Antimicrobial resistance and molecular epidemiology of *Staphylococcus aureus* from Ulaanbaatar, Mongolia

This study aimed to characterize *Staphylococcus aureus* (*S. aureus*) strains isolated from human infections in Mongolia. Infection samples were collected at two time periods (2007-08 and 2011) by the National Center for Communicable Diseases (NCCD) in Ulaanbaatar, Mongolia. *S. aureus* isolates were characterized using polymerase chain reaction (PCR) for *mecA*, PVL, and *sasX* genes and tested for *agr* functionality. All isolates were also *spa* typed. A subset of isolates selected by frequency of *spa* types was subjected to antimicrobial susceptibility testing and multilocus sequence typing. Among 251 *S. aureus* isolates, genotyping demonstrated methicillin resistance in 8.8% of isolates (22/251). Approximately 28% of the tested *S. aureus* isolates were observed to be multidrug resistant (MDR). Sequence type (ST) 154 (*spa* t667) was observed to be a strain with high virulence potential, as all isolates for this *spa* type were positive for PVL, had a functional *agr* system and 78% were MDR. *S. aureus* isolates of ST239 (*spa* t037) were observed to cause infections and roughly 60% had functional *agr* system with a greater proportion being MDR. Additionally, new multilocus sequence types and new *spa* types were identified, warranting continued surveillance for *S. aureus* in this region.

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37 **Introduction**

38   In the past fifty years, *Staphylococcus aureus* (*S. aureus*) has established itself as one of the most

39   frequent antibiotic resistant bacterial pathogens in hospitals and communities (Boucher & Corey

40   2008). *S. aureus* typically causes skin and soft tissue infections, but can also cause invasive

41   infections such as bacteremia, sepsis, endocarditis, pneumonia, osteomyelitis, etc. (Hidron et al.

42   2008; Liu et al. 2011). In earlier years, *S. aureus* infections were commonly observed in

43   individuals with a history of exposure to hospitals (David & Daum 2010). There has been a major

44   epidemiologic transition since the mid-1990s when *S. aureus* was observed to cause infections in

45   population with no known risk exposures. The emergence of such community-associated *S.*

46   *aureus* has further magnified the challenge of *S. aureus* prevention and treatment practices

47   (David & Daum 2010). Methicillin-resistant *S. aureus* (MRSA) is associated with the rise in

48   attributable mortality due to staphylococcal infections (Cooper et al. 2004).

49   Surveillance studies have observed a considerable difference in proportions of MRSA invasive

50   infections in Europe ranging from < 1% in Denmark and the Netherlands to 44% in the United

51   Kingdom and Greece (Cooper et al. 2004; Kock et al. 2010). Nationwide surveillance for

52   invasive MRSA infections conducted in the United States reported about 94,000 cases resulting

53   in approximately 18,000 deaths (Klevens et al. 2007). Worldwide, rates of MRSA have been

54   increasing as observed from data obtained via surveillance initiatives by the National Nosocomial

55   Surveillance System (NNIS) and the European Antimicrobial Resistance Surveillance System

56   (EARSS) (Fridkin et al. 2002; Grundmann et al. 2006; Tiemersma et al. 2004; Turnidge & Bell

57   2000). Nevertheless, a major concern is the lack of data in many countries, particularly the

58   developing countries, as this could potentially result in global transmission of undetected MRSA

59   strains (Azeez-Akande 2010; Molton et al. 2013).

60   Mongolia is a relatively small country in North-East Asia locked for the most part between China

61   in the south and Russia in the north with a population of approximately 2.8 million (Bataar et al.

62   2010; Ider et al. 2010). The capital city of Ulaanbaatar is home to roughly half of the country's

63   population. Infectious diseases still figure in the top 10 causes of death in the country, with sepsis

64   being a common diagnosis among ICU patients (Bataar et al. 2010). The evolving political and

65   economic changes in Mongolia have impacted the working of laboratory networks, data

66   collection and management systems, and training of healthcare professionals in identification and

67   prevention of hospital-acquired infections (Ider et al. 2010). There has also been a disruption of

68   funds to hospitals to conduct surveillance for multidrug-resistant organisms. Due to insufficient

69   laboratory capacity, hospitals in Mongolia use culture testing methods only when empiric therapy

70   fails (Mongolia 2007). This process could potentially propagate antimicrobial resistance in

71   pathogens such as *S. aureus*. In addition, a large herder population in Mongolia with a livestock

72   population of 43 million animals increases the risk of transmission of zoonotic infections

73   (Mongolia 2010).

74   A community-based survey conducted by the World Health Organization (WHO) in Ulaanbaatar

75   between March and April 2009 observed a prevalence of 42% in the use of non-prescription

76   antibiotics among children less than 5 years of age. This proportion is much higher than other

77   regions such as rural communities in Vietnam (12%) and a Chinese city (36%) (Togoobaatar et al.

78   2010). The study found approximately 50% of the children in participating households were

79   prescribed antibiotics, of which roughly 51% children were given both prescribed and non-

80   prescribed antibiotics by their caregiver (Togoobaatar et al. 2010). In a developing country such

81   as Mongolia, unconditional use of antibiotics is of particular concern (Stefani & Goglio 2010).

