

1 **Title: Methicillin-resistant *Staphylococcus haemolyticus* (MRSH) in fish samples**  
2 **harboring atypical staphylococcal cassette chromosome *mec* (SCC*mec*) elements.**

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31 **Abstract**

32 Incidence of *Staphylococcus haemolyticus* in fish is a sign of post-harvesting  
33 contamination. The main objective of the present study was to monitor the prevalence of  
34 methicillin-resistant *Staphylococcus haemolyticus* (MRS<sub>H</sub>) in fishes and its genotypic  
35 characterization. Fish samples (n=79) were collected from retail markets in three pre-decided  
36 sites in Assam. The collected fish samples were subjected to microbial analysis and as a result  
37 13 MRS<sub>H</sub> were isolated. Susceptibility of the isolates towards 13 classes of antibiotics was  
38 assessed by employing BD Phoenix M50 system. Polymerase chain reaction (PCR) was  
39 performed for the molecular detection of methicillin-resistance determinant. Molecular typing  
40 of the isolates was attempted by recruiting staphylococcal cassette chromosome *mec*  
41 (SCC*mec*) and pulsed-field gel electrophoresis (PFGE). The studied isolates showed varying  
42 levels of resistance to different classes of antibiotics such as cephalosporin (100%),  
43 lincosamide (30.76%), macrolides (30.76%), aminoglycoside (53.85%), quinolones (38.46%)  
44 and sulfonamides (61.54%). BD Phoenix M50 instrument recognized a few (30.76%) isolates  
45 as inducible macrolide-lincosamide-streptogramin B (iMLS<sub>B</sub>) phenotype. Remarkably, all the  
46 isolates were reported as multi-drug resistant (MDR) as they showed resistance to  $\geq 3$  classes  
47 of antibiotics. All the studied isolates tested positive for *mecA* gene and were carrying multiple  
48 SCC*mec* elements. PFGE cluster analysis grouped the isolates into two major clusters and  
49 seven individual lineages. In short, the current study documented the incidence of multi-drug  
50 resistant, multiple SCC*mec* elements carrying *S. haemolyticus* in fish samples. The present  
51 study underpinned the significance of enhanced surveillance of MRS<sub>H</sub> and also the role of the  
52 hygiene to mitigate the AMR in fisheries.

53 **Key words:** multi-drug resistance, PFGE, SCC*mec* elements, methicillin-resistant  
54 *Staphylococcus haemolyticus*, Northeast India.

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## 66 Introduction

67 *Staphylococcus haemolyticus* is a coagulase-negative bacterium found commonly in  
68 anterior nares, ear canals, inguinal areas, respiratory and gastrointestinal mucosal membranes  
69 of humans as well as animals (Piette and Verschraegen 2009). Among coagulase-negative  
70 staphylococci (CoNS), *S. haemolyticus* is only second to *S. epidermidis* in causing bloodstream  
71 infections (Barros et al., 2012). Generally, CoNS is considered as less virulent or sometimes  
72 non-virulent since it lacks coagulase, an enzyme-like protein which determines the invasive  
73 pathogenic potential of staphylococci (Prasad et al., 2012). Nevertheless, many studies  
74 documented the emergence of *S. haemolyticus* as an opportunistic pathogen which can cause  
75 severe infections in immunocompromised, immunosuppressed patients and those with  
76 underlying diseases (Soumya et al., 2017). Additionally, the potential of *S. haemolyticus* to  
77 develop resistance against conventional drugs is also a growing concern. Antimicrobial  
78 resistance (AMR), the ability of bacteria to encompass resistance against drugs, is one of the  
79 hot topics of recent times and has gained momentum as annual mortality rates have  
80 surprisingly elevated to unprecedented levels (Hendriksen et al., 2019). It is estimated that the  
81 mortality attributed to AMR may hit 10 Million by 2050 if the scenario is poorly addressed  
82 (Cassini et al., 2019). Methicillin-resistance in staphylococci is greatly conferred by the  
83 acquisition of *mecA* gene which is carried on a mobile genetic element, namely Staphylococcal  
84 Cassette Chromosome *mec* (*SCCmec*) (Sekizuka et al., 2020). However, in addition to the  
85 *mecA*, a homologue namely *mecA<sub>LGA251</sub>* has also been reported (Stegger et al., 2012). *S.*  
86 *haemolyticus* is the best example of a pathogen that has evolved spectacularly to acquire  
87 multi-drug resistance owing to the extreme plasticity of its genome (Chiew et al., 2007).  
88 *SCCmec* elements, owing to their high transmissibility, contribute greatly to the dissemination  
89 of methicillin resistance among susceptible staphylococci by horizontal gene transfer  
90 (Chongtrakool et al., 2006). Delineation of closely related bacterial isolates is essential for  
91 epidemiological surveillance. Pulsed-field gel electrophoresis (PFGE), a gold standard used  
92 predominantly in molecular typing is a reliable method for the delineation of staphylococci.  
93 PFGE is essentially employed when there is a disease outbreak and it enables source tracking  
94 among outbreak isolates (Bannerman et al., 1995). PFGE also helps to explicate the genetic  
95 diversities among isolates (Ruiz et al., 2008).

96 In Assam, fishery plays very crucial role as it provides livelihood for billions of people  
97 and to pursue their daily requirements. Considering the fact that more than 2% of gross state  
98 domestic product (GSDP) to the state economy is contributed by the fishery, significance of  
99 the sector particularly in Assam is well understood. In fact, the peculiar sub-tropical climate  
100 and vast piscine diversity makes the state congenial for establishing the freshwater fish  
101 cultures (Gogoi et al., 2015). It was noted with surprise that the 95% of total population in  
102 Assam are fish eaters and that underlined the high demand of fishes in markets. Hence, it has  
103 been forced to increase the fishery production and as a result the state has secured sixth  
104 position in the country as inland fish producers. As far as the aquatic resources of Assam as a

105 source of food is concerned, the piscine diversity is commendable since out of 216 species of  
106 fishes reported till now, 210 have nutritive value (Gogoi et al., 2015). On the other hand,  
107 scarcity of people's involvement, unsustainable utilization of aquatic resources and  
108 inappropriate farming system approaches (FSA) pose challenges to the growing fishery sector  
109 in Assam. Unscientific farming methods can provide ideal environments for bacterial  
110 contamination.

111 MRS in context of nosocomial as well as community-associated infections has been  
112 discussed widely in the literature. Studies are outnumbered that indicated the prevalence of  
113 staphylococcus in hospitals and health-care sectors (Becker et al., 2014). Similarly, many  
114 articles documented the prevalence of MRSH in companion animals and its dissemination to  
115 humans (Ruzauskas et al., 2014). Reports are not less, when comes to its prevalence in  
116 environmental samples (Dziri et al., 2016). However, all these studies are reflecting the  
117 incidence of CoNS in different sectors and potentiality of being reservoir for the intra- and  
118 inter-sectoral dissemination of the bacteria. On the contrary, only a few studies have  
119 demonstrated the incidence of methicillin-resistant staphylococci other than *S. aureus*,  
120 particularly in environmental samples (Pokhrel et al., 2018). Thus, considering these facts, it  
121 appeared rational to monitor the prevalence of MRS in fish samples from natural water bodies  
122 and local markets. In this study, we investigated the incidence of MRSH and its molecular  
123 characterization in the selected sites in Assam, India.

