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Article

Analysis of antibiotic resistance genes in publicly accessible *Staphylococcus aureus* genomes

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SUMMARY

***Staphylococcus aureus* is a versatile bacterium commonly found in the human microbiota that can also cause a wide range of infections, from minor skin conditions to life-threatening diseases. Among its strains, methicillin-resistant *Staphylococcus aureus* (MRSA) is rapidly developing resistance to many antibiotics, including methicillin, penicillin, and other beta-lactam antibiotics. While MRSA incidence has declined in some areas, it remains a clinical threat due to its extensive resistance. Eradicating MRSA will take time, but a pressing question remains: can genetic diversity among MRSA strains guide the development of more effective treatments? We hypothesized that strains containing *mecA* and *blaZ* would show high antibiotic resistance, while strains with tetM would be least resistant. To test this, we used NCBI, PathogenWatch, and BLAST to identify and analyze 51 *S. aureus* strains and investigate their antibiotic resistance profiles. Our results showed that genetic diversity regarding resistance genes is present in all but six strains—those six lacked resistance genes entirely, making them highly susceptible to treatment. Our findings partially supported the hypothesis: *mecA* was strongly associated with resistance, *tetM* surprisingly also conferred resistance, and *blaZ* showed less resistance than expected. Our study underscores the need to examine genetic variability when designing treatments for MRSA. While no universal solution currently exists, understanding gene-based resistance patterns may eventually guide individualized treatment plans. Until then, combination antibiotic therapies may remain the most effective option against MRSA.**

INTRODUCTION

Staphylococcus aureus is a normal skin colonizer, but it can lead to various infections (1). *S. aureus* infections are a global health concern, with high prevalence rates in North America, Europe, and Asia, especially in healthcare settings. In particular, countries like Brazil, China, and Taiwan have reported some of the highest rates of *S. aureus* infections, likely due to widespread antibiotic use and limited infection control in certain healthcare environments. Beta-lactam antibiotics, like penicillin, are the usual treatment for *S. aureus*; however, methicillin-resistant *S. aureus* (MRSA) is becoming more and more widespread, which greatly reduces the spectrum of antibiotics that can be used for the infection (2). Left untreated, MRSA infections can result in pneumonia, or even sepsis, and may be fatal in some cases if a patient's immune system is not functioning properly (3). Infection with MRSA can occur when healthy individuals touch objects that have been contaminated by infected people or touch an infected person (4). Those who are at higher risk for contracting MRSA are athletes, the elderly, daycare and school students, and military personnel in barracks because the risk of contracting MRSA increases in areas or activities that involve crowding, skin-to-skin contact, and shared equipment or supplies (5). Every 2 in 100 people carry the MRSA strain and MRSA is highly prevalent in hospitals throughout the world – especially in regions in East Asia where an excessive amount of antibiotics is used to treat staph infections (6, 7). However, most people who contract MRSA are asymptomatic, but if they do end up getting an infection due to *S. aureus,* treatment is more difficult because a lot of the traditional antibiotics are ineffective against MRSA. MRSA presents a large threat to society, especially to those who are in the hospital or nursing homes and are at higher risk of contracting this infection (8). The global resistance rate for strains of *S. aureus* to penicillin is 85.8%, to erythromycin 87.2%, and ciprofloxacin 90.8% (9). The mortality rate for those who are infected with hospital-acquired MRSA is 29% while those who have been infected with community-acquired MRSA is 18%. This amounted to a rate of 6.3 deaths per 100,000 people in the United States in 2005 (10). In addition, not only are there physical impacts for those with MRSA, but there are also psychological impacts on patients due to fear, discrimination, and isolation (11).

