environmental microbiology reports

Environmental Microbiology Reports (2022) **14**(3), 391–399 doi:10.1111/1758-2229.12995

Brief Report

Genotypes and phenotypes of methicillin-resistant staphylococci isolated from shrimp aquaculture farms

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Summary

The population of methicillin-resistant (MR) staphylococci in aquatic environment is rarely investigated. Here, we characterized a collection of MR staphylococci recovered from shrimp aquaculture farms ($n = 37$) in Kerala, India. A total of 261 samples yielded 47 MR isolates (16 *S. aureus*, 13 *S. haemolyticus*, 11 *S. epidermidis*, 3 *S. saprophytics* and 2 each of *S.intermedius* and *S. kloosii*). Multi-drug resistance was evident in 72.3% of the isolates, with resistance mainly towards erythromycin (78.7%), norfloxacin and trimethoprim-sulfamethoxazole (53.2%), and gentamicin (34%). Major resistance genes identified included *mecA* (100%), *ermC* (38.3%), *aacA-aphD* (21.3%), *tetK* (14.9%) and *tetM* (21.3%). Almost 60% of the isolates carried type V SCC*mec* (Staphylococcal Cassette Chromosome *mec*), and the remaining harboured untypeable SCC*mec* elements. Comprehensive genotyping of the methicillin-resistant *Staphylococcus aureus* isolates revealed high prevalence of ST772-t345-V (sequence type-*spa* type-SCC*mec* type) (75%), followed by minor representations of ST6657-t345-V and ST3190-t12353. The isolates of *S. haemolyticus* and *S. epidermidis* were genotypically diverse as shown by their pulsed-field gel electrophoresis (PFGE) profiles. Genes encoding staphylococcal enterotoxins were observed in 53.2% of the isolates. Various genes involved in adhesion and biofilm formation were also identified. In conclusion, our findings provide evidence that shrimp aquaculture settings can act as reservoirs of methicillin-resistant staphylococci.

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Introduction

The public health importance of methicillin-resistant *Staphylococcus aureus* (MRSA) cannot be overemphasized considering the significant morbidity and healthcare costs associated with this pathogen. MRSA is implicated in a plethora of illnesses, ranging from mild skin and soft tissue infections to life-threatening diseases such as endocarditis, sepsis and osteomyelitis. The genetic basis of methicillin resistance is the chromosomally located *mecA* or *mecC* gene harboured on a mobile genetic element, namely Staphylococcal Cassette Chromosome *mec* (SCC*mec*) (Lee *et al*., 2018). Recently, a plasmid-borne *mec* gene designated as *mecB* has also been described in clinical MRSA (Becker *et al*., 2018). Once confined largely to hospitals (healthcare-associated, HA-MRSA), MRSA later made its presence in community settings, infecting young and otherwise healthy people with no prior healthcare contacts. This version of MRSA, known as the community-associated (CA) MRSA, is generally less resistant to antibiotics, carries smaller SCC*mec* elements IV or V and produces the characteristic toxin Panton-Valentine leukocidin (PVL). While HA-MRSA exhibits less heterogeneity, with only a handful of clones accounting for the majority of infections globally, CA-MRSA clones are highly diverse in nature. The major lineages of CA-MRSA prevailing in Asia-Pacific region are the sequence types ST30, ST59, ST72, ST22 and ST772 (Chen and Huang, 2014). However, the epidemiology of CA-MRSA has changed considerably in the last decade, with many clones infiltrating hospitals and causing healthcare-associated infections.

Besides MRSA, methicillin-resistant coagulase-negative staphylococci (MR-CoNS) are also increasingly being recognized as important nosocomial pathogens, with

S. epidermidis and *S. haemolyticus* being the major players. Although less aggressive compared to *S. aureus*, CoNS are frequently implicated in biofilm-associated infections on indwelling medical devices and associated complications such as bacteremia (Zheng *et al*., 2018). Once believed to be innocuous commensals, CoNS are now known as important reservoirs of resistance genes not only to the beta-lactam drugs but also to other classes of antibiotics (Fišarová *et al*., 2019).