82   Selective pressures such as the unrestricted use of antibiotics and inadequate compliance to

83   antibiotic regime in conjunction with inadequate surveillance for antimicrobial resistance are

84   some of the important reasons for the emergence of highly resistant *S. aureus* strains (Grundmann

85   et al. 2006; Stefani & Goglio 2010).

86   High population density, urbanization, inadequate infection control policies, exploding antibiotic

87   use, and lack of appropriate healthcare delivery are some of the established social risk factors for

88 colonization and transmission of *S. aureus* strains in hospitals and communities ([Charlebois et al.](#)

89 [2002](#); [Chen et al. 2011](#); [Clements et al. 2008](#); [Henderson 2006](#); [Rehm & Tice 2010](#)). There are

90 very few studies in the published literature on the epidemiology of *S. aureus* in Mongolia. A

91 study conducted in 2006 in Ulaanbaatar analyzed *S. aureus* infection isolates obtained from four

92 university hospitals ([Orth et al. 2006](#)). Analysis using molecular methods and antibiotic

93 susceptibility testing in isolates from this study determined the prevalence of MRSA to be very

94 low (2.9%) ([Orth et al. 2006](#)). However, this study only included isolates collected between 2000

95 and 2002 and characterized only the six MRSA isolates identified in their cohort of *S. aureus*

96 isolates. The aim of our study is to bridge the gap in *S. aureus* literature from Mongolia, and

97 determine the *S. aureus* molecular epidemiology and antimicrobial resistance patterns in

98 Mongolia.

99 **Materials and Methods**

100 This is an observational study conducted during two time periods (2007-08 and 2011) to

101 investigate the prevalence of MRSA infections, and to characterize the *S. aureus* strains causing

102 these infections in Mongolia. The University of Iowa IRB evaluated this project and determined

103 that it did not qualify as human subjects research. To accomplish the study objective, we

104 collaborated with the National Center for Communicable Diseases (NCCD) in Ulaanbaatar. The

105 NCCD has an established Hospital Related Infection Surveillance and Research Unit (HRISRU)

106 ([Ider et al. 2010](#)). This study characterized *S. aureus* isolated from human infections in a

107 convenience sample obtained from the NCCD.

108 In 2007-08, the NCCD transported a convenience sample of "potentially confirmed" *S. aureus*

109 isolates to the Centers for Emerging Infectious Diseases (CEID) at Coralville, IA as a

110 collaborative project to characterize these infection isolates. Additionally, a study team member

111 who traveled to Mongolia in 2011 tested potential *S. aureus* infection isolates banked in

112 Ulaanbaatar and shipped back frozen isolates to the CEID. Patient information such as age,

113 gender, and sample type were available only for the 2007-08 infection samples. Isolates were

114 labeled "wound" if collected from surgical site infections or wound samples. All urine samples

115 collected in 2007-08 were obtained from voided urine i.e. none of these patients were

116 catheterized.

117 Biochemical testing and DNA isolation

118 Isolates were grown and re-confirmed to be *S. aureus* at the CEID, as described previously

119 ([O'Brien et al. 2012](#)). All *S. aureus* isolates were frozen and stored in glycerol broth solution at

120 -80^0C for future use. *S. aureus* DNA was extracted using the Wizard Genomic DNA Purification

121 Kit (Promega, Madison WI) following manufacturer's instructions.

122 Antimicrobial Susceptibility Testing (AST)

123 The antimicrobial susceptibility of isolates were tested by the broth microdilution method in

124 accordance with the Clinical Laboratory Standards Institute (CLSI) standards ([CLSI 2012](#)).

125 Isolates were tested for susceptibility to the following 11 antimicrobials: oxacillin, gentamicin,

126 erythromycin, clindamycin, tetracycline, trimethoprim/sulfamethaxozole (TMP/SMX),

127 imipenam, levofloxacin, linezolid, vancomycin, and daptomycin. Resistance to high-level

128 mupirocin and inducible clindamycin resistance (ICR) were also examined. All AST-confirmed

129 MRSA isolates were considered to be multidrug resistant (MDR). MSSA isolates non-susceptible

130 to $\geq$ 1 antimicrobial agent in $\geq$3 discrete antimicrobial categories were also classified as MDR, as

131 per a recently published report on standardization of bacterial antimicrobial resistance profiles

132 ([Magiorakos et al. 2012](#)).

133 *S. aureus* genetic analysis

134 Amplification of the *spa* fragment was performed using methods and primers described

135 previously ([Shopsin et al. 1999](#)). Identification of the *spa* type for each isolate, the Based Upon

136 Repeat Pattern (BURP) analysis to identify *spa* cluster complexes (*spa*CCs), and calculation of

137   the diversity index (Hunter & Gaston 1988) and corresponding confidence intervals (Grundmann

138   et al. 2001) was performed using the Ridom StaphType software (version2.2.1; Ridom GmbH,

139   Wǖrzburg, Germany) (Harmsen et al. 2003; Mellmann et al. 2008; Mellmann et al. 2007;

140   Strommenger et al. 2008). All isolates were tested for the Panton-Valentine leukocidin (PVL)

141   (*luk*S-PV and *luk*F-PV) (Lina et al. 1999), *mecA* (Bosgelmez-Tinaz et al. 2006), and the *sasX*

142   gene as previously described (Holden et al. 2010; Li et al. 2012). Identified positive and negative

143   controls were used in all molecular assays.