There are seven common antibiotics used against MRSA: vancomycin, daptomycin, linezolid, trimethoprim-sulfamethoxazole (TMP-SMX), quinupristin-dalfopristin, clindamycin and tigecycline (12). Treatment of MRSA at home usually includes a 7-10-day course of an oral antibiotic such as trimethoprim-sulfamethoxazole, clindamycin, minocycline, linezolid, or doxycycline (13). Right now, the most effective antibiotic to treat MRSA is vancomycin or daptomycin (14). However, MRSA is quickly developing resistance even to these antibiotics, so some healthcare providers have turned to experimental treatments, such as quorum sensing inhibition, lectin inhibition, phage therapy, and beta-lactam antibiotics like ceftaroline or cefazolin (14). Researchers have also turned to using combination therapy – where multiple treatments/medications are used in conjunction with one another to eradicate an infection – using vancomycin or daptomycin with beta-lactam antibiotics (e.g., ceftaroline) in order to see if there is successful clearance of persistent bacteremia caused by *S. aureus* strains (15).

MRSA is slowly becoming more widespread throughout the world, so it is imperative that some sort of antibiotic

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treatment be developed that can provide individuals with relief and circumvent the resistance of MRSA (16). Treating MRSA costs about $10 billion per year, which averages about $60,000 per patient (17). The high cost may prevent many people in developing countries who are affected by MRSA from getting the treatment they need (18). Therefore, we aimed to investigate the presence and diversity of antibiotic resistance genes in *S. aureus* strains. We hypothesized that most *S. aureus* strains would carry the *mecA* and *blaZ* resistance genes, making them highly resistant to penicillin and methicillin, but they would be less resistant to tetracycline, given our expectation of a lower presence of *tetM*. This expectation was based on the frequent use of penicillin and methicillin to treat MRSA, compared to the less common use of tetracycline, given that there is little data to guide clinicians in how to properly use tetracycline (19). Our results partially supported this hypothesis: while *mecA* was frequently present, *tetM* was also more common than anticipated, and *blaZ* appeared less often than expected. This information will be useful in that it can provide more current and updated information about the antibiotic resistance profile of MRSA, and this can be used by biotech companies to develop a new third-generation antibiotic that can work on MRSA.

RESULTS

Our data was composed of 51 genomes that were taken from the NCBI database on *S. aureus* assemblies. The reason for choosing only 51 genomes out of 103,184 genomes (all genomes available on NCBI as of July 9, 2023) was to balance both the accuracy of an assessment of genetic diversity along with practical considerations regarding computational resources. The genomes came from 13 different countries, though our dataset contained no samples from Africa (**Figure 1**). This wasn't intentional – we simply didn't come across African samples in the database we were using, which might reflect the lower number of publicly available genome sequences from that region. In addition, most of our samples came from various isolation sources including blood, an ATCC isolate, a nasal swab, bone, urine, peritoneal fluid, etc (**Figure 2**).

We analyzed the 51 genomes against antibiotic resistance genes using BLASTn – a program that compares nucleotide sequences (**Figures 3-5**). We examined the hits that the BLAST generated as well as the description table. Across all of the hits, the E-value was always 0 and the percentage identity ranged from 96% to as high as 100% in all 51 strains. The E-value, or Expectation value, is a parameter used in sequence alignment to indicate the number of hits that would be expected by chance when searching a database. An E-value of 0 indicates a highly significant match with no expected false positives, meaning that the sequence matches identified were extremely unlikely to occur by random chance.

BLASTn returned no hits for any of the searched genes for the following six strains, written as strain name (accession number): NCTC 8325 (CP000253), DSM 20231 (CP104478), DSM 20231 (CP011526), ATCC 12600 (CP035101), FDAARGOS_773 (CP040998), and PartF-Saureus-RM8376 (CP064365). When we looked on PathogenWatch – a web-based platform that uses genome sequencing to monitor and analyze pathogens – to examine our results from the BLASTn, we noticed that all of these strains were susceptible to amikacin, gentamicin, tobramycin, kanamycin, methicillin, penicillin, erythromycin, and tetracycline, which are the targets of the antibiotic resistance genes we tested. Thus, we concluded that there are still *S. aureus* strains analyzed in this study that do not have antibiotic resistance.