## 124 **Materials and methods**

### 125 **Sampling strategy**

126 Sampling was performed in August, 2019 at three distinct locations in Assam (Northeast  
127 India) viz., Silagrang (Site 1, 26.176570° N, 91.689732° E), Garchuk (Site 2, 26.104791° N,  
128 91.712418° E) and North Guwahati Township Committee (Site 3, 26.193736° N, 91.721780°  
129 E). All the three sites were at a distance of less than 26 km radius from the center of the city  
130 (Fig 1).

131 A total of 79 samples comprising diverse species of fish fauna, are collected from retail  
132 markets and natural water bodies in these sites. Of 79 fish samples, 17 were collected from  
133 site 1, 21 from site 2 and remaining 41 from site 3. Samples were preserved in a sterile  
134 polythene bag on ice and transported to the laboratory in chilled condition.

### 135 **Sample processing and bacterial isolation**

136 Samples were initially processed at Quality Control lab, ICAR- National Research Centre  
137 on Pig (ICAR-NRCP), Rani, Assam, India. Microbial identification and further analyses were  
138 performed at Microbiology Fermentation and Biotechnology Division of ICAR-Central Institute  
139 of Fisheries Technology (ICAR-CIFT), Cochin, Kerala, India.

140 Standard protocol of United States Food and Drugs Administration (USFDA) were  
141 followed to process fish samples for the isolation of Staphylococci with slight modifications

142 (Bennet and Lancet 2001). Briefly, 25 grams of each sample were aseptically transferred to  
143 225 mL Trypticase soy broth (TSB) (BD BBL and Difco, USA) supplemented with 10% (w/v)  
144 sodium chloride and 1% (w/v) sodium pyruvate and incubated at 37°C for overnight. Enriched  
145 cultures were then serially diluted and 200 µL inoculated on mannitol salt agar (MSA) (BD BBL  
146 and Difco, USA) by spread plate method followed by incubation at 37°C for 48 hours. Mannitol  
147 non-fermenting colonies (pink color) with colony characteristics of CoNS were picked to screen  
148 the methicillin resistance as stated by The Clinical and Laboratory Standards Institute (CLSI)  
149 guidelines (CLSI 2020). Presumptive CoNS (n=5) colonies from each MSA plate were  
150 inoculated separately to Brain Heart Infusion broth (BHI) (BD BBL and Difco, USA) to prepare  
151 the samples for spot test. Each sample (10 µL) were spot inoculated on Muller-Hinton agar  
152 (MHA) (BD BBL and Difco, USA) supplemented with 4% (w/v) sodium chloride and Oxacillin  
153 (6µg/mL) (Sigma-Aldrich, USA) followed by overnight incubation at 35°C. Those isolates with  
154 reduced susceptibility to oxacillin and grown on MHA+ Oxacillin plate were discerned to carry  
155 methicillin resistance determinant and considered for further studies.

### 156 **Biochemical characterization and Antimicrobial Susceptibility Test (AST)**

157 BD Phoenix™ M50 system (BD Diagnostic Systems, Sparks, MD) was employed for the  
158 bacterial identification and to study AST profile of the selected isolates according to the  
159 protocol described elsewhere (Hong et al., 2019). Briefly, Bacterial colonies were inoculated  
160 into the bacterial identification broth (ID broth) at a concentration of 0.5 McFarland. Twenty-  
161 five microliters of the adjusted ID broth were transferred into the AST broth (BD Diagnostic  
162 Systems) with the AST indicator (methylene blue and resazurin). The prepared suspension was  
163 poured through the fill port on both sides of the BD Phoenix™ PMIC/ID combo panel (BD  
164 Diagnostic Systems) and is sealed, then loaded to the instrument. Test results were analyzed  
165 using EpiCentre™ software. BDXpert infers the resistance level based on minimum inhibitory  
166 concentration (MIC) mentioned in CLSI guidelines. Quality controls were performed according  
167 to the manufacturer's recommendations using reference isolate, *S. aureus* ATCC 25923.

### 168 **Molecular detection of methicillin resistant determinant: *mecA* gene**

169 All amplifications were performed on supernatants from DNA lysate. To prepare the  
170 lysate, 200 µL of bacterial suspension was initially heated at 94°C in AccuBlock™ digital dry  
171 bath (Labnet international, USA) for exactly 10 minutes followed by immediate cooling at -  
172 80°C for at least one hour. Samples after incubation were centrifuged for 5 minutes at 8500  
173 rpm and the supernatant was used as DNA template. PCR was performed in a 50 µL reaction  
174 volume with Red Taq®Ready Mix™PCR reaction mix (Sigma-Aldrich, USA), 800 nM  
175 concentrations of *mecP4* and *mecP7* primers (Table 1) and 5 µL of DNA lysate. PCR  
176 amplifications were performed in Veriti™ 96-well Thermal cycler (Applied Biosystems) with the  
177 PCR conditions mentioned in table 1. PCR products (10 µL) were resolved on a 2% agarose  
178 (Sigma-Aldrich, USA) gel in 1X Tris-Acetate-EDTA (TAE) buffer (Bio-Rad, Hercules, Calif) to  
179 which added EtBr (0.5 µg/mL) to visualize the amplified product.

## 180 **Molecular typing of MRSH**

### 181 **SCCmec typing**

182 Multiplex PCR was employed for SCCmec typing (Rajan et al., 2015). Amplification was  
183 performed using Red Taq® Ready Mix™ PCR reaction mix (Sigma-Aldrich, USA). Primers used  
184 in this study are listed in Table 1. Amplifications were performed with the PCR condition  
185 mentioned in table 1.

### 186 **Pulsed-Field Gel Electrophoresis (PFGE)**

187 All the 13 isolates used in the current study were genotyped by *SmaI*-pulsed field gel  
188 electrophoresis. Briefly, genomic DNA was prepared in agarose plugs as described previously  
189 (Rajan et al., 2015) and were subjected to restriction digestion by 40 U of *SmaI* (New England  
190 Biolab, UK) for 18 h at 25°C. To ensure successful digestion, additional 20 U of *SmaI* (NEB,  
191 UK) was added and incubated for 4 h at 25°C whereas marker strain was restriction digested  
192 with *XbaI* (NEB, UK). PFGE was carried out in contour-clamped homogenous electric field  
193 (CHEF) system (Bio-Rad, USA) with following parameter; Temperature at 14°C, initial switch  
194 time for 5 seconds, final switch time for 40 seconds and for a duration of 21 hours. The gel  
195 was stained with ethidium bromide (1 µg/ml) for 20 min and documented with UV imaging  
196 system. *Salmonella* serotype strain H9812 of known banding pattern was used as the marker  
197 strain. The total DNA was digested with *SmaI* restriction enzyme (New England Biolabs,  
198 Beverly, Mass.) into several fragments separated on agarose gel. Cluster analysis was  
199 performed using BioNumerics 7.6.1 software with a similarity co-efficient optimization of 0.5%  
200 and band matching tolerance of 1%. The dendrogram was constructed based on un-weighted  
201 pair group method with arithmetic mean (UPGMA) algorithm. Genetic diversity among MRSH  
202 isolates were assessed based on dice similarity index.