Aquatic environment is generally considered an ideal setting for acquisition and dissemination of antibiotic resistance genes (ARGs) (Zhao *et al*., 2020). This is particularly true in the case of aquaculture settings where diverse microbial populations co-exist in an environment which often has an abundance of nutrients, probiotics, prebiotics, other supplements and more importantly antibiotics. Although the concentration of antibiotics in such settings might be too low to be lethal to any natural bacterial flora in water, it will be sufficient enough to select for resistance (Karkman *et al*., 2018). Besides antibiotics, heavy metals (such as cadmium, silver, copper etc.), disinfectants and biocides used in shrimp farming have also been shown to select antibiotic resistance genes (Seiler and Berendonk, 2012; Wales and Davies, 2015). Concerning the co-selection of methicillin resistance, studies have shown that SCC*mec* elements can be selected by the use of certain heavy metals and metalloids in addition to beta-lactam drugs (Argudín and Butaye 2016; van Alen *et al*., 2018). More recently, it has been shown that *mecA* can be selected by diverse compounds, including various antibiotics, non-antibiotic drugs, natural and synthetic compounds, suggesting a greater role for complex natural environments in the selection process of this gene (Snitser *et al*., 2020).

MRSA has been isolated from farm-cultured fishes and shrimps, raising food safety concerns (Arfatahery *et al*., 2016; Murugadas *et al*., 2016; Sivaraman *et al*., 2021). India is one of the leading producers of aquaculture; however, there were incidences of rejection of Indian shrimps by EU owing to the presence of prohibited antibiotics, raising concerns on the usage of antibiotics in shrimp farms in India (Thornber *et al*., 2020). Aquaculture, being a fast-expanding food production sector with rampant use of antibiotics in it, has serious implications for antimicrobial resistance (AMR) developing and spreading locally and even globally through trade. Despite all these, not much is known about the prevalence and characteristics of major human pathogens like methicillin-resistant staphylococci from aquaculture environments. In the present study, we sought to describe the prevalence, antibiotic resistance, virulence and clonal diversity of methicillin-resistant staphylococci isolated from selected shrimp aquaculture farms in the southern state of Kerala, India.

Results and discussion

Bacterial isolates and phenotypic resistance

The isolates of methicillin-resistant staphylococci ($n = 47$) analysed in this study were recovered from samples of shrimp (77), water (92) and sediment (92) collected from 37 aquaculture farms (See Supporting Information for details of sample collection and processing). BD Phoenix™ M50 automated system was employed for bacterial identification and susceptibility testing. Isolates comprised *S. aureus* ($n = 16$), *S. haemolyticus* ($n = 13$), *S. epidermidis* ($n = 11$), *S. saprophyticus* ($n = 3$), *S. intermedius* ($n = 2$) and *S. kloosii* ($n = 2$). Among the species recovered, *S. aureus* and *S. intermedius* are coagulase-positive, whereas the remaining are all coagulase-negative staphylococci. At the farm level, at least one sample from 13 farms yielded methicillin-resistant staphylococcal isolate. A low frequency of isolation was observed, with 24.7% ($n = 19$), 14.1% ($n = 13$) and 16.3% ($n = 15$) of shrimp, water and sediment samples, respectively, yielding methicillin-resistant staphylococcal isolates. Concerning the incidence of MRSA, 16 samples (five shrimps, six water and five sediment samples) collected from eight different farms tested positive. Detection of MRSA from environmental reservoirs such as aquatic settings is rare compared to the isolation of drug-resistant Gram-negative bacteria (Galler *et al*., 2018). However, MRSA and coagulase-negative staphylococci have been shown to survive in surface water (Sood *et al*., 2014; Čuvalová *et al*., 2015; Silva et al., 2020).