In addition, when looking at our results, we noticed that *mecA*, *tetM*, and *ermA* were present in most of the genomes while the other sequences were not as common. In all the genomes that we searched, 42/51 had *mecA*, 41/51 had *tetM*, and 39/51 had *ermA*, showing that MRSA would be resistant to methicillin, tetracycline, and erythromycin. Moreover, there were several strains in our collection with multidrug resistance, having the sequence of almost every gene we blasted against them. To help confirm the accuracy of our BLAST results, we compared them with the resistance gene profiles listed in PathogenWatch for the same strains. In other words, we used PathogenWatch to check whether the genes identified through our BLAST searches matched the known resistance genes already reported in the database.

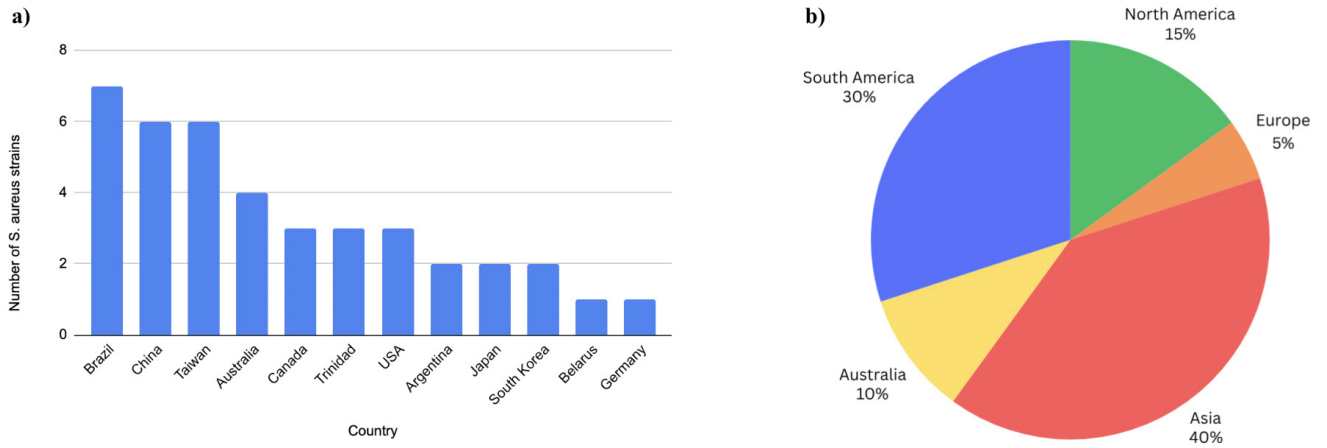

Figure 1: Relative abundance of *S. aureus* strains around the globe. Metadata from 51 *S. aureus* strains was analyzed to see where the strains were most common. **a)** Metadata from 51 *S. aureus*strains depicting the number of strains isolated from different countries in 2023. **b)** A more generalized depiction of the metadata in Figure 1a - instead of countries, the chart shows the continents where each strain was found.

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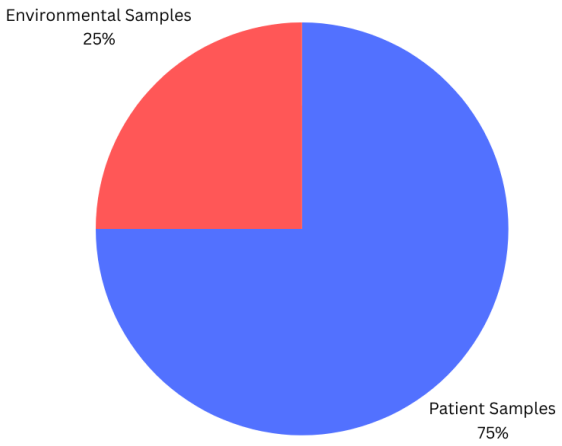

Figure 2: Strain source type. Seventy-five percent of strains were isolated from patient samples, while the remaining 25% were isolated from environmental samples. Data from metadata gathered from each of the 51 downloaded genomes.