### 203 **Statistical analysis**

204 Data were statistically analyzed using one-way Analysis of Variance (ANOVA) followed  
205 by Duncan's test for testing the significance of difference.

## 206 **Results**

### 207 **Demography of fish population in Assam**

208 It is evident from many literatures that the Assam bears a spectacular piscine diversity.  
209 Basically, as far as sources were concerned; the samples were classified into three such  
210 categories as river-caught, cultured fishes and imported varieties. Certain fish varieties such as  
211 singara (*Mystus tengara*), singhi (*Heteropneustes fossilis*) and puthi (*Puntius* spp.) are  
212 indigenous to Assam and represents typical examples of river-caught fish varieties.  
213 Aquaculture settings are well established in villages such as Nagaon and Hajo. The fish  
214 varieties such as *Pangasius*, *Labeo rohitha* and *L. catla* were imported from different states. In  
215 our study, the fish samples collected from natural water bodies were largely endemic to

216 Northeast India, while the majority of those collected from retail markets were imported from  
217 different states of the country.

### 218 **Bacterial identification and resistance pattern**

219 Thirty non-duplicate MRS were recovered from 79 tested fish samples and 13 (43.33%)  
220 were identified as MRS<sup>H</sup>. At the site level, prevalence of MRS<sup>H</sup> (12/13, 92.31%) was found to  
221 be very high at site 3. Only 1 fish sample collected from site 1 were found to harbor MRS<sup>H</sup>.  
222 The MICs of antibiotics to each studied isolates are represented in Table 2. BD Phoenix M50  
223 was employed to test the isolates against a panel of 13 classes comprising 21 antibiotics. All  
224 13 (100%) isolates were resistant to ampicillin, cefazolin, methicillin and penicillin, 4 (30.76%)  
225 to clindamycin, 4 (30.76%) to erythromycin, 7 (53.85%) to gentamicin, 5 (38.46%) to  
226 norfloxacin and 8 (61.54%) to trimethoprim-sulfamethoxazole (Fig 2). Additionally, 5  
227 (38.46%) isolates showed intermediate resistance to gentamicin and 1 (7.69%) to  
228 tetracycline. Based on clindamycin and erythromycin resistance, 4 (30.76%) isolates were  
229 reported as inducible macrolide-lincosamide-streptogramin B (iMLS<sub>B</sub>) phenotype. On the  
230 brighter side, neither vancomycin nor linezolid resistance was observed. Surprisingly, all 13  
231 (100%) *S. haemolyticus* isolates were reported as MDR (resistant to ≥3 classes) (Table 2).

### 232 **Determination of *mecA* gene and SCC*mec* type**

233 Isolates were subjected to PCR for the molecular detection of *mecA* gene. All the 13  
234 (100%) isolates of MRS<sup>H</sup> were tested positive of *mecA* gene. Furthermore, in contrast to  
235 previously reported SCC*mec* types, all the isolates used in the present study revealed an  
236 unusual combination of SCC*mec* cassette. Twelve (92.3%) isolates exhibited similar SCC*mec*  
237 type having the amplicons corresponding to internal control; *mecA* gene (162 bp), *kdp* gene  
238 (284 bp), *RIF5* gene (414 bp), *ccrC* gene (449 bp) and *CIF2* gene (495 bp) which indicated a  
239 possible combination of type I, type II, type III and type V. In contrast, the 13<sup>th</sup> isolate had  
240 the combination of *mecA* gene (162 bp), *RIF5* gene (414 bp) and *ccrC* (449 bp) gene,  
241 indicated a mixture of type III and type IV SCC*mec* elements.

### 242 **PFGE typing**

243 Four isolates recovered from *Macragnathus aral*, *Puntius sophore*, *Ompok bimaculatus*  
244 and *Pangasium pangasius* showed 100% similarity and grouped into a single cluster. Similarity  
245 of those recovered from *Channa punctatus* and *Hypophthalmichthys molitrix* also found to be  
246 100% and grouped into another cluster. However, these two clusters showed only 50%  
247 similarity. Major cluster had four isolates whereas the other cluster had only two. Though  
248 remaining isolates were existed as single lineages, similarity among them found to be nearly  
249 90%. The isolate recovered from site 1 showed 94.75% similarity with major cluster. The  
250 PFGE band patterns of the isolates and PFGE clustering are depicted in fig 3.

### 251 **Discussion**

252 It is not surprising to observe the occurrence of *S. haemolyticus* in humans and animals  
253 since it is a ubiquitous commensal organism of normal skin flora. Nevertheless, their  
254 prevalence in processed fishes is an indication of post-harvest bacterial contamination  
255 (Sergelidis et al., 2014). According to Sergelidis et al, the incidence of CoNS in ready to eat  
256 fishes was high mainly because of poor hygiene of fish handlers. Another study where the  
257 incidence of staphylococci in edible portion of fishes was examined before and after cleaning  
258 the gut, observed that the staphylococcal load was reduced after cleaning the gut (Sahoo et  
259 al., 2009). The above observations further emphasized the pivotal role of cleanliness to avoid  
260 post-harvest contamination. In our study, all *S. haemolyticus* were recovered from retail  
261 market fishes, possibly indicating either post-harvesting contamination or poor hygienic  
262 practices followed by fish vendors. In addition to *S. haemolyticus*, other staphylococcal species  
263 such as *S. sciuri*, *S. xylosus*, *S. gallinarum*, *S. warneri* etc. were also identified from market  
264 fishes as well as those collected from natural water bodies which are reported already as  
265 major contaminants of meat and fish products (Regecová et al., 2014).

266 Susceptibility status against oxacillin revealed that all the studied MRSH isolates  
267 consistently showed resistance to oxacillin. To support the credibility, our findings were  
268 compared with a study conducted in Japan where reported the occurrence of methicillin-  
269 resistant coagulase-negative particularly *S. haemolyticus* in retail ready to eat raw fish  
270 (Hammad et al., 2012). Historically, the first case of methicillin resistance was reported in  
271 1961 from United Kingdom, soon after the introduction of methicillin into clinical practice  
272 (Enright 2003). In contrast to wild type staphylococci, methicillin resistant isolates have a  
273 modified form of penicillin binding protein (PBP) namely PBP2a, encoded by *mecA* gene which  
274 has low affinity for beta-lactam antibiotics (Stapleton and Taylor 2002). In our study, we  
275 explicated the presence of gene that confers methicillin-resistance in all the MRSH isolates,  
276 confirming the drug resistance is mediated by *mecA* gene. Increasing prevalence of methicillin  
277 resistance necessitated the introduction of vancomycin (Kirby 1984). However, in our study  
278 neither vancomycin nor linezolid resistance was encountered. In addition to oxacillin,  
279 resistance to clindamycin (30.76%), erythromycin (30.76%), gentamicin (53.86%), norfloxacin  
280 (38.46%) and trimethoprim-sulfamethoxazole (61.54%) was also observed. The findings were  
281 in agreement with the resistance pattern of nosocomial MRSH reported elsewhere (Szcuka et  
282 al., 2016). Staphylococcal isolates, when found resistant to erythromycin, can be declared as  
283 clindamycin-resistant (Magiorakos et al., 2012). Similarly, when found resistant to oxacillin or  
284 cefoxitin, the isolates are considered to be resistant to all beta-lactam antibiotics except anti-  
285 MRSA cephalosporin. Thus, as far as the definition of MDR with respect to MRS is concerned,  
286 all isolates which are resistant to methicillin is generally treated as MDR (Magiorakos et al.,  
287 2012). It is noteworthy that all MRSH strains in the present study were characterized as MDR  
288 not only by the definition of Magiorakos et al, 2012 but also as the isolates showed remarkable  
289 resistance against more than 3 classes of antibiotics. Innate ability of *S. haemolyticus* to  
290 acquire MDR is greatly attributed to its genome plasticity. Additionally, predominance of the