144   Accessory Gene Regulator (*agr*) testing

145   *agr* functionality (functional or dysfunctional) was measured using the level of δ-hemolysin

146   production, as described previously (Sakoulas et al. 2002; Schweizer et al. 2011; Traber &

147   Novick 2006). *agr* positive and negative reference strains were used as controls to ensure validity

148   of our findings.

149   Multi-locus sequence typing (MLST)

150   The allelic profile of *S. aureus* isolates were determined as described previously (Enright et al.

151   2000). At least one representative isolate from the most frequent *spa*CCs, rare *spa*CCs, *spa* types

152   such as t037 that could potentially have two sequence types, or when available a MRSA isolate

153   was selected for MLST analysis from each time period.

154   **Statistical analysis**

155   Data analysis was performed using the SAS statistical software (Version 9.3, SAS Institute Inc.,

156   Cary, NC). We used the 2-tailed Fisher's exact test, and the Wilcoxon signed-rank test to analyze

157   categorical and continuous variables, respectively. *P* values ≤ 0.05 were considered statistically

158   significant for associations between explanatory variables such as age, gender, and type of

159   infection and *S. aureus spa* type, *mecA*, PVL and *agr* functionality. Antimicrobial susceptibility

160   results were analyzed by year of data collection and methicillin-resistance status to observe trends

161   in resistance for each tested antibiotic. Association between *S. aureus* antimicrobial susceptibility,

162   MDR, *mecA*, PVL, *agr* functionality, and *spa* types were also assessed. Odds ratio (OR) and 95%

163   confidence interval (CI) was reported for significant associations.

164   **Results**

165   Patient and sample characteristics

166   In total we analyzed 252 potential *S. aureus* isolates, 198 from 2011 and 54 from 2007-08 isolate

167   collections. Of these, 251 were confirmed to be *S. aureus* isolates. Patient demographics and

168   sample characteristics were available only for the 53 isolates collected in 2007-08. The age of

169   patients ranged from 1day- 82 years (median: 24 years). Of the 53 patients, 31 (58.5%) were

170   females and 22 (41.5%) males. Approximately 43% of the *S. aureus* were isolated from wound

171   samples.

172   Molecular typing

173   We observed a greater proportion of isolates from the 2007-08 collection to be positive for the

174   *mecA* gene. The cohort of *S. aureus* isolates from 2007-08 had greater proportion of PVL

175   positivity in both the MSSA and MRSA isolates, with 59% of MRSA isolates being positive for

176   the PVL gene (**Table 1**). *S. aureus* isolates were observed to have a greater proportion of

177   functional *agr* in both time periods, albeit the proportions were higher for MSSA and MRSA in

178   the 2011 collection. We observed that *mecA* positive *S. aureus* isolates had a 77% less likelihood

179   of having a functional *agr* (OR=0.23, 95% CI 0.067, 0.79; p=0.033). A borderline significance

180   was observed between age and PVL positivity in that most of our PVL-positive *S. aureus* isolates

181   were obtained from older individuals (p=0.045, other data not shown). All isolates tested negative

182   for the *sasX* gene.

183   Antimicrobial susceptibility patterns

184   A subset of *S. aureus* isolates (80/251, ~32%) were tested for antimicrobial susceptibility, based

185   on the frequency of *spa* types. The proportion of *S. aureus* isolates that were MRSA (MDR) was

186   greater in 2011 (**Fig 1A**). Interestingly, we did not observe any *S. aureus* isolates that belonged to

187   the MSSA-MDR category in 2011. Isolates in both years have comparable proportions of MSSA

188   that do not meet the MDR criteria. There was no significant difference in the proportion of MDR

189   isolates between the two study periods (p=0.092).

190   Overall, the proportion of antibiotic resistance in tested *S. aureus* isolates was 38.8%. Greater

191   proportion of MRSA isolates in 2011 was observed to be resistant to erythromycin, tetracycline,

192   levofloxacin and TMP/SMX (**Fig 1B**). We also observed a wider spectrum of resistance in the

193   2011 MRSA isolates as there is additional resistance to gentamicin, imipenam, and ICR. MSSA

194   isolates were observed to be proportionately more resistant to erythromycin and tetracycline in

195   2011 while resistance to levofloxacin, erythromycin and TMP-SMX was observed in 2007

196   isolates (**Fig 1C**). We did not identify high-level mupirocin resistance in our isolates. There was

197   good concordance in *mecA* positivity and phenotypic expression of oxacillin resistance among

198   tested *S. aureus* isolates. Three isolates were observed to have discordant oxacillin-resistance

199   phenotype-genotype (data not shown).

200   We observed that *S. aureus* isolates resistant to 3 or more antimicrobials had significantly lower

201   odds of having a functional *agr* system (OR=0.166, 95% CI 0.046-0.59, p=0.014). Of the 22

202   MDR isolates, roughly 55% (12/22) were positive for the PVL gene, about 86% (19/22) positive

203   for the *mecA* gene, and approximately 64% (14/22) had a functional *agr* phenotype.