This helped ensure that the strains we labeled as resistant (based on the presence of specific genes) were consistent with previously published data. When we looked at our metadata, we noticed that most of these strains were from South America, in areas such as Brazil and Argentina, or they were from East Asia, near Taiwan (**Figure 1**), meaning that these areas correlate with high rates of resistant strains. Thus, the results produced from our BLAST are consistent with previous findings regarding those areas having higher cases of resistance.

After analyzing the antibiotic resistance genes in several bacterial strains linked to six key genes – *mecA* (MW682923, *S. aureus* SA-28), *aphA-3* (CP003194, *Aeromonas salmonicida* 01-B526), *aacA-aphD* (CP010526, *Enterococcus faecium* EnGen0383), *blaZ* (MT536162, *S. aureus* SA-84), *ermA* (CP002120, *Streptococcus pneumoniae* Hungary19A_6), and *tetM* (M21136, *Enterococcus faecalis* pIP501) – we found some interesting patterns related to beta-lactam resistance. PathogenWatch suggested that the *S. aureus* strains SA-28 and SA-84 carried the *blaZ* resistance gene. But after a closer look, it seems these strains might actually have *blaR1* instead, which can be confused with *blaZ* because they are closely related genes in the beta-lactamase operon. Our analysis reveals that the presence of *blaZ* or *blaR1* in *S. aureus* strains SA-28 and SA-84 suggests their resistance to beta-lactam antibiotics while the absence of these genes in *A. salmonicida*, *E. faecium, S. pneumoniae,* and *E. faecalis* points to species-specific resistance mechanisms. Since both *blaZ* and *blaR1* provide resistance to beta-lactam antibiotics but differ in sequence, correctly identifying them is important for understanding how resistance develops. The lack of these genes in the other strains makes sense, as these species use different resistance strategies. This difference underscores the need for accurate gene identification to clarify resistance profiles (**Table 1**) (20).

We also noticed some differences in tetracycline resistance genes. Some strains, possibly including *S. aureus* SA-28 or SA-84, had *tetK* but not *tetM*. Since *tetM* provides resistance to all tetracycline drugs while *tetK* only gives limited resistance, this could affect how well tetracycline treatments work. For example, *E. faecalis* carrying *tetM* (M21136) is likely more resistant to tetracycline than strains with only *tetK* (21). Doing more detailed sequencing on *S. aureus* SA-28 and SA-84 to confirm the presence of *blaZ*, *blaR1*, and *tetK* could help us better understand their resistance and how it might affect treatment (21).

DISCUSSION

Ultimately, this study aimed to explore the genetic basis of antibiotic resistance in *S. aureus* by analyzing the presence and conservation of six key resistance genes: *mecA, aphA-3, aacA-aphD, blaZ, ermA,* and *tetM*. Our results showed varying gene conservation across strains, with notable patterns in the presence of beta-lactam resistance genes. Additionally, discrepancies between *blaZ* and *blaR1* highlighted the need for further investigation into their roles in resistance.

Single nucleotide polymorphisms (SNPs) are variations in a single nucleotide that occur at a specific position in the genome among individuals or populations. While analyzing our genome collection, we observed that both *mecA* and *ermA* lacked SNPs. However, due to time constraints, we chose to focus on the absence of SNPs in *mecA*, as it was the more prevalent resistance gene in our results. Essentially, *mecA* appeared to be uniform across the samples tested without any SNPs at the positions studied. This uniformity

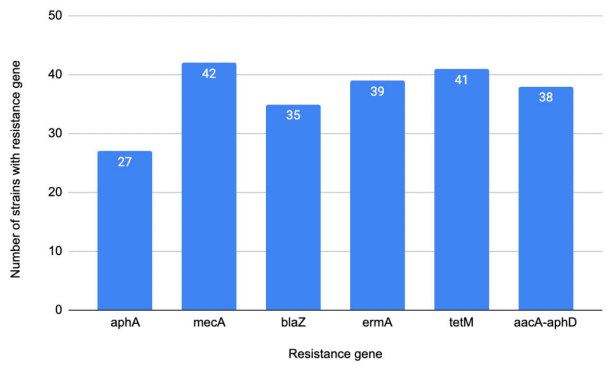

Figure 3: Number of strains with each resistance gene. Number of times a resistance gene was found in one of our 51 strains of MRSA.