291 insertion elements (IS elements) in *S. haemolyticus* are significantly contributing to the  
292 emerging drug resistance simply by activation or inactivation of resistance determinants  
293 (Takeuchi et al., 2005).

294 The present study used the primers that could group the isolates only up to six *SCCmec*  
295 types. Unusual combination of *SCCmec*, documented in our study in all MRSH isolates, was  
296 tempting to recognize the isolates as either non-typeable or the mixture of multiple *mec*  
297 elements. It is indeed reported that the *SCCmec* diversities among CoNS are remarkably high  
298 and those non-typeable isolates can act as a reservoir for *SCCmec* elements (McManus et al.,  
299 2015). Several studies, over the years substantiated our findings by reporting atypical  
300 combination of *SCCmec* elements particularly in CoNS (Otto 2013; Hannson and Sollid 2007).  
301 Besides, the new combination of *SCCmec* in MRSH may contribute to epidemiological  
302 importance of the strain as they are speculated to be the reservoir of methicillin-resistance  
303 determinants which can easily be disseminated to virulent isolates of *S. aureus*. However,  
304 whole genome sequencing (WGS) of the isolates should be recruited to achieve more insight  
305 to the structural characterization of *SCCmec* elements.

306 Bacterial typing in general, plays a crucial role in phenotypic and genotypic  
307 discrimination of isolates especially when an outbreak is reported (Neoh et al., 2019).  
308 Previously bacterial typing was performed by implementing phage typing, serotyping and  
309 analyzing susceptibility pattern. However, PFGE gained tremendous acceptance over these  
310 techniques owing to the reproducibility since the technique is DNA based, rapidity and  
311 robustness (Adzitey et al., 2013). In this study, we employed PFGE to investigate the genetic  
312 diversity among MRSH isolates. Isolates were grouped into 2 distinct clusters showing genetic  
313 relatedness among the isolates. It is noteworthy that all isolates, recovered from one particular  
314 site (site 3) has grouped to different clusters, probably indicating the prevalence of multiple  
315 clones at this area. Our observations were comparable to a previously reported study where  
316 seven *S. haemolyticus* isolates from a hospital were grouped to same cluster (Chamon et al.,  
317 2014). Importantly, comparing DNA fingerprint of PFGE with that of *SCCmec* recognized one  
318 particular isolate as an outlier since it showed different band pattern from that of other  
319 isolates in both PFGE and *SCCmec* typing. It was also notable to observe the isolates  
320 belonging to the same cluster showed different antibiotic resistance pattern. It may be  
321 attributable to phenotypic plasticity which refers to changes in the traits of an organism in  
322 response to its microenvironment. In our study, though isolates are basically from fish  
323 samples, each fish belongs to different niches and provides a different micro-environment for  
324 the bacteria. Such micro-environment can trigger certain phenotypic changes in the organism.  
325 A recent study reported elsewhere perceived the phenotypic plasticity of *S. epidermidis*  
326 isolated from three different niches (Garcia-Gutierrez et al., 2020).

## 327 **Conclusions**

328 The present study outlined the incidence of multidrug resistant *S. haemolyticus* in  
329 market fishes. The antibiogram revealed the potential resistance to penicillins, cephalosporins,  
330 lincosamides, aminoglycosides, marcolides, quinolones and sulfonamides. The unusual  
331 combination of *SCCmec* elements in the isolates contributed to the epidemiological  
332 significance. PFGE analysis documented the prevalence of multiple clones at one site. Presence  
333 of MRSH in market fish samples pointed at the hand contamination among food handlers as  
334 the major source. Thus, the current study emphasized the paramount importance of food  
335 handlers to follow strict hygienic practices. Moreover, the enhanced surveillance of antibiotic  
336 use in aquaculture settings should be ensured as the imprudent antibiotic usage may  
337 contribute to the emergence of AMR. However, the study has to be expanded in terms of  
338 number of samples and duration of sampling that covers all the major climates of the country  
339 in order to achieve more comprehension to the prevalence of AMR in fish and its dissemination  
340 to different sectors.

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##### 354 **Conflict of Interest**

355 Authors declare no conflict of interests

##### 356 **Ethics and Approval**

357 Not applicable

##### 358 **Consent for publication**

359 Consent has been obtained from all the authors regarding the submission of this  
360 manuscript.

##### 361 **Availability of data and material**

362 Not applicable.

363

### Author's contribution

364 Conceptualization and supervision: G. K Sivaraman. Fund acquisition: Bibek Shome and  
365 Mark Holmes. Jennifer Cole edited and reviewed the manuscript. K. H Muneeb and Sudha S  
366 were involved in sample collection and Microbial processing. K. H Muneeb performed the  
367 experiments and prepared first draft of the manuscript.