Among our MRSA isolates, multidrug resistance phenotype (MDR, resistance to ≥3 drugs of different classes) was observed in 68.8%, with resistance mainly towards erythromycin (13, 81.3%), norfloxacin (11, 68.8%), trimethoprim–sulfamethoxazole (10, 62.5%) and gentamicin (9, 56.3%). In regard to the non-aureus isolates, MDR was evident in 74.2% with the highest resistance observed for erythromycin (24, 77.4%) followed by trimethoprim–sulfamethoxazole (15, 48.4%), norfloxacin and tetracycline (14, 45.2%), gentamicin (7, 22.6%) and clindamycin (2, 6.5%). This is not surprising as multi-drug resistance is increasingly becoming a typical feature of methicillin-resistant isolates recovered from various settings including the aquatic and fisheries environment (Arfatahery *et al*., 2016; Ramessar and Olaniran, 2019; Fri *et al*., 2020). Incidence of MRSA in ready-to-eat shrimps has been reported previously, suggesting an important public health risk (Beshiru *et al*., 2021). As most of the farm-raised shrimps are intended for export, improved hygiene practises throughout the supply chain starting from the farms is crucial in preventing the dissemination of bugs like MRSA. On a positive note, all our

isolates were sensitive to the following drugs: daptomycin, levofloxacin, linezolid, moxifloxacin, nitrofurantoin, rifampicin, teicoplanin and vancomycin.

Detection of mecA *and SCC*mec *typing*

All methicillin-resistant isolates carried *mecA* gene, as assessed by PCR. Further, by employing the multiplex PCR-based SCC*mec* typing proposed by Kondo *et al*. (2007), 28 (59.6%) isolates were found to harbour type V SCC*mec* element (*ccrC* complex + class C2 *mec* complex) (Fig. 1). This included 13 *S. aureus*, 11 *S. haemolyticus*, 2 *S. epidermidis*, and 1 each of *S. intermedius* and *S. kloosii*. SCC*mec* V is often considered a feature of community-associated MRSA and is frequently associated with lineages such as ST5, ST672, and ST772 (Monecke *et al*., 2011). A specific SCC*mec* type could not be assigned for the remaining isolates ($n = 19$) as either they possessed an untypeable *mec* gene complex ($n = 10$); or had untypeable *ccr* complex ($n = 5$); or had untypeable *mec* complex as well as *ccr* complex ($n = 5$); or had multiple *ccr* complexes ($n = 9$) (Fig. 1, Table 1). Among our CoNS isolates, 51.7% carried untypeable SCC*mec* elements. It is known from previous studies that heterogeneity in SCC*mec* elements is more frequently encountered in coagulase-negative staphylococci than in MRSA, probably due to high degree of recombination events in these species (Zhang *et al*., 2009). SCC*mec* typing is the fundamental and the most widely employed typing method for methicillin-resistant isolates. This typing scheme looks at variations in the two essential parts of the SCC*mec* element – the *mec* and the *ccr* gene complexes – and gives useful information on the probable origin of the isolate, i.e., healthcare, community or livestock. Moreover, it is imperative from an epidemiological point of view to determine the SCC*mec* element carried by an MRSA isolate as two MRSA strains harbouring different SCC*mec* elements are considered different even if they belong to the same sequence type (ST) or pulsotype as determined by MLST and PFGE respectively.