204   *spa* type distribution (BURP) and Multilocus sequence type (MLST)

205   Eleven confirmed *S. aureus* isolates were identified as "non-typeable" after at least two attempts

206   to sequence the *spa* gene. Of these, 8 isolates demonstrated bands on amplification of the *spa*

207   fragment with the published forward primer as well as an alternate forward primer (Molla et al.

208   2012). However, the RIDOM StaphType software could not assign *spa* types, possibly due to

209   deviating repeats as demonstrated in previous studies (Baum et al. 2009; Sorum et al. 2013). The

210   remaining 3 isolates demonstrated double bands for the *spa* gene as observed in a study

211 conducted by Shakeri et al (Shakeri et al. 2010), which could not give *spa* types even after three

212 attempts on PCR or purification of bands from an agarose gel.

213 We identified 63 distinct *spa* types among the 251 *S. aureus* isolates (**Table 2**). The most

214 common *spa* types in 2007-08 were t589 (13%), t3465 (13%), and t435 (11%). *spa* types t435

215 (10%), t589 (8%), t5288 (7.5%), and 7% each t1460 and t8677 were the most frequently

216 occurring strains in 2011. *spa* types belonging to *spa*CC 667 was observed to be the most

217 frequently isolated MRSA strain while MSSA isolates were frequently of the *spa*CC 589 type. *S.*

218 *aureus* isolates of *spa* types t589, t435, t037 and t667 were observed in both MRSA and MSSA

219 groups (**Table 2A & 2B**). We identified 8 different *spa* types among the MRSA isolates and 58

220 *spa* types among the MSSA isolates. The diversity index was higher for MSSA isolates (0.952)

221 compared to MRSA isolates (0.865), but due to the small sample size of MRSA isolates the

222 confidence intervals overlap (**Table 3**).

223 BURP analysis revealed clustering around putative founder *spa* types t589 (2011), t435 (2007-08

224 and 2011), t3465 (2007-08), and t8677 (2011) (**Fig 2**). We identified three new MSSA *spa* types

225 (t10064, t10066, and t10358) in the 2011 collection. *spa*CC 667 constituted only 5% of all the

226 strains. Nevertheless, isolates in this group had high PVL prevalence (100%), high *mecA*

227 prevalence (72.7%), 100% functional *agr* isolates, and high multi-drug resistance. *spa*CC 037

228 also had high multidrug resistance but had lower presence of PVL (10%), *mecA* (50%) and

229 functional *agr* (60%). Singleton *spa* types were t002, t126, t156, t521, t647, t803, t1451, t3329

230 and t8039. None of the tested singletons appeared to be MDR.

231 A subset of *S. aureus* isolates from each time period was tested by MLST revealing the following

232 information: t021 (ST30), t037 (ST239), t084 (ST15), t435 (ST121) and t667 (ST154). *spa*

233 types t1460, t5288, and t589 were observed to be ST45 in our study. One t589 MRSA isolate

234 from the 2011 collection was identified to be a new sequence type ST2600, a double-locus

235   variant (DLV) of ST45 as indicated by MLST. In addition, isolates belonging to *spa* types t8677

236   and t3465 in our sample set have novel *arcC* and *aroE* alleles and was categorized as ST2737 by

237   the MLST database curator.

238   **Discussion**

239   The prevalence of MRSA among clinical *S. aureus* isolates obtained from Mongolia was 8.8%. A

240   study conducted in 2006 in Mongolia analyzing *S. aureus* isolates collected between 2000-02

241   found a low prevalence of MRSA (2.9%) by susceptibility testing (Orth et al. 2006). Our study is

242   based on a convenience sample of Mongolian *S. aureus* isolates. Nevertheless, we could infer

243   with caution that there may be an increase in the prevalence of MRSA in Mongolia, reflected by

244   the increase in MDR-MRSA with the relative absence of MDR-MSSA isolates in our sample set.

245   Due to lack of published *S. aureus* data from Mongolia we compared our results to studies from

246   China and Russia since there is a potential for transmission given its geographical proximity.

247   China reported a prevalence of 50.4% for MRSA in 2005 with considerable variations even

248   within the country (Chu et al. 2013; Song et al. 2013; Wang et al. 2008; Yu et al. 2012; Zhao et

249   al. 2012). The proportion of methicillin resistance reported from *S. aureus* in Russia varied from

250   18% (Vorobieva et al. 2008) to 48% (Baranovich et al. 2010). Our study data observed a lower

251   prevalence of MRSA relative to the neighboring countries. Nevertheless, given the convenience

252   sample this may be an underestimate of the 'true' prevalence of MRSA in Mongolia.

253   Our study observed genetic diversity in the MSSA isolates compared to the MRSA isolates.

254   However, we note that the observed diversity could not be concluded to be statistically significant

255   given the small sample size of MRSA isolates resulting in overlap of confidence intervals. A

256   limited number of *spa* types were common in both groups and these are not genetically unrelated

257   strains. This observation of common *spa* types is consistent with findings from other studies

258   (Hallin et al. 2007; Strommenger et al. 2008), and supports the view that MRSA could

259   potentially emerge from existing MSSA clones by acquisition of the SCC*mec* complex (Enright

260   et al. 2002; Robinson & Enright 2003). It is more likely, however, that in Mongolia, MRSA

261   infections are increasing in prevalence due to strain replacement, not SCC*mec* acquisition, which

262   is supported by the presence of MDR-MRSA without any MDR-MSSA in 2011. We are unable to

263   conclude this hypothesis of strain replacement with certainty, hence it is crucial to implement

264   surveillance protocols for *S. aureus* in Mongolia, as there are a myriad of factors contributing to

265   antimicrobial resistance.