### 368 REFERENCES

- 369 1. Adzitey F, Huda N, Ali GRR (2013) Molecular techniques for detecting and typing of  
370 bacteria, advantages and application to foodborne pathogens isolated from ducks. 3  
371 Biotech 3:97-107. doi: 10.1007/s13205-012-0074-4
- 372 2. Bannerman TL, Hancock GA, Tenover FC, Miller JM (1995) Pulsed-field gel  
373 electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. J  
374 Clin Microbiol. 33:551-555.
- 375 3. Barros EM, Ceotto H, Bastos MCF, Dos Santos KRN, Giambiagi-deMarval M (2012)  
376 *Staphylococcus haemolyticus* as an important hospital pathogen and carrier of  
377 methicillin resistance genes. J Clin Microbiol. 50:166-168. doi: 10.1128/JCM.05563-11
- 378 4. Becker K, Heilmann C, Peters G (2014). Coagulase-negative staphylococci. Clin Microbiol  
379 Rev. 27(4): 870-926. DOI: 10.1128/CMR.00109-13
- 380 5. Bennett RW, Lancette GA (2001) *Staphylococcus aureus*. In: Merker, R. L. (Ed.) Food  
381 and Drug Administration Bacteriological Analytical Manual, 8th edn. (revision A), AOAC  
382 International, Gaithersburg, MD.
- 383 6. Cassini A, Högberg LD, Plachouras D, Quattrocchi A, Hoxha A, Simonsen GS, Colomb-  
384 Cotinat M, Kretzschmar ME, Devleeschauwer B, Cecchini M, et al (2019) Attributable  
385 deaths and disability-adjusted life-years caused by infections with antibiotic-resistant  
386 bacteria in the EU and the European Economic Area in 2015: a population-level  
387 modelling analysis. Lancet Infect Dis. 19:56-66. DOI:https://doi.org/10.1016/S1473-  
388 3099(18)30605-4
- 389 7. Chamon RC, Iorio NLP, Cavalcante FS, Teodoro CR, de Oliveira APC, Maia F, dos Santos  
390 KRN (2014) Linezolid-resistant *Staphylococcus haemolyticus* and *Staphylococcus*  
391 *hominis*: single and double mutations at the domain V of 23S rRNA among isolates from  
392 a Rio de Janeiro hospital. Diagn Micr Infec Dis. 80:307-310. doi:  
393 10.1016/j.diagmicrobio.2014.09.011
- 394 8. Chiew YF, Charles M, Johnstone MC, Thompson KM, Parnell KD, Penno EC (2007)  
395 Detection of vancomycin heteroresistant *Staphylococcus haemolyticus* and vancomycin  
396 intermediate resistant *Staphylococcus epidermidis* by means of vancomycin screening  
397 agar. Pathol. 39:375-377. https://doi.org/10.1080/00313020701330441
- 398 9. Chongtrakool P, Ito T, Ma XX, Kondo Y, Trakulsomboon S, Tiensasitorn C, Jamklang M,  
399 Chavalit T, Song JH, Hiramatsu K (2006) Staphylococcal cassette chromosome mec  
400 (SCCmec) typing of methicillin-resistant *Staphylococcus aureus* isolates isolated in 11

- 401 Asian countries: a proposal for a new nomenclature for SCCmec elements. Antimicrob  
402 Agents Chemother. 50:1001-1012. doi: 10.1128/AAC.50.3.1001-1012.2006
- 403 10. Clinical and Laboratory Standards Institute (2020) Performance standards for  
404 antimicrobial susceptibility testing-30th edition. M100. Clinical and Laboratory Standards  
405 Institute, Wayne, PA.
- 406 11. Dziri, Raoudha, Naouel Klibi, Carmen Lozano, Leila Ben Said, Ridha Bellaaj, Carmen  
407 Tenorio, Abdellatif Boudabous, Karim Ben Slama, Carmen Torres (2016). High  
408 prevalence of *Staphylococcus haemolyticus* and *Staphylococcus saprophyticus* in  
409 environmental samples of a Tunisian hospital. Diagn Microbiol Infect Dis. 85: 136-140.  
410 doi: 10.1016/j.diagmicrobio.2016.03.006.
- 411 12. Enright MC (2003). The evolution of a resistant pathogen—the case of MRSA. Curr Opin  
412 Pharmacol. 3:474-479. DOI: 10.1016/s1471-4892(03)00109-7
- 413 13. Garcia-Gutierrez E, Walsh CJ, Sayavedra L, Diaz-Calvo T, Thapa D, Ruas-Madiedo P,  
414 Mayer MJ, Cotter PD, Narbad A (2020) Genotypic and Phenotypic Characterization of  
415 Fecal *Staphylococcus epidermidis* Isolates Suggests Plasticity to Adapt to Different  
416 Human Body Sites. Front Microbiol. 11:688. <https://doi.org/10.3389/fmicb.2020.00688>
- 417 14. Gogoi B, Kachari A, Dutta R, Darshan A, Narayan D (2015) Fishery based livelihood  
418 approaches and management of fishery resources in Assam, India. Forest. 5:2-514.
- 419 15. Hammad AM, Watanabe W, Fujii T, Shimamoto T (2012) Occurrence and characteristics  
420 of methicillin-resistant and-susceptible *Staphylococcus aureus* and methicillin-resistant  
421 coagulase-negative staphylococci from Japanese retail ready-to-eat raw fish. Int J Food  
422 Microbiol. 156:286-289. DOI: 10.1016/j.ijfoodmicro.2012.03.022
- 423 16. Hendriksen RS, Munk P, Njage P, Van Bunnik B, McNally L, Lukjancenko O, Röder T,  
424 Nieuwenhuijse D, Pedersen SK, Kjeldgaard J, et al (2019) Global monitoring of  
425 antimicrobial resistance based on metagenomics analyses of urban sewage. Nat  
426 Commun. 10:1124.
- 427 17. Hong JS, Kim D, Kang DY, Park BY, Yang S, Yoon EJ, Lee H, Jeong SH (2019)  
428 Evaluation of the BD Phoenix M50 automated microbiology system for antimicrobial  
429 susceptibility testing with clinical isolates in Korea. Microb Drug Resist. 25:1142-1148.  
430 doi: 10.1089/mdr.2018.0370.
- 431 18. Kirby WM (1984) Vancomycin therapy of severe staphylococcal infections. J Antimicrob  
432 Chemother. 14:73-78. DOI: 10.1093/jac/14.suppl\_d.73
- 433 19. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S,  
434 Hindler JF, Kahlmeter G, Olsson-Liljequist B, et al (2012) Multidrug-resistant, extensively  
435 drug-resistant and pandrug-resistant bacteria: an international expert proposal for  
436 interim standard definitions for acquired resistance. Clin Microbiol Infect. 18:268-281.  
437 DOI: 10.1111/j.1469-0691.2011.03570.x
- 438 20. McManus BA, Coleman DC, Deasy EC, Brennan GI, O'Connell B, Monecke S, Ehricht R,  
439 Leggett B, Leonard N, Shore AC (2015) Comparative genotypes, staphylococcal cassette  
440 chromosome mec (SCCmec) genes and antimicrobial resistance amongst