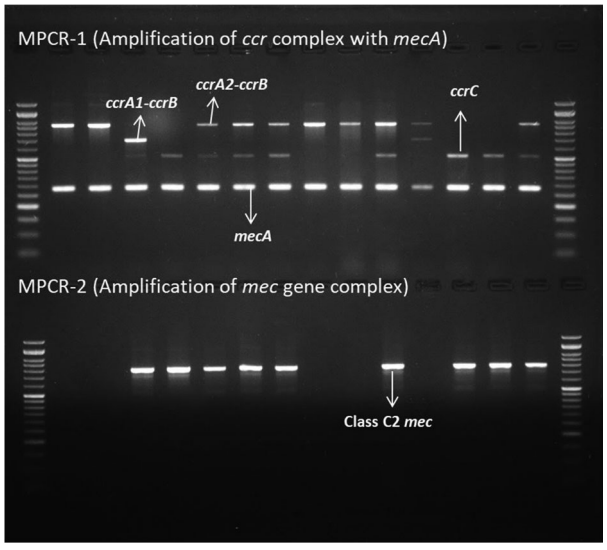

Fig. 1. Gel image showing different amplification patterns obtained from SCC*mec* typing PCRs. MPCR-1 detects the type of *ccr* complex (*ccrC*, 518 bp; *ccrA1-ccrB*, 695 bp; *ccrA2-ccrB*, 937 bp) present in the isolate; amplification of *mecA* (286 bp) is served as the internal control. MPCR-2 is employed for the identification of the type of *mec* gene complex (class C2, 804 bp); blank lanes indicate non-typeable *mec* gene complex. The DNA ladder used here has 17 fragments ranging from 50 bp to 1500 bp.

Prevalence of other resistance genes

Besides *mecA*, other resistance genes were also detected among the isolates: *ermC* (18, 38.3%), *aacA-aphD* (10, 21.3%), *tetK* (7, 14.9%) and *tetM* (10, 21.3%) (Supplementary Table S1). All the four resistance genes were found in isolates of *S. haemolyticus*, whereas, in the case of *S. aureus*, all these genes except *tetK* were detected. With regard to the isolates of other species, the following distribution of resistance genes was observed: *S. epidermidis* (*ermC*, *tetM*, *tetK*), *S. saprophyticus* (*tetM*, *tetK*), *S. kloosii* (*ermC*). All these genes have known to be associated with plasmids or transposons found in both MRSA and MSSA (Turner *et al*., 2019). The *ermC* gene, which confers the MLS_B (macrolides, lincosamides and streptogramin B) resistance phenotype, is the most predominant *erm* gene in staphylococci isolated from humans and animals and is often located on small plasmids (Feßler *et al*., 2018). MRSA or MR-CoNS isolates carrying the gentamicin resistance gene *aacA-aphD* and the tetracycline genes tetK/M are reported from various sources including livestock and ready-to-eat foods (Zehra *et al*., 2017; Wang *et al*., 2019). In our study, co-occurrence of *tetK* and *tetM* was observed in *S. haemolyticus*, *S. epidermidis* and *S. saprophyticus*, whereas only *tetM* was detected in the single tetracycline-resistant isolate of *S. aureus*. It has been shown that most *tetM*-positive isolates tend to also carry *tetK* gene and MRSA isolates usually exhibit a *tetM* or *tetKM* genotype (Trzcinski *et al*., 2000). With regard to aquaculture sources, diverse tetracycline resistance genes have been described in both Gram-negative and Gram-positive isolates including staphylococcal species (Akinbowale *et al*., 2007; Liyanage and Manage, 2019). Tetracycline is one among the widely used antibiotics in aquaculture and *tet* genes have been shown to persist for years in aquaculture farms even after antibiotic usage is discontinued (Tamminen *et al*., 2011).

Table 1. Characteristics of SCC*mec* elements present in the investigated methicillin-resistant staphylococcal isolates.

Staphylococcal species	Source (no. of isolates)	*mec* complex	*ccr* complex	SCC*mec* type
S. aureus ($n = 16$)	Shrimp (5)	C2	*ccrC*	SCC*mec* V
	water (5)			
	sediment (3)			
	sediment (2)	C2	*ccrA2-ccrB*, *ccrC*	Untypeable
	water (1)	Untypeable	Untypeable	Untypeable
S. haemolyticus ($n = 13$)	Shrimp (4)	C2	*ccrC*	SCC*mec* V
	water (3)			
	sediment (4)			
	shrimp (2)	Untypeable	Untypeable	Untypeable
S. epidermidis ($n = 11$)	Shrimp (2)	C2	*ccrC*	SCC*mec* V
	water (1)	C2	*ccrA2-ccrB*, *ccrC*	Untypeable
	sediment (3)			
	Shrimp (1)	C2	*ccrA1-ccrB*, *ccrA2-ccrB*, *ccrC*	Untypeable
	Sediment (1)	A	*ccrA2-ccrB*	Untypeable
	Shrimp (2)	Untypeable	*ccrA2-ccrB*	Untypeable
	water (1)			
S. saprophyticus ($n = 3$)	Shrimp (1)	C2	*ccrA1-ccrB, ccrC*	untypeable
	shrimp (1)	Untypeable	Untypeable	Untypeable
	water (1)			
S. intermedius ($n = 2$)	Sediment (1)	C2	*ccrC*	SCC*mec* V
	Water (1)	Untypeable	*ccrA1-ccrB*	Untypeable
S. kloosii ($n = 2$)	Shrimp (1)	C2	*ccrC*	SCC*mec* V
	Sediment (1)	Untypeable	*ccrA1-ccrB, ccrA2-ccrB*	Untypeable

Molecular subtyping of the isolates

Multi-locus sequence typing (MLST) and staphylococcal protein A (*spa*) typing identified three major genotypes of MRSA: ST772-t345 (sequence type-*spa* type) (12, 75%), ST3190-t12353 (3, 18.8%) and ST6657-t345 (1, 6.3%). ST772 (allelic profile: 1-1-1-1-22-1-1) and ST6657 (1-1-1-1-22-99-1) belong to the clonal complex 1 (CC1), whereas ST3190 (1-3-1-1-1-4-13) belongs to CC8. ST772, dubbed as the 'Bengal Bay clone' is a multi-resistant community-associated lineage first described in India and Bangladesh, and later identified in many countries (Blomfeldt *et al*., 2017). According to PubMLST database, ST3190 has been reported only from China; however, further details on this are not available. ST6657 is a novel ST and a single locus variant (SLV) of ST772 with respect to *pta* allele. All ST772 and ST6657 isolates carried SCC*mec* type V, whereas the isolates of ST3190 had untypeable SCC*mec* elements. ST772 has become epidemic in India and replaced many traditional nosocomial MRSA lineages in healthcare settings (D'Souza *et al*., 2010; Shambat *et al*., 2012; Bouchiat *et al*., 2015; Dhawan *et al*., 2015). A combination of high-level drug resistance and virulence, attributed mainly to multiple mobile genetic elements (MGEs) and resistance-conferring chromosomal mutations is the hallmark of ST772 (Steinig *et al*., 2015; Baktavatchalam *et al*., 2020). In particular, resistance genes for aminoglycosides and co-trimoxazole, which are not usually found in CA-MRSA genomes, have been described in ST772 (Steinig *et al*., 2015). Resistance to these agents was conspicuous in the ST772 isolates from this study too. The predominant *spa* types reported for ST772 are t657 and t345 (Shambat *et al*., 2012; Bouchiat *et al*., 2015; Blomfeldt *et al*., 2017). A recent study by Murugadas *et al*. (2017) which analysed clones of MRSA from seafood and aquatic environment from Kerala, India reported, though in small numbers, the prevalence of ST772 and all these isolates were of the *spa* type t657.