266   We observed a significant association between antimicrobial resistance and functionality of the

267   *agr* system suggesting a potential influence of antimicrobial resistance on the fitness of the

268   pathogen via the *agr* system, or vice versa (Paulander et al. 2013). In addition, there appears to be

269   a significant association between presence of the gene for methicillin resistance and having a

270   dysfunctional *agr* system. Evidence from our study on the impact of drug resistance on the

271   regulation of *S. aureus* virulence is consistent with findings from previous studies that observed

272   changes in *S. aureus* cell wall induced by methicillin. This process was observed to affect the

273   bacterial quorum sensing system leading to reduced virulence by suppression of toxins (Hao et al.

274   2012; Rudkin et al. 2012). These findings could potentially influence treatment options for *S.*

275   *aureus* infections in Mongolia by considering the trade-off between fitness of the strain and its

276   range of antimicrobial resistance.

277   Our data suggests that the MRSA clone ST239-*spa* t037 is being transmitted amongst the

278   population. This MLST type was also reported in the previous study from Mongolia, suggesting

279   that ST239 could potentially be the dominant MRSA clone circulating in the country (Orth et al.

280   2006). ST239, a *S. aureus* bacterial hybrid formed by the admixture of MRSA clonal complexes

281   ST30 and ST8 has been reported to be the dominant hospital clone in Asia (Aires de Sousa et al.

282   2003; Baranovich et al. 2010; Song et al. 2013; Xu et al. 2009; Yamamoto et al. 2012; Yu et al.

283   2012), Europe (Alp et al. 2009; Szczepanik et al. 2007; Wisplinghoff et al. 2005), South America

284   (Carvalho et al. 2010; Vivoni et al. 2006), and the Middle East (Cirlan et al. 2005) and even

285 responsible for an outbreak of device-associated bacteremia in Europe (Edgeworth et al. 2007). In

286 accordance with previous reports, all three identified ST239 *S. aureus* strains in our study were

287 observed to be MDR (Smyth et al. 2010). We also observed *S. aureus* sequence types ST45 and

288 ST121, which have previously been observed to be to be associated with *S. aureus* infections in

289 other regions of the world, circulating in Mongolia.

290 The proportion of multidrug resistance was observed to be higher in 2011 relative to 2007-08,

291 albeit this difference was not statistically significant. Reports on *S. aureus* multidrug resistance

292 observed variable rates ranging from ~29% - 100% from China (Chao et al. 2013; Chen et al.

293 2009; Wang et al. 2012) and about 90% in Russian MRSA isolates (Baranovich et al. 2010)..

294 Studies observed that presence of the *sasX* gene in *S. aureus* potentially increased its virulence

295 capacity by boosting the bacterial defense mechanism, particularly in the ST239 clones (Li et al.

296 2012). Furthermore, testing of *S. aureus* isolates from three teaching hospitals in eastern China

297 demonstrated that the proportion of *S. aureus* strains that were non-ST239 and positive for the

298 *sasX* gene increased from 5% to 28% between 2003-05 and 2009-11 (Holden et al. 2010; Li et al.

299 2012). None of our isolates, including the identified ST239 isolates, exhibited the presence of the

300 *sasX* gene, suggesting its prevalence may be low or absent in Mongolia. We did not identify any

301 known livestock-associated strains in our isolate collection, although several reports from China

302 have observed the presence of these strains (Wagenaar et al. 2009; Zhao et al. 2012).

303 Our study has several limitations. *S. aureus* isolates were collected as a convenience sample and

304 could not be consistently linked to important patient information, particularly the 2011 collection

305 that had a larger sample size for *S. aureus* isolates. This could have potentially resulted in

306 inclusion of duplicate patient isolates and over-representation of *S. aureus* strains. In addition,

307 isolates from both time periods were not collected in a systematic manner adding to the potential

308 selection bias. Hence, results from this study may reflect only a snapshot of the 'true' estimate of

309 *S. aureus* infections in Mongolia. Conclusions drawn from this study could be used as

310 preliminary results to design studies that are sufficiently powered to validate the observed

311 associations. Nevertheless, there are not many studies from Mongolia and our study adds

312 valuable information on the molecular epidemiology of *S. aureus* infections in Mongolia.

313 Another drawback of our study was the inability to differentiate the potential origin of *S. aureus*

314 strains as healthcare associated (HCA-) versus community associated (CA-) since we did not

315 have access to the date of admission before *S. aureus* isolation from the infection. Given that in

316 recent times there has been a gradual blurring in the origin of *S. aureus* strains, the reliability of

317 this differentiation may be questionable (Mera et al. 2011). In addition, we also did not test all *S.*

318 *aureus* isolates in our collection for antimicrobial susceptibility that could have resulted in loss of

319 information on 'true' antimicrobial susceptibility trends.