- 441 Staphylococcus epidermidis and *Staphylococcus haemolyticus* isolates from infections in  
442 humans and companion animals. PloS one. 10. doi: 10.1371/journal.pone.0138079
- 443 21. Milheirico C, Oliveira DC, de Lencastre H (2007) Update to the multiplex PCR strategy  
444 for assignment of mec element types in Staphylococcus aureus. Antimicrob Agents  
445 Chemother. 51:3374-3377. DOI: 10.1128/AAC.00275-07
- 446 22. Neoh HM, Tan XE, Sapri HF, Tan TL (2019) Pulsed-field gel electrophoresis (PFGE): A  
447 review of the "gold standard" for bacteria typing and current alternatives. Infec Genet  
448 Evol. 74:103935. <https://doi.org/10.1016/j.meegid.2019.103935>
- 449 23. Otto M (2013) Coagulase-negative staphylococci as reservoirs of genes facilitating MRSA  
450 infection: Staphylococcal commensal species such as Staphylococcus epidermidis are  
451 being recognized as important sources of genes promoting MRSA colonization and  
452 virulence. Bioessays 35:4-11. DOI: 10.1002/bies.201200112
- 453 24. Piette A, Verschraegen G (2009) Role of coagulase-negative staphylococci in human  
454 disease. Vet Microbiol. 134:45-54. DOI: 10.1016/j.vetmic.2008.09.009
- 455 25. Pokhrel H, Krishnan R, Pillai D, Rakesh CG, Sneha KG (2018) Identification and  
456 Characterization of Methicillin Resistant Bacterial Isolates from Fish and Shellfish  
457 Collected from Cochin Backwaters, Kerala, India. Int J Curr Microbiol App Sci. 7: 916-  
458 922. DOI:10.20546/ijcmas.2018.707.110
- 459 26. Prasad S, Nayak N, Satpathy G, Nag HL, Venkatesh P, Ramakrishnan S, Ghose S, Nag  
460 TC (2012) Molecular & phenotypic characterization of Staphylococcus epidermidis in  
461 implant related infections. Indian J Med Res. 136:483.
- 462 27. Rajan V, Schoenfelder SM, Ziebuhr W, Gopal S (2015) Genotyping of community-  
463 associated methicillin resistant Staphylococcus aureus (CA-MRSA) in a tertiary care  
464 centre in Mysore, South India: ST2371-SCCmec IV emerges as the major clone. Infec  
465 Genet Evol. 34:230-235. <https://doi.org/10.1016/j.meegid.2015.05.032>
- 466 28. Regecová I, Pipová M, Jevinová P, Marušková K, Kmeť V, Popelka P (2014) Species  
467 identification and antimicrobial resistance of coagulase-negative staphylococci isolated  
468 from the meat of sea fish. J Food Sci 79:M898-902. [https://doi.org/10.1111/1750-  
469 3841.12429](https://doi.org/10.1111/1750-3841.12429)
- 470 29. Ruiz P, Izquierdo PM, Seseña S, Palop ML (2008) Intraspecific genetic diversity of lactic  
471 acid bacteria from malolactic fermentation of Cencibel wines as derived from combined  
472 analysis of RAPD-PCR and PFGE patterns. Food Microbiol. 25:942-948.  
473 <https://doi.org/10.1016/j.fm.2008.06.007>
- 474 30. Ruzauskas M, Siugzdinien, R, Klimiene I, Virgailis M, Mockeliunas R, Vaskeviciute L,  
475 Zienius D (2014). Prevalence of methicillin-resistant *Staphylococcus haemolyticus* in  
476 companion animals: a cross-sectional study. Ann Clin Microbiol Antimicrob. 13(1): 56.  
477 10.1186/s12941-014-0056-y
- 478 31. Sahoo RS, Sahu Jyotirmayee, Prasad MM (2009) Antibiotic susceptibility of staphylococci  
479 isolated from labeo rohita sold in burla fish market, orissa.  
480 <http://hdl.handle.net/123456789/1132>

- 481 32. Sekizuka T, Niwa H, Kinoshita Y, Uchida-Fujii E, Inamine Y, Hashino M, Kuroda M  
482 (2020) Identification of a *mecA/mecC*-positive MRSA ST1-t127 isolate from a racehorse  
483 in Japan. *J Antimicrob Chemother.* 75:292-295. <https://doi.org/10.1093/jac/dkz459>  
484 33. Sergelidis D, Abraham A, Papadopoulos T, Soultos N, Martziou E, Koulourida V, Govaris  
485 A, Pexara A, Zdragas A, Papa A (2014) Isolation of methicillin-resistant *S. taphylococcus*  
486 spp. from ready-to-eat fish products. *Lett Appl Microbiol.* 59:500-506.  
487 DOI: 10.1111/lam.12304  
488 34. Soumya KR, Philip S, Sugathan S, Mathew J, Radhakrishnan EK (2017) Virulence factors  
489 associated with Coagulase-negative Staphylococci isolated from human infections. *3*  
490 *Biotech.* 7:140. DOI: 10.1007/s13205-017-0753-2  
491 35. Stapleton PD, Taylor PW (2002) Methicillin resistance in *Staphylococcus aureus*:  
492 mechanisms and modulation. *Sci Prog.* 85:57-72. DOI: 10.3184/003685002783238870  
493 36. Stegger Á, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale  
494 C, Skov R, Larsen AR (2012) Rapid detection, differentiation and typing of methicillin-  
495 resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue  
496 *mecALGA251*. *Clin Microbiol Infect.* 18:395-400. DOI: 10.1111/j.1469-  
497 0691.2011.03715.x  
498 37. Szczuka E, Krajewska M, Lijewska D, Bosacka K, Kaznowski A (2016) Diversity of  
499 staphylococcal cassette chromosome *mec* elements in nosocomial multiresistant  
500 *Staphylococcus haemolyticus* isolates. *J Appl Genet.* 57:543-547.  
501 38. Takeuchi F, Watanabe S, Baba T, Yuzawa H, Ito T, Morimoto Y, Kuroda M, Cui L,  
502 Takahashi M, Ankai A, et al (2005) Whole-genome sequencing of *Staphylococcus*  
503 *haemolyticus* uncovers the extreme plasticity of its genome and the evolution of  
504 human-colonizing staphylococcal species. *J Bacteriol.* 187:7292-7308.  
505 DOI: 10.1128/JB.187.21.7292-7308.2005

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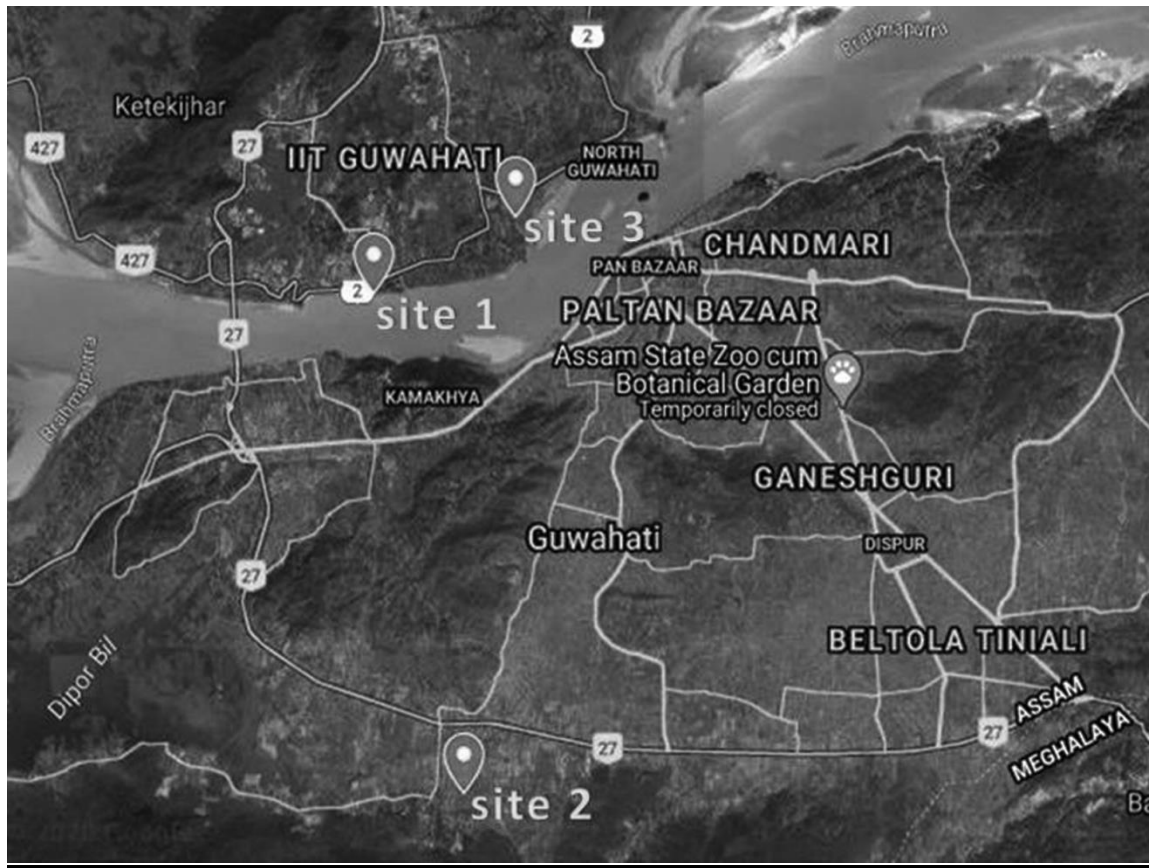
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## **Figures**