We also analysed the *agr* (*accessory gene regulator*) types present in the MRSA isolates and found that all ST772 and ST6657 had type II *agr* (*accessory gene regulator*) element. However, the *agr* types of ST3190 isolates could not be determined as no amplification was observed with the primer set we employed to detect the known *agr* specificity groups (I–IV). Similar to our finding, many previous studies reported type II *agr* as the most common *agr* element in ST772 strains (Rajan *et al*., 2015; Steinig *et al*., 2015; Pokhrel *et al*., 2016). *Agr* is one of the best-characterized quorum-sensing systems in staphylococci and is implicated in invasiveness by upregulating secreted virulence factors and down regulating surface proteins (Singh and Ray, 2014).

While MRSA from our study showed the predominance of a single clone, isolates of *S. haemolyticus* and *S. epidermidis* exhibited heterogeneity, as evidenced by their diverse *Sma*I-PFGE profiles (Fig. 2). By applying a similarity cut-off value of 80%, isolates of *S. haemolyticus* ($n = 13$; 12 pulsotypes) and *S. epidermidis* ($n = 11$; 10 pulsotypes) were grouped into 7 and 5 clusters respectively. In the case of *S. haemolyticus*, isolates from shrimp fell into four separate clusters, whereas both water- and

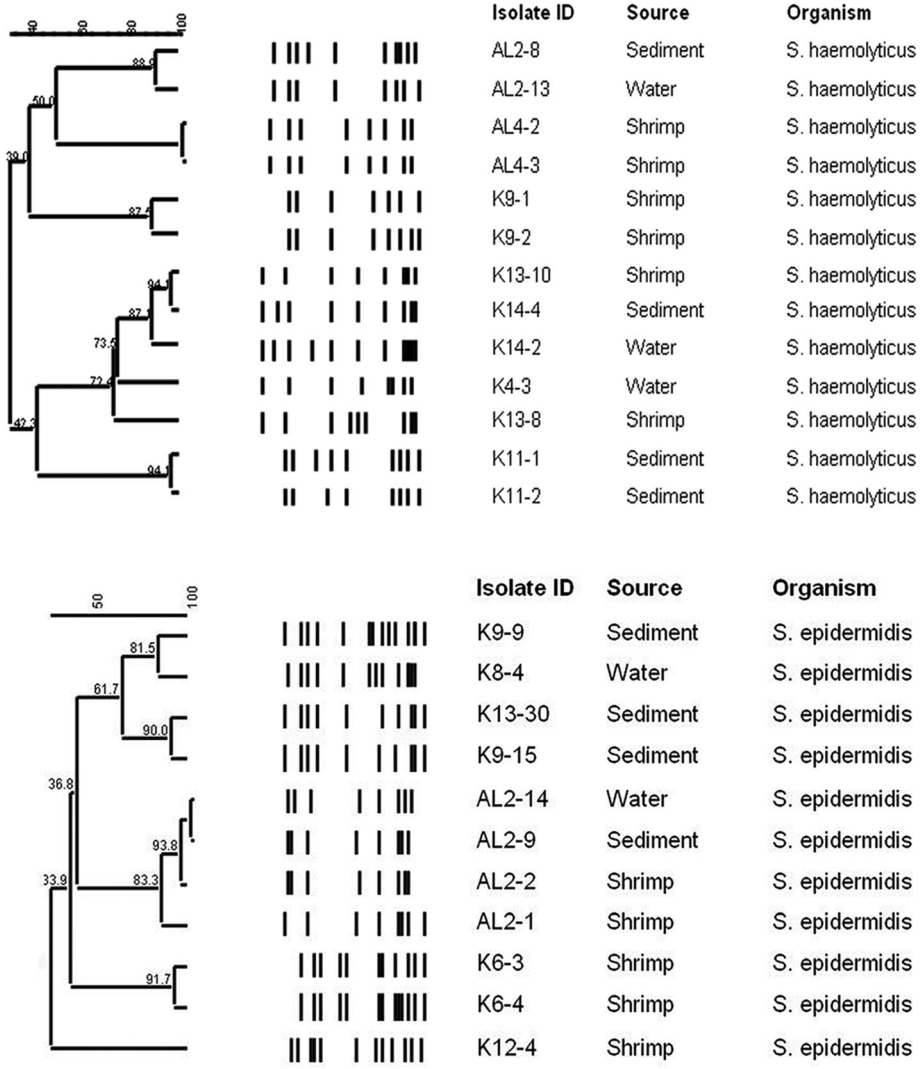

Fig. 2. Pulsed-field gel electrophoresis (PFGE) dendrograms of methicillin-resistant *S. haemolyticus* (top) and *S. epidermidis* (bottom) isolates recovered from shrimp, water and sediment samples.