320 **Conclusions**

321 In summary, our study observed an increasing prevalence of MRSA (8.8%) by AST, and recorded

322 MDR rate of 28% in *S. aureus* isolates from Mongolia. We also observed the presence of

323 previously identified *S. aureus* strains such as ST239 and ST30 adding its virulence potential to

324 an existing burden of antimicrobial resistance. Regular surveillance and implementation of

325 stricter policies for antimicrobial use is warranted to prevent further transmission of *S. aureus* in

326 Mongolia.

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332 shipping, assimilation and communication of patient data, and review of the manuscript.

333 **Figure Legends**

334 **Figure 1A: Illustration for multidrug resistance in Mongolia *S. aureus* isolates.**
335 MRSA = methicillin-resistant *S. aureus*, MSSA = methicillin-susceptible *S. aureus*, MDR =
336 multidrug resistant, MSSA-not MDR category include MSSA isolates that are non-susceptible to
337 ≥ 1 antimicrobial agent in < 3 discrete antimicrobial categories

338 **Figure 1B: Graph for antimicrobial resistance in MRSA isolates.**
339 *TMP-SMX = trimethoprim/sulfamethaxozole, MRSA = methicillin-resistant *S. aureus*, ICR =
340 inducible clindamycin resistance
341 Resistance to rifampicin and complete resistance to clindamycin were not observed

342 **Figure 1C: Graph for antimicrobial resistance in MSSA isolates.**
343 *TMP-SMX = trimethoprim/sulfamethaxozole, MSSA = methicillin-susceptible *S. aureus*
344 No resistance was observed to gentamicin, rifampicin, imipenam, clindamycin or inducible
345 clindamycin resistance

346 **Figure 2: Population snapshot for MRSA & MSSA Based-Upon Repeat Pattern (BURP)**
347 **analysis.**
348 BURP grouping using default parameters resulted in 8 *spa*CCs and excluded 5 *spa* types (t026,
349 t132, t517, t2493, and t10358). Each dot represents a unique *spa* type. Diameter of a dot is
350 proportional to the quantity of corresponding *spa* type. Blue dots = group putative founders (i.e.
351 *spa* type with the highest score within the CC). Yellow dots = putative subfounders with second
352 highest score. If two or more *spa* types have the same highest founder score they are illustrated in
353 blue. The distance between linked and/or unlinked *spa* types do not concern the genetic distance
354 between them.

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Prevalence of *S. aureus* genes in Mongolia MRSA and MSSA isolates

*Seven *S. aureus* isolates from 2007-08 did not grow for *agr* testing. Significant if p ≤0.05.

Data for 2x2 table presented as frequency (%).

MRSA = methicillin-resistant *S. aureus*, MSSA = methicillin-susceptible *S. aureus.*