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516 **Fig 1** showing such sites as site 1 (Silagranti), site 2 (Garchuk) and site 3 (North Guwahati  
517 Township Committee) from where samples were drawn. Pin map symbols on the map indicate  
518 the three study sites as mentioned.

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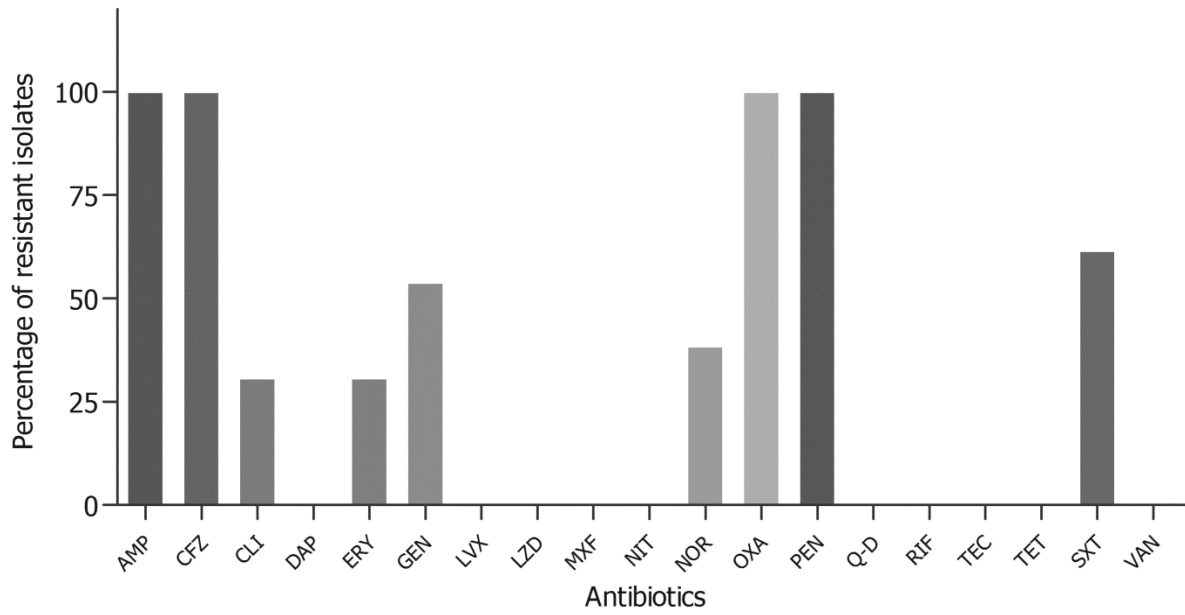
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534 **Fig 2** showing the percentage of non-susceptible isolates of methicillin resistant  
 535 *Staphylococcus haemolyticus* against various antibiotics.

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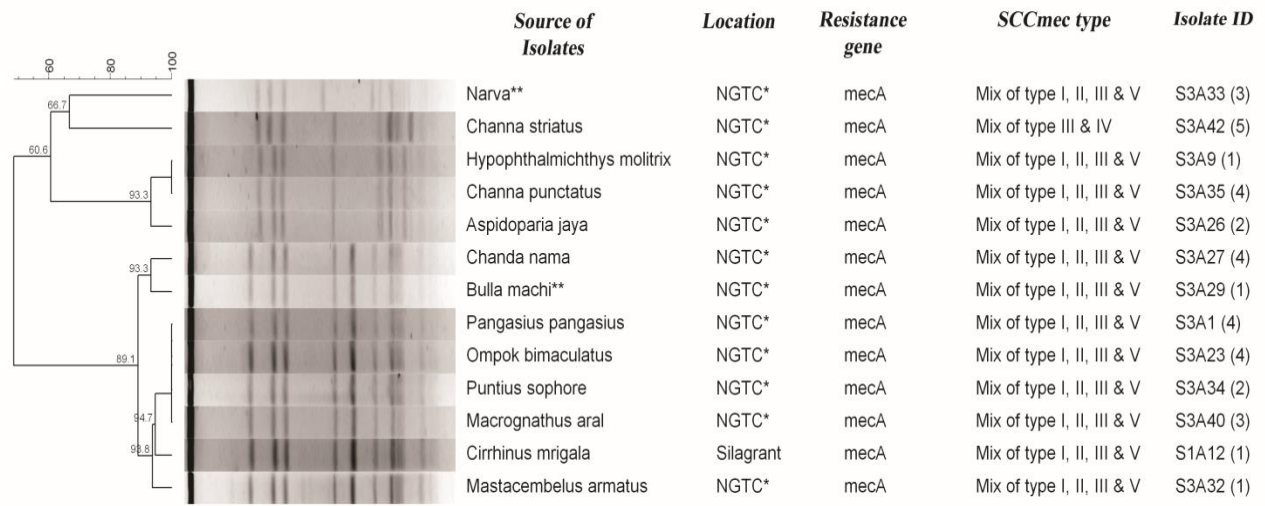
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555 **Fig 3** PFGE cluster analysis of methicillin resistant *Staphylococcus haemolyticus*.