sediment-derived isolates were scattered across three clusters. Isolates of *S. epidermidis* from shrimp, sediment and water were distributed in 4, 3 and 2 clusters respectively. Moreover, single clustercontaining isolates from all the three sources, i.e., shrimp, sediment and water, were observed in *S. haemolyticus* as well as in *S. epidermidis*. It is noteworthy that, in most cases, isolates recovered from the same farm were found clustered; however, exclusively in two events, one involving *S. haemolyticus* and the other involving *S. epidermidis*, same farm harboured isolates with unrelated PFGE profiles. This suggests multiple sources contaminating the farms in question. There were also instances where samples from different farms yielded isolates with closely related PFGE profiles, probably representing the genotypes, which are well adapted to this kind of environment.

Prevalence of toxin genes and biofilm-associated genes

All MRSA isolates were found to harbour the leukotoxin gene *pvl* and the majority (14, 87.5%) also had the enterotoxin genes *sea*, *seg* and *sei* (refer Supplementary Table S1). Among the non-aureus isolates, *S. haemolyticus* ($n = 2$) and *S. epidermidis* ($n = 4$) carried *sea*, *seg* and *sei*, whereas *S. saprophyticus* ($n = 3$), *S. intermedius* ($n = 1$) and *S. kloosii* ($n = 1$) harboured *seg* and *sei*. None of the isolates were positive for the tested exfoliative toxin genes (*eta* and *etb*) or the toxic shock syndrome toxin gene (*tst*). PVL is often considered a marker of community-associated lineages of MRSA and also an important virulence determinant. Among the enterotoxin genes detected here, *sea* is known to be frequently associated with staphylococcal food poisoning (Pinchuk *et al*., 2010). A recent

study from India which analysed a collection of MRSA isolates of environmental origin reported *sea* as the most prevalent staphylococcal toxin gene followed by *tst*, suggesting the environmental reservoirs of toxin genes (Bhowmik *et al*., 2021). *S. aureus* isolates harbouring various toxin and virulence genes have been recovered from retail aquatic products including shrimp (Rong *et al*., 2017; Beshiru *et al*., 2021). The enterotoxigenic potential of coagulase-negative staphylococci has been reported previously in isolates recovered from various sources including ready-to-eat food and bovine milk (Chajęcka-Wierzchowska *et al*., 2020; Helek *et al*., 2020). However, in general, the ability of CoNS to secrete classical staphylococcal enterotoxins (SEs) or toxic shock syndrome toxins (TSST) in significant amount to cause food poisoning or TSS has not been proven (Becker *et al*., 2014).

Various genes associated with adhesion and biofilm formation were identified in the isolates (detailed in Supporting Information Table S1). This mainly included genes encoding Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) and the *ica* genes which mediate the synthesis of polysaccharide intercellular adhesin (PIA), a major component of staphylococcal biofilm. All MRSA isolates ($n = 16$) carried *icaA*, *icaD*, *fib*, *fnbpA*, *clfB*, *eno* and *ebps* genes. However, varied distribution was observed for other genes such as *icaC* (8, 50%), *sdrC* (14, 87.5%) and *SdrE* (8, 50%). In the case of non-aureus isolates ($n = 31$), *clfB and eno* were present invariably, whereas *fib* and *fnbpA* (24, 77.4%), *icaAD* (8, 25.8%), *icaA* (6, 19.4%), *icaD* (2, 6.5%), *SdrC* (6, 19.4%) and *sdrE* (4, 13%) were found distributed unevenly. None of our isolates carried *icaB*, *bap*, *clfA*, *cna* or *sdrD* genes. Although MSCRAMM genes are found ubiquitously in *S. aureus*, variations in their occurrence and expression, which are mainly lineage-associated have been observed (McCarthy and Lindsay, 2010; Atshan *et al*., 2012). All the MSCRAMM genes identified in the ST772 isolates from this study have been reported previously in isolates belonging to this lineage (Pokhrel *et al*., 2016; Firoozeh *et al*., 2020); however, the *cna* gene that was identified invariably in all the ST772 isolates from those studies, has not been found in our isolates. The most common MSCRAMM genes in the non-aureus isolates from our study were *fib*, *fnbpA*, *clfB* and *eno*. It is noteworthy here that the biofilm-associated genes may not have conserved sequences across various CoNS species, and thus the primer sets employed may not detect all the gene variants (Simojoki *et al*., 2012). Due to scarcity of studies with this type of samples, there is little scope to compare our results with that of others in the literature. Nevertheless, similar to our observation, high prevalence of *eno* gene has been reported in various CoNS species including standard strains, isolates of animal origin and air-borne isolates (Seo *et al*., 2008; Simojoki *et al*., 2012).

In our study, we found *ica* genes (of the operon *icaADBC*) either singly or in different combinations: *icaAD* genotype was observed in *S. aureus* ($n = 16$), *S. haemolyticus* ($n = 2$), *S. epidermidis* ($n = 5$) and *S. saprophyticus* ($n = 1$); *icaADC* in *S. aureus* ($n = 8$); *icaA* in *S. epidermidis* ($n = 4$) and *S. kloosii* ($n = 2$); and *icaD* in *S. saprophyticus* ($n = 2$). It has been shown that *icaADC* is sufficient to direct the biosynthesis of PIA, but expression of all four genes is required for PIA to become fully functional (Gerke *et al*., 1998; Vuong *et al*., 2004). However, we have not investigated the biofilm-forming capability of these isolates to correlate the genotypes with biofilm formation. Many studies have found *icaA* and *icaD* to be similar in incidence (Pereyra *et al*., 2016; Zhang *et al*., 2018); yet, differences in frequency of these genes (which belong to the same operon) have also been reported (Gajewska and Chajęcka-Wierzchowska, 2020).

Conclusion

In conclusion, our results indicate that aquaculture farms can be reservoirs of methicillin-resistant staphylococci and aid in the dissemination of such strains. The presence of toxin and virulence genes in the isolates has important implications for food safety, if proper hygiene and storage are not ensured in the supply chain. Particularly worrisome is the high prevalence of ST772, a multi-resistant epidemic clone with a history of great epidemiological success. Moreover, the incidence of MDR strains of coagulase-negative staphylococci is a matter of concern, as evidence is mounting on such strains in environment being important donors of resistance and virulence to the more pathogenic MRSA. More surveillance studies are required in aquaculture and similar aquatic environments to monitor the emergence of drug-resistant staphylococci and other bacteria.

Acknowledgements

The authors are grateful to all the shrimp farmers for participating in this study. The authors would also like to thank Dr. Sudha and Mr. Muneeb Hamza for their technical assistance. The present study was completed as part of the DOSA Project (Diagnostics for One Health and User Driven Solutions for AMR, www.dosa-diagnostics.org). DOSA is funded by the Government of India's Department of Biotechnology, UK Research and Innovation/Economic Social Science Research Council, and the Newton Fund.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Experimental procedures

Supplementary Table S1 Resistance and virulence profile of the methicillin-resistant staphylococcal isolates recovered from shrimp aquaculture farms

Supplementary Table S2 Details of the primers and PCR conditions employed in the present study