clumping factor may allow bacteria to stay together in groups, generating a greater capability to resist antibiotics, share genetic information, and spread infections faster which are all qualities observed in S. aureus and S. pseudintermedius.

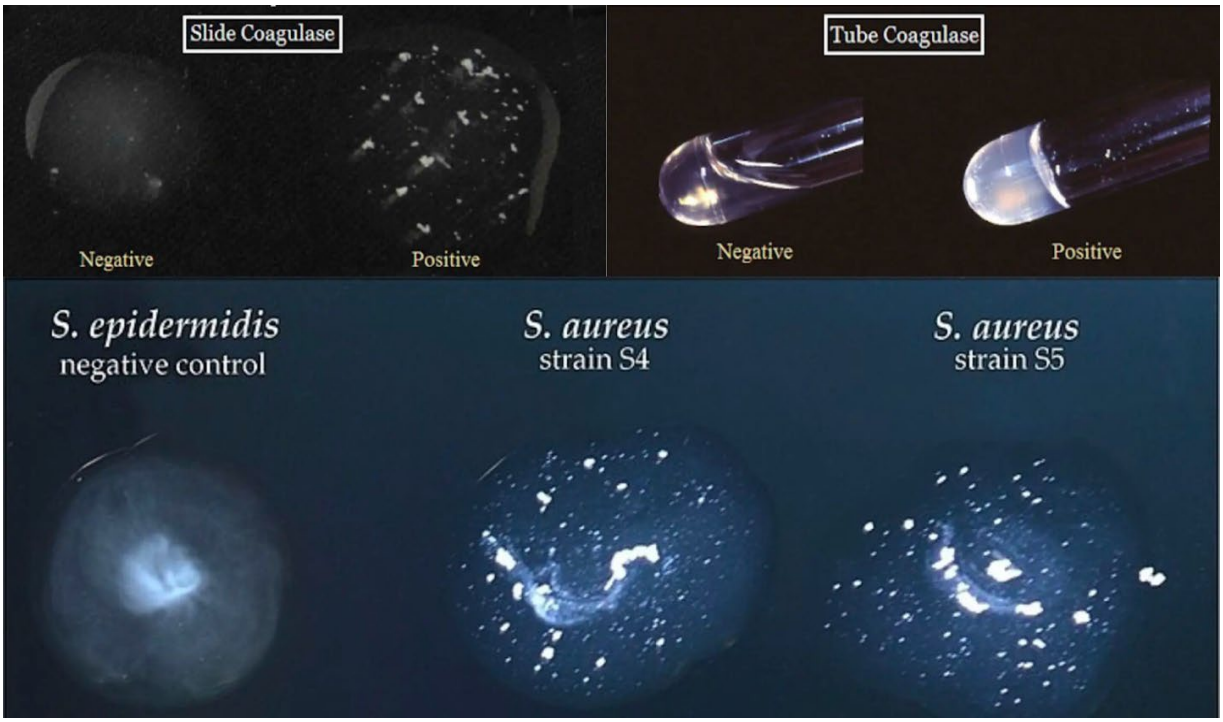

Figure 9. Image depicting how slide and tube tests help distinguish between coagulase positive forms of staphylococcus vs coagulase negative forms.
Adapted from Coagulase Test Principle, Procedure, Application, Result. (n.d.). Jan 2022, from https://microbiologynote.com/coagulase-test/

Discussion

Antibiotic resistance has drastically spiked in recent decades and hospitals are one of the largest breeding grounds for antibiotic-resistant bacteria. The analysis of Staphylococcal species has largely indicated antibiotic resistance patterns due to the presence of SCCmec, a product of evolution from a harmless genetic component to a cassette vital for bacterial cell survival in the face of beta-lactam antibiotics. The differences in S. sciuri and S. aureus as indicated by the Clustal Omega analysis are clear indicators of evolutionary differences in specific residue patterns in both the transpeptidase and dimerization domains. Some of the residue differences

are structural while others are functional and further research with more experimental validation in this area would be vital in confirming which of the residue differences are most important in the expression of antibiotic resistance. For instance, testing strains of MRSA with modified aspartic acid at residue 220 could clarify the role of aspartic acid and asparagine in metabolic reactions that may be connected to antibiotic resistance mechanisms (Fig 6).

The analysis of S. psuedintermedius done is also insightful to the patterns of cross-species infection from animals to humans that is possible in Staphylococcal species. S. sciuri is an animal-based bacterium but carries ccrA7 unlike S. pseudintermedius carrying ccrA5. This difference may stop the full jump of modified S. sciuri to human infections, but further research is needed to ensure that scientists can be prepared for an uprise in S. sciuri infections. As S. sciuri is already showing signs of antibiotic resistance it is imperative to pinpoint problematic residues that are being modified in similar patterns to S. aureus and experiment with various classes of antibiotics to see which are most effective against these infections.

Another area of exploration may lie in examining homologs to mecA such as mecC. MecC has also begun showing methicillin resistance and shares a 70% nucleotide identity with mecA. (24) However, they differ in binding characteristics toward beta-lactam antibiotics and temperature-dependent activities. (24) Examining the alternative steps of evolution that led to mecC could uncover new facets of antibiotic-resistant processes. The PBP protein of mecC (PBP2c) shares only a 63% similar nucleotide identity with PBP2a and unlike PBP2a, its activity decreased at 37°C. (24) This raises new questions of whether strains harboring modified PBP proteins differ in resistance levels as a consequence of structural, functional, or transcriptional differences compared to their homologs. Further research is needed to compare mecA to its homologs and them further analyze mecC-carrying species with S. aureus and S. sciuri to develop more data regarding the expression of antibiotic resistance in the Staphylococcal species that can aid the production of new antibiotics targeting the new areas of interest.

Through analyzing S. sciuri in comparison to S. aureus the ancestral evolution becomes clear, indicating a potential for further research on MRSA infections that continue to be an issue, especially in hospital settings. Key residue differences between the homologous mecA of various staphylococcus species underscore the mechanism for beta-lactam resistance, and further study is needed to verify these residue differences and brainstorm new approaches for treatment of MRSA.

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