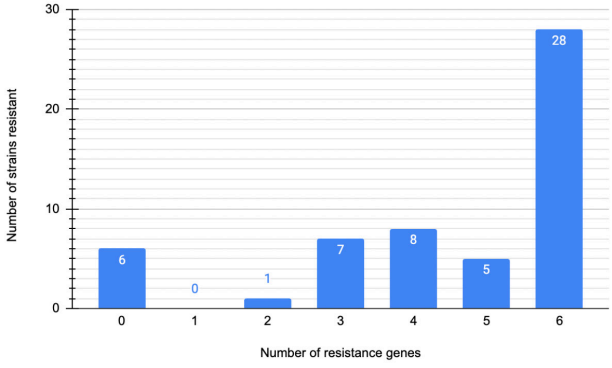

Figure 4: Quantification of the number of resistance genes each strain has. Number of strains that contain different numbers of resistance genes. For example, 28 strains contain 6 resistant genes while 0 strains contain only 1 resistance gene.

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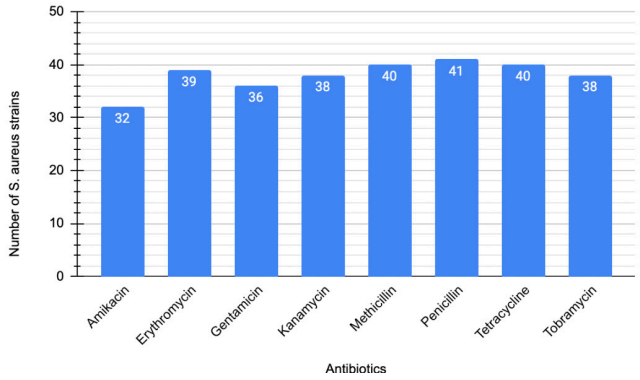

Figure 5: Number of *S. aureus* strains containing genes encoding resistance to antibiotics. Number of strains that exhibit resistance to different antibiotics due to the presence of various genes involved in antibiotic resistance.

Accession #	Strain/Species	Gene	blaZ Present?	blaR1 Present?
MW682923	*S. aureus* SA-28	*mecA*	Yes	Yes
CP003194	*A. salmonicida* 01-B526	*aphA-3*	No	No
CP010526	*E. faecium* EnGen0383	*aacA-aphD*	No	No
MT536162	*S. aureus* SA-84	*blaZ*	Yes	Yes
CP002120	*S. pneumoniae* Hungary19A_6	*ermA*	No	No
M21136	*E. faecalis* (pIP501)	*tetM*	No	No

Table 1: Presence of *blaZ* and *blaR1* resistance genes in selected bacterial strains. Results indicate the presence of *blaZ* and *blaR1* resistance genes in *S. aureus* SA-28 and SA-84, with no detection in *A. salmonicida* 01-B526, *E. faecium* EnGen0383, *S. pneumoniae* Hungary19A_6, and *E. faecalis* pIP50.

across our samples was notable because previous studies have identified that SNPs in *mecA* were associated with resistance to various antibiotics (22). This finding suggests that *mecA* in our samples lacked these genetic variations that could potentially confer resistance, particularly to beta-lactam antibiotics, as seen in some strains of *S. aureus* (23, 24). This may have happened because we only included 51 genomes in this study, which is a small sample size, so a large sample may have yielded some SNPs in *mecA.* This would affect the treatment of patients with MRSA because if these strains had SNPs, then researchers and doctors could compare the genetic make-up of a bacteria and an antibiotic in order to provide them with treatment that is effective and safer (12). With SNPs, it would be easier to determine an individual's risk of contracting various illnesses as well as predict their responses to drugs (25).