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576 **Tables**577 **Table 1.** List of primer sequences used in the study for SCCmec typing of methicillin resistant *Staphylococcus haemolyticus* (MRSH)

SI No	Primer name	Primer sequence	Product size (in bp)	SCCmec region	PCR conditions	Reference
1	CIF F2	TTCGAGTTGCTGATGAAGAAGG	495	I, J1 region	Initial denaturation at 94°C for 15 minutes followed by 30 cycles of 30 sec at 94°C, 30 sec at 53°C and 1 min at 72°C. Final extension at 72°C for 7 min.	(Milherico et al. 2007)
	CIF R2	ATTTACCACAAGGACTACCAGC				
2	ccrC F2	GTA CTCTGTTACAATGTTTGG	449	V, ccr complex		
	ccrC R2	ATAATGGCTTCATGCTTCAC				
3	RIF5 F10	TTCTTAAGTACACGCTGAATCG	414	III, J3 region		
	RIF5 F13	ATGGAGATGAATTACAAGGG				
4	SCCmec VJ1F	TTCTCCATTCTTGTTTCATCC	377	V, J1 region		
	SCCmec VJ1R	AGAGACTACTGACTTAAGTGG				
5	dcs F2	CATCCATATGATAGCTTGGTC	342	I, II, IV & VI, J3 region		
	dcs R1	CTAAATCATAGCCATGACCG				
6	ccrB2 F2	AGTTTCTCAGAATTCGAACG	311	II & IV, ccr complex		
	ccrB2 R2	CCGATATAGAAWGGGTTAGC				
7	kdp F1	AATCATGTGCCATTGGTGATG	284	II, J1 region		
	kdp R1	CGAATGAAGTGAAAGAAAGTGG				
8	SCCmec III J1F	CATTTGTGAAACACAGTACG	243	III, J1 region		
	SCCmec III J1R	GTTATTGAGACTCCTAAAGC				
9	mec I P2	ATCAAGACTTGCATT CAGGC	209	II & III, mec complex		
	mec I P3	GCGGTTTCAATTC ACTTGTC				
10	mecA P4	TCCAGATTACA ACTTCCCAGG	162	Internal control		
	mecA P7	CCACTTCATATCTTGTAACG				

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Isolate ID	β-Lactams				Non β-Lactams																MDR	Source of Isolates		Resistant gene
	Cephems		Penicillin derivatives		Fluroquinolones	Amino glycosides	Ansamycin	Glycopeptides		Folate pathway antagonists	Lincomide	Lipopeptides	Macrolides	Nitroheterocycles	Oxazolidinones	Streptogramin	Tetracyclines							
	Cephalosporin I	Cephamycins	Penicillinase-labile penicillins	Penicillinase stable penicillins				Moxifloxacin	Norfloxacin									Levofloxacin	Gentamicin	Rifamycin		Glycopeptide	Lipoglycopeptide	
S1A12 (1)	R	R	R	R	>2 R	≤0.25 S	≤1 S	≤0.5 S	>8 R	≤0.5 S	≤1 S	2 S	>4/76 R	≤0.25 S	≤0.5 S	≤0.25 S	≤16 S	≤1 S	≤0.5 S	2 S	Yes	Mrigal	<i>Cirrhinus mrigala</i>	<i>mecA</i>
S3A1 (4)	R	R	R	R	>2 R	1 S	2 S	>2 X**	8 I	≤0.5 S	≤1 S	2 S	≤1/19 S	≤0.25 R	≤0.5 S	>4 R	≤16 S	≤1 S	≤0.5 S	2 S	Yes	Kos	<i>Pangasius pangasius</i>	<i>mecA</i>
S3A9 (1)	R	R	R	R	>2 R	≤0.25 S	≤1 S	≤0.5 S	>8 R	≤0.5 S	≤1 S	2 S	>4/76 R	≤0.25 S	≤0.5 S	≤0.25 S	≤16 S	≤1 S	≤0.5 S	2 S	Yes	Silver Karp	<i>Hypophthalmichthys molitrix</i>	<i>mecA</i>
S3A23 (4)	R	R	R	R	>2 R	≤0.25 S	≤1 S	≤0.5 S	>8 R	≤0.5 S	≤1 S	4 S	>4/76 R	≤0.25 S	≤0.5 S	≤0.25 S	≤16 S	≤1 S	≤0.5 S	2 S	Yes	Kajali	<i>Ompok bimaculatus</i>	<i>mecA</i>
S3A26 (2)	R	R	R	R	>2 R	1 S	>8 R	>2 X**	4 S	≤0.5 S	≤1 S	4 S	≤1/19 S	≤0.25 R	≤0.5 S	>4 R	≤16 S	≤1 S	≤0.5 S	8 I	Yes	Borela	<i>Aspidoparia jaya</i>	<i>mecA</i>

S3A27 (4)	R	R	R	R	>2	<=0.25	<=1	<=0.5	>8	<= 0.5	<= 1	<=1	>4/76	<= 0.25	<=0.5	<= 0.25	<=16	<=1	<=0.5	2	Yes	Chanda	<i>Chanda nama</i>	581 582 583 584 585
S3A29 (1)	R	R	R	R	>2	1	>8	>2	8	<= 0.5	<= 1	4	<=1/19	<= 0.25	<=0.5	>4	<=16	<=1	<=0.5	8	Yes	Bulla machi	Not available	586 587 588 589 590
S3A32 (1)	R	R	R	R	>2	<=0.25	<=1	<=0.5	>8	<= 0.5	<= 1	2	>4/76	<= 0.25	<=0.5	<= 0.25	<=16	<=1	<=0.5	2	Yes	Bami	<i>Mastacembelus armatus</i>	591 592 593 594 595
S3A33 (3)	R	R	R	R	>2	<=0.25	<=1	<=0.5	>8	<= 0.5	<= 1	<=1	>4/76	<= 0.25	<=0.5	<= 0.25	<=16	<=1	<=0.5	2	Yes	Narva	Not available	596 597 598 599 600
S3A34 (2)	R	R	R	R	0.5	1	>8	>2	8	<= 0.5	<= 1	<=1	<=1/19	<= 0.25	<=0.5	<= 0.25	<=16	<=1	<=0.5	<=0.5	Yes	Puti	<i>Puntius sophore</i>	601 602 603 604 605
S3A35 (4)	R	R	R	R	>2	1	>8	>2	8	<= 0.5	<= 1	4	<=1/19	<= 0.25	<=0.5	>4	<=16	<=1	<=0.5	8	Yes	Khorai	<i>Channa punctatus</i>	606 607 608 609 610 611
S3A40 (3)	R	R	R	R	0.5	1	>8	>2	<=2	<= 0.5	<= 1	<=1	<=1/19	<= 0.25	<=0.5	<= 0.25	<=16	<=1	<=0.5	<=0.5	Yes	Tura	<i>Macroglyphus aral</i>	612 613 614 615 616
S3A42 (5)	R	R	R	R	>2	<=0.25	<=1	<=0.5	8	<= 0.5	<= 1	<=1	>4/76	<= 0.25	<=0.5	<= 0.25	<=16	<=1	<=0.5	2	Yes	Sol machi	<i>Channa striatus</i>	617 618 619 620 621 622

\*Isolates resistant to oxacillin are inevitably considered as resistant to all classes of beta-lactam antibiotics except anti-MRSA beta lactam antibiotics. Thus MIC value for beta-lactam antibiotics are not mentioned in the table.

\*\*MIC value is tempting to speculate the isolate to be resistant. However, further confirmation by any other alternative method is advised.

\*\*\* According to CLSI guidelines, MIC <= 0.25 is considered as sensitive. But when isolate showed erythromycin resistance, it is speculated the isolates to be resistant to clindamycin as well (iMLSb phenotype)