1 **Table 1**

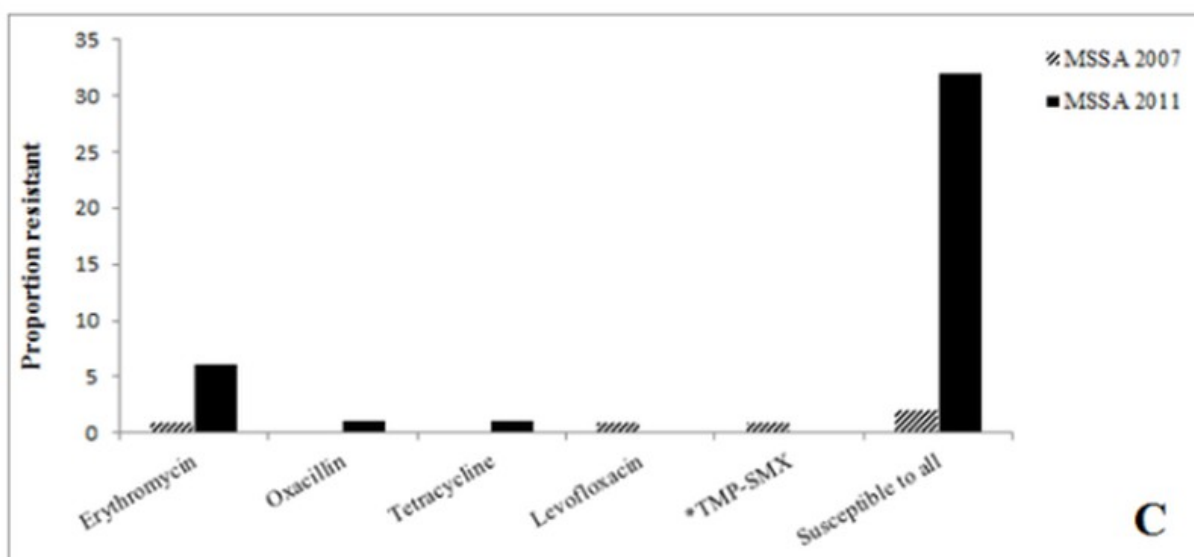
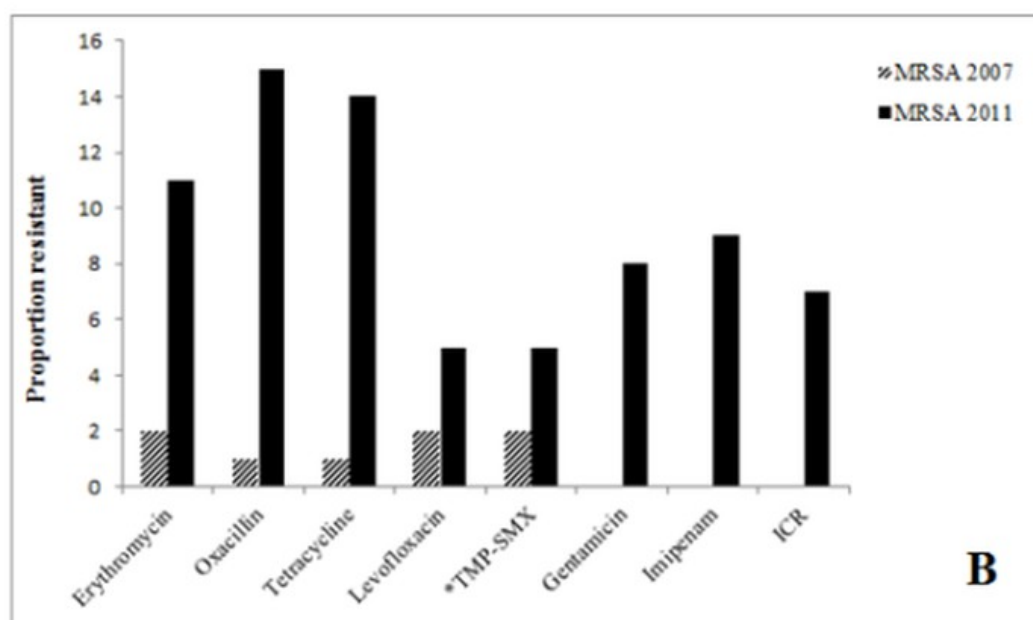
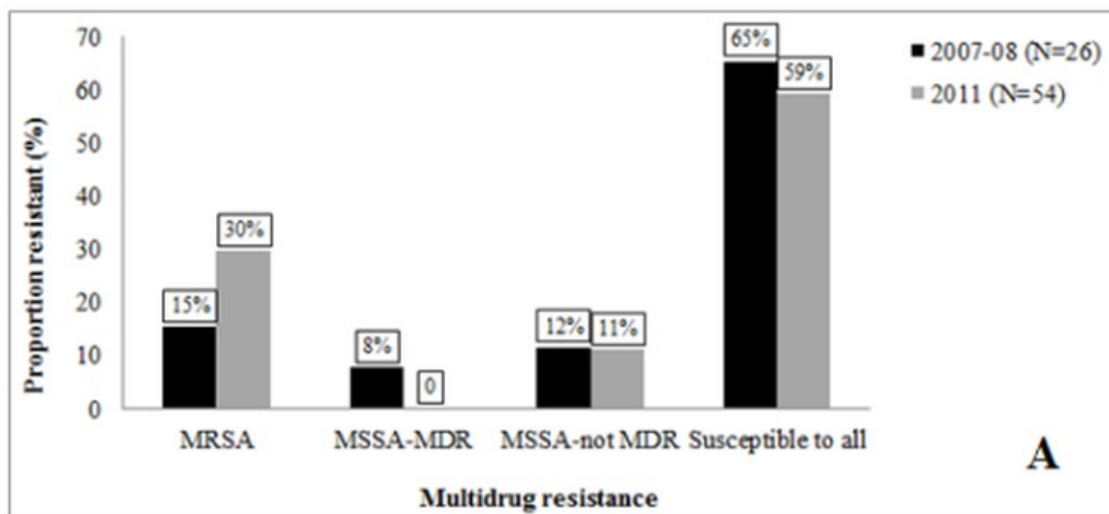
2 **Prevalence of S. aureus genes in Mongolia MRSA and MSSA isolates**

Factor tested	2007-08 (N=53)			2011(N=198)		
	MSSA	MRSA	p-value	MSSA	MRSA	p-value
PVL						
Positive	40 (85.1)	5 (83.3)	1.00	55 (30.2)	8 (50)	0.16
Negative	7 (14.9)	1 (16.7)		127 (69.8)	8 (50)	
*agr**						
Functional	37 (86.1)	2 (66.7)	0.39	175 (96.2)	13 (81.3)	0.037
Dysfunctional	6 (13.9)	1 (33.3)		7 (3.9)	3 (18.8)	

3 *Seven *S. aureus* isolates from 2007-08 did not grow for *agr* testing
4 Significant if p≤0.05
5 Data for 2x2 table presented as frequency (%)
6 MRSA = methicillin-resistant *S. aureus*, MSSA = methicillin-susceptible *S. aureus*

Figure 1

Antimicrobial resistance in Mongolia *S. aureus* isolates

Figure 1A: Illustration for multidrug resistance in Mongolia *S. aureus* isolates. MRSA = methicillin-resistant *S. aureus*, MSSA = methicillin-susceptible *S. aureus*, MDR = multidrug resistant, MSSA-not MDR category include MSSA isolates that are non-susceptible to ≥ 1 antimicrobial agent in < 3 discrete antimicrobial categories. **Figure 1B: Graph for antimicrobial resistance in MRSA isolates.** *TMP-SMX = trimethoprim/sulfamethaxozole, ICR = inducible clindamycin resistance. Resistance to rifampicin and complete resistance to clindamycin were not observed. **Figure 1C: Graph for antimicrobial resistance in MSSA isolates.** No resistance was observed to gentamicin, rifampicin, imipenam, clindamycin or inducible clindamycin resistance.