When we looked at the metadata for our strains, we noticed that most strains with multidrug resistance were from South America, in areas such as Brazil and Argentina, or they were from East Asia, near Taiwan, meaning that these areas correlate with high rates of resistant strains (**Figure 1**). Thus, the results produced from our BLAST are consistent with previous findings regarding those areas having higher cases of resistance. During our analysis using BLAST, we also identified six genomes lacking active resistance genes. Verification on PathogenWatch confirmed these strains exhibited no resistance to any antibiotics, highlighting their high susceptibility. Plasmids, which are small circular DNA molecules that exist independently of the chromosomal DNA in bacteria, can facilitate the spread of resistance. While it is known that plasmids can enhance survival under selective pressures, strains are able to survive without plasmids and being more vulnerable to compounds, such as antibiotics. One hypothesis is that genomes carrying fewer plasmids might experience less frequent horizontal gene transfer of resistance genes, potentially leading to greater overall fitness compared to strains burdened with more plasmids (26). While the direct relationship between plasmid number and vulnerability requires further investigation, this idea has been proposed in other contexts. Alternatively, it is possible that while these genomes initially harbor a limited number of plasmids, when exposed to antibiotics, these genomes could temporarily acquire additional plasmids through horizontal gene transfer, thereby gaining short-term resistance and survival capability (27, 28). After the antibiotic has been cleared from the body, the genomes may shed these surplus plasmids, possibly returning to a vulnerable state while enhancing their adaptability by reducing metabolic burden and increasing mutation rates (29, 30). This suggests a dynamic relationship where plasmids aid survival temporarily, but their absence may foster greater adaptability overall. The reduced genomic load may allow for increased mutagenesis, enabling faster evolution in response to environmental pressures. Although studies directly linking plasmid loss to enhanced adaptability are limited, previous research has shown that certain bacteria, such as *Escherichia coli*, exhibit increased genetic diversity following the loss of plasmids, which could suggest a potential mechanism for increased adaptability in fluctuating environments (29). For example, research in *E. coli,* showed that the loss of plasmids could enhance the bacterium's adaptability and genetic diversity, allowing for more rapid evolution in response to changing conditions (31). Further studies are needed to explore the full implications of plasmid loss on bacterial adaptability and mutagenesis. It is worth noting that antibiotic resistance genes can reside not only on plasmids but also within the bacterial chromosome, ensuring their retention even after antibiotics have been eliminated from the system. For example, in *S. aureus* TW20 (CP015447), *blaZ* is typically plasmid-borne, while *mecA* is chromosomal (31). Some *S. aureus* strains maintain resistance genes without ever encountering antibiotics, as these genes may confer survival advantages, such as stress resistance, in non-antibiotic environments (32). This is supported by studies indicating that certain bacteria possess resistance genes that provide survival benefits in environments lacking antibiotics (33). Further research is needed to understand these strains and how they survive.

Another factor that may contribute to the resistance of the bacteria could be the source from which the strain was isolated. There was a strain in our sample that was isolated from a nasal swab (CP015447, *S. aureus* TW20) and one was isolated from bone (LR822061, *S. aureus*). *S. aureus* TW20 exhibits multidrug resistance, including *blaZ*, *mecA*, and *ermA*, likely due to frequent antibiotic exposure in the nasal microbiome, while the bone isolate shows methicillin resistance, likely via *mecA*, reflecting lower antibiotic exposure (34, 35). Bacteria

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from a nasal swab might be more resistant to antibiotics than those from bone due to the nasal cavity's open environment, which is rich in microorganisms and connected to the oral cavity, potentially allowing crossover of resistance traits from oral antibiotic exposure. Because bacteria isolated from a nasal swab originate in an environment that is typically non-sterile and linked to the oral cavity – where antibiotics are frequently administered – this setting provides numerous opportunities for bacteria to encounter resistance traits, either directly or through exposure to other resistant microorganisms (34, 35). In contrast, exposure to antibiotics does not happen often in the bone, reducing the possibility of resistance should bacteria make their way there.

Our study, however, does have some limitations. We only tested 51 different strains of MRSA out of tens of thousands that are present in the NCBI database, which is a small sample size if we want to apply the results to the global population (36). If we had tested more, our results could be more accurate. This also means that future researchers should approach our results with caution – although there is much diversity in terms of isolation source and geographical location, genetic lineages or resistance mechanisms may be underrepresented.

The collection of genomes we investigated in this study is important because it can be used to compare and analyze the different MRSA genomes, which can help us understand the evolving history of resistance and genetic diversity of the various strains. The reason that we selected these genomes is because we were looking for MRSA strains and wanted to have some diversity between the strains, meaning they would not be resistant to the same antibiotics. By selecting the first 51 human-associated *S. aureus* genomes in the NCBI database, we ensured some genetic diversity among our strains, though this was not truly random. In addition, the samples were taken mainly from patients in order to be more accurate in terms of effects on the human body (**Figure 2**). Scientists could take this information and apply it to create an effective multi-drug therapy regimen for patients. This data could help doctors to select from existing treatment options, without creating new treatment methods, because if they are able to identify the strain of MRSA in a patient, they may be able to know how to treat them accordingly with the proper antibiotics that will have a positive effect on eradicating the disease from their body.

MATERIALS AND METHODS

Data collection

Both the genomes and the genes to be tested with the software BLAST were downloaded from the NCBI database. BLAST was used to search for *S. aureus* genomes, and the first 51 assemblies, sorted by NCBI's default order of human-associated isolates, were downloaded to ensure clinical relevance and diversity. The accession number, strain, host, collection date, isolation source, and geographical location were noted. FASTA files for each genome were uploaded to PathogenWatch (version 21.2.0, PathogenWatch Team, 2021). The six most common resistance genes, *mecA, blaZ, ermA, tetM, aaca-aphD*, and *aphA-3*, were selected for further analysis. Sequences for these genes were obtained from GenBank (version 257.0, Sayers et al., 2022), EMBL (version 130.0; EMBL-EBI, 2021), and DDJB (version 130.0, DDBJ Center, 2021) and compared (37). Although our dataset includes genomes from 13 different countries, we did not include samples from Africa due to limited availability of publicly accessible *S. aureus* genomes from the region in available genomic databases.

Gene presence and absence analysis

We took the sequence of each gene - *mecA, ermA, aacA-aphD, aphA-3, blaZ,* and *tetM* - and we ran our set of genomes through BLAST with each of those genes. The protein IDs of the genes that we searched with BLAST are QTW05967.1 for *mecA*, AEW64313.1 for *aphA-3*, AJE63499.1 for *aacA-aphD*, QKF95755*.1* for *blaZ*, ADL64887.1 for *ermA*, and AAA26678.1 for *tetM*. These sequences were from various strains and they were picked by searching in the NCBI database for genomes that contained this specific gene. We used the program BLASTn in the NCBI database (version 2.14.1). We blasted three genomes at once to ensure the BLASTn program was not overburdened and could run smoothly.

Nucleotide and amino acid diversity

Our final step was to check and see if there were any SNPs in the genes or any variants in the genes present in the genomes. We aligned each genome to reference sequences using BLAST and closely examined the aligned regions for differences at the nucleotide level. We focused on finding any SNPs or amino acid changes that could be associated with antibiotic resistance or other traits, which could influence the function of the corresponding proteins. Variants were compared across the different genomes to assess their diversity and potential impact on antibiotic resistance or other functional traits.

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