Distribution of *spa* types and *spaCC* among Mongolia MRSA and MSSA isolates

*Bolded *spa* type is the putative founder for that *spa*CC.

BURP clusters formed of 22 MRSA isolates.

3 non-typeable isolates and one *spa* type (2 isolates) excluded from BURP clusters.

MRSA= methicillin-resistant *S. aureus.*

*Bolded *spa* type is the putative founder for that *spa*CC.

€ Identified as new *spa* types.

BURP clusters formed of 229 MSSA isolates.

8 non-typeable isolates and four *spa* types (6 isolates) excluded from BURP clusters.

MSSA= methicillin-susceptible *S. aureus.*

 Table 2

2 **Distribution of *spa* types and *spaCC* among Mongolia MRSA and MSSA isolates**

3 **(2A). MRSA**

*spa*CC	Study assigned *spa*CC	No. (%) of strains	*spa* types*
*spa*CC 589	CC1	2 (9.1)	**t589**, t2397
*spa*CC 435	CC2	1 (4.5)	t435
*spa*CC 037	CC4	5 (22.7)	**t037**, t074
*spa*CC 667	CC7	8 (36.4)	**t667**, t2832
*spa*CC 8	CC9	1 (4.5)	**t008**
Total		**17**	

4 *Bolded *spa* type is the putative founder for that *spa*CC
5 BURP clusters formed of 22 MRSA isolates
6 3 non-typeable isolates and one *spa* type (2 isolates) excluded from BURP clusters
7 MRSA= methicillin-resistant *S. aureus*

8 **(2B). MSSA**

*spa*CC	Study assigned *spa*CC	No. (%) of strains	*spa* types*
*spa*CC 589	CC1	76 (33.2)	**t5288**, t3126, t1460, t589, t4153, t6242, t3103, t630, t073, t3219, t7043, t102, t550, t722, t908, t10064$^\epsilon$
*spa*CC 435	CC2	64 (27.9)	**t435**, t270, t2392, t169, t272, t284, t159, t308, t8762, t6870, t2087, t1441
*spa*CC 8677	CC3	34 (14.8)	**t8677**, t4049, t3156, t10066$^\epsilon$, t4473, t3465
*spa*CC 037	CC4	5 (2.2)	**t037**, t021
*spa*CC 084	CC5	4 (1.7)	**t084**, t085, t1038
*spa*CC 1194	CC6	3 (1.3)	**t1194**, t1710
*spa*CC 667	CC7	3 (1.3)	t667
*spa*CC 1010	CC8	2 (0.9)	**t1010**, t081
*spa*CC 8	CC9	1 (0.4)	t024
Total		**192**	

9 *Bolded *spa* type is the putative founder for that *spa*CC
10 $^\epsilon$ Identified as new *spa* types
11 BURP clusters formed of 229 MSSA isolates
12 8 non-typeable isolates and four *spa* types (6 isolates) excluded from BURP clusters
13 MSSA= methicillin-susceptible *S. aureus*

Table 3_(on next page)

Diversity index for Mongolia MRSA and MSSA isolates by *spa* typing and BURP

CI: Confidence Interval, BURP: Based Upon Repeat Pattern analysis using the RIDOM StaphType.

MRSA: methicillin-resistant *S. aureus*; MSSA: methicillin-susceptible *S. aureus.*

1 **Table 3**

2 **Diversity index for Mongolia MRSA and MSSA isolates by spa typing and BURP**

Organism groups	No. of isolates	No. of isolates rejected by BURP	Number of different spa types	Typeability (%)	Diversity/discriminatory index (95% CI)
MRSA (n=22)	19	3	8	95	0.865 (0.767-0.964)
MSSA (n=229)	222	7	58	99.11	0.952 (0.942-0.962)
MRSA+MSSA (n=251)	241	10	63	98.77	0.956 (0.947-0.965)

3 CI: Confidence Interval, BURP: Based Upon Repeat Pattern analysis using the RIDOM StaphType
4 MRSA: methicillin-resistant *S. aureus*; MSSA: methicillin-susceptible *S. aureus*

Figure 2

Population snapshot for MRSA & MSSA Based-Upon Repeat Pattern (BURP) analysis

BURP grouping using default parameters resulted in 8 *spa*CCs and excluded 5 *spa* types (t026, t132, t517, t2493, and t10358). Each dot represents a unique *spa* type. Diameter of a dot is proportional to the quantity of corresponding *spa* type. Blue dots = group putative founders (i.e. *spa* type with the highest score within the CC). Yellow dots = putative subfounders with second highest score. If two or more *spa* types have the same highest founder score they are illustrated in blue. The distance between linked and/or unlinked *spa* types do not concern the genetic distance between them.

Figure 2: