Antibiotic resistance and typing of the methicillin-resistant Staphylococcus aureus clones in Kuwait hospitals, 2016–2017

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Abstract

Background: Methicillin-resistant Staphylococcus aureus (MRSA) belong to diverse genetic backgrounds that differ in antibiotic resistance. Knowledge of the local clonal composition of MRSA strains is important for patients’ management and for designing effective control and eradication methods. The aim of this study was to compare the antibiotic resistance patterns and genotypic characteristics of MRSA isolates obtained in public hospitals in Kuwait in 2016 and 2017 for changes in their resistance patterns and clonal composition.

Methods: A total of 4726 MRSA isolates obtained in 2016–2017 from clinical specimens in Kuwait public hospitals were characterized using antibiogram, SCCmec typing, spa typing and DNA microarray.

Results: The isolates expressed resistance to fusidic acid (52.9%), kanamycin (41.6%), gentamicin (32.5%) and erythromycin (36.2%). The prevalence of high-level mupirocin resistance decreased from 3.7% in 2016 to 2.4% in 2017, while the proportion of resistance to other antibiotics remained relatively stable. A total of 382 spa types were detected with eight spa types, t688 (N = 547), t304 (N = 428), t860 (N = 394), t127 (N = 306), t044 (N = 230), t311 (N = 243), t223 (N = 184) and t002 (N = 181) constituting 53.1% of the MRSA isolates in 2016–2017. Of the 3004 MRSA isolates obtained in 2016 (N = 1327) and 2017 (N = 1677) selected for DNA microarray analysis, 26 clonal complexes (CCs) were identified. Most of the isolates belonged to CC1 (N = 248), CC5 (N = 833), CC6 (N = 241), CC8 (N = 292), CC22 (N = 421), CC30 (N = 177), CC80 (N = 177) and CC97 (N = 171). The prevalence of CC5 isolates has significantly (p ≤ 0.05) increased from 294 isolates in 2016 to 539 isolates in 2017. Although CC22 increased from 196 isolates in 2016 to 225 isolates in 2017, CC1 increased from 112 isolates in 2016 to 136 isolates in 2017, CC6 increased from 103 isolates in 2016 to 138 isolates in 2017, these changes were not significant (p ≥ 0.05).

Conclusion: These results revealed the diversity in the genetic backgrounds of MRSA isolates and the stable maintenance of the dominant MRSA clones in Kuwait hospitals in 2016 and 2017 suggesting an on-going transmission of these clones. Novel and creative infection prevention and control measures are required to curtail further transmission.

Keywords: MRSA, DNA microarray, Antibiotic resistance, Genotypes, Clonal complex
**Background**

Since its report in 1961 in England [1], methicillin-resistant *Staphylococcus aureus* (MRSA) has spread to many countries causing serious infections that are sometimes difficult to treat [2]. Initially, MRSA was a well-established pathogen among elderly patients with previous admission to healthcare settings and with history of previous antibiotic usage. This type of MRSA was known as healthcare-associated or healthcare-acquired MRSA (HA-MRSA) [3]. Then in the 1990s, a new lineage of MRSA emerged in people with no previous history of hospitalization or exposure to the healthcare system and previous antibiotic use which was designated as community-associated or community-acquired MRSA (CA-MRSA) [4].

Molecular epidemiological typing techniques, such as SCCmec typing, staphylococcal protein A (*spa*) typing, multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE) and DNA microarray have been used to study the genetic background of MRSA. HA-MRSA isolates carry relatively large SCCmec genetic element belonging to type I, II, or III, and are usually resistant to multiple non-beta-lactam antibiotics [2]. In contrast, CA-MRSA isolates carry smaller sized SCCmec elements belonging to SCCmec type IV, V or VI and are usually sensitive to most non-beta-lactam antibiotics [2]. MRSA isolates carrying different SCCmec genetic elements have been further differentiated using MLST and eBURST which grouped them into sequence types and clonal complexes (CCs) [2].

Studies have shown that CA-MRSA have replaced HA-MRSA in many countries including Kuwait [5], Singapore [6], United Arab Emirates [7] and Portugal [8]. A previous study in Kuwait showed that the clonal composition of the MRSA has changed significantly from 1992 to 2010 [5] with the emergence of different CA-MRSA clones. The same report revealed that CC8/ST239-III remained the most common clone in Kuwait hospitals from 1992 to 2010, although their prevalence decreased overtime [5]. During the same period, the prevalence of CA-MRSA clones including CC5/ST5-IV/V, CC80/ST80-IV and ST1-IV/V were increasing [5]. To provide an update on the clonal composition of MRSA strains circulating in Kuwait hospitals, this study compared the antibiotic resistance patterns and genotypic characteristics of MRSA isolates obtained in public hospitals in Kuwait in 2016 and 2017 for changes in clonal composition and resistance profile.

**Results**

**Specimen source and antibiotic susceptibility profile of MRSA isolates**

MRSA isolates obtained in 2016 and 2017 were mostly from skin and soft tissue infection specimens (1983; 41.9%). This was followed by nasal swabs (1276; 26.9%), endotracheal specimens (271; 5.7%), blood (200; 4.2%), urine (102; 2.1%), throat (89; 1.8%) and ear (64; 1.3%). The clinical sources for 741 (15.6%) of the isolates were not provided.

The distribution of resistance phenotype among MRSA isolates in 2016–2017 is shown in Table 1. All isolates were sensitive to vancomycin (MIC: ≤ 2 μg/ml), teicoplanin (MIC: ≤ 2 μg/ml) and linezolid. Besides beta-lactam resistance, most of the MRSA isolates obtained in 2016 and 2017 were resistant to penicillin G (PG) (92% in 2016 and 98% in 2017), gentamicin (GM) (33% in 2016 and 32% in 2017), and kanamycin (KM) (42% in 2016 and 41% in 2017). The prevalence of the different resistance phenotypes was similar in both years, except for the resistance to tetracycline (EM), clindamycin (CC), trimethoprim (TP), rifampicin (RF), fusidic acid (FA), chloramphenicol (CM), and ciprofloxacin (CIP), which showed some variations over the years.

**Table 1** Distribution of resistance phenotype among MRSA isolates in 2016–2017

| Resistance phenotype | 2016 (N = 2305) | 2017 (N = 2421) | Total (N = 4726) | p-value |
|----------------------|----------------|----------------|-----------------|---------|
|                      | No. | %  | No. | %  | No. | %  |       |         |
| PG                   | 2118| 92 | 2363| 98 | 4481| 94.8|
| GM                   | 763 | 33 | 775 | 32 | 1538| 32.5|
| KM                   | 974 | 42 | 992 | 41 | 1966| 41.6|
| EM                   | 912 | 40 | 800 | 33 | 1712| 36.2|
| CC(I)                | 435 | 19 | 366 | 15 | 801 | 16.9| ≤ 0.05|
| CC(C)                | 404 | 18 | 365 | 15 | 769 | 16.2| ≤ 0.02|
| CM                   | 290 | 13 | 376 | 16 | 666 | 14.1| ≤ 0.05|
| TET                  | 832 | 36 | 943 | 39 | 1775| 37.5|         |
| TP                   | 861 | 37 | 835 | 35 | 1696| 35.8|         |
| RF                   | 12  | 0.5| 10  | 0.4| 22  | 0.46|         |
| FA                   | 1178| 51 | 1324| 55 | 2502| 52.9|         |
| CIP                  | 854 | 37 | 835 | 35 | 1689| 35.7|         |
| MUP(L)               | 189 | 8  | 221 | 9  | 410 | 8.6 |         |
| MUP(H)               | 87  | 3.7| 59  | 2.4| 146 | 3.1 | ≤ 0.05 |

*Abbreviations: PG penicillin G, GM gentamicin, KM kanamycin, TET tetracycline, EM erythromycin, CC (I) induced-resistance clindamycin, CC (C) constitutive-resistance clindamycin, TP trimethoprim, RF rifampicin, FA fusidic acid, CM chloramphenicol, CIP ciprofloxacin, MUP (H) mupirocin high- level resistance, MUP (L) mupirocin low- level resistance*
2016 and 2017 were resistant to fusidic acid (52.9%). This was followed by resistance to kanamycin (41.6%), tetracycline (37.5%), erythromycin (36.2%), trimethoprim (35.8%), ciprofloxacin (35.7%), gentamicin (32.5%), clindamycin (16.9%), and chloramphenicol (14.1%). Only a small proportion of the isolates expressed high-level resistance (HLR) to mupirocin (3.1%).

Comparison of the distribution of antibiotic resistance in MRSA isolates obtained between 2016 and 2017 is shown in Table 1. During 2016–2017, there were significant (p ≤ 0.05) decrease in the prevalence of resistance to erythromycin, clindamycin and high-level mupirocin resistance, while resistance to chloramphenicol increased significantly (Table 1).

Results of DNA microarray analysis revealed that the high-level mupirocin-resistant isolates were positive for mupA that encodes an alternative isoleucyl-tRNA synthetase (ileS2) which is unaffected by mupirocin. Similarly, resistance to gentamicin, erythromycin and clindamycin, and tetracycline corresponded with the presence of their respective determinants, aacA-aphD, ermA/ermC, and tet(K)/tet(M). Fusidic acid resistance was mediated by fusC in most of the isolates and by fusB/faR1 in CC80 isolates.

Prevalence of SCCmec types in MRSA isolates in 2016–2017

The dominant SCCmec type among the MRSA isolates obtained in 2016–2017 was SCCmec type IV (47.0%) (Table 2). This was followed by SCCmec type V (29.8%), type III (12.1%) and type VI (9.3%). SCCmec type II (0.5%) and type I (0.08%) were detected in small numbers. Thirty-four isolates carried a new combination of SCCmec types (SCCmec IV + V).

There were no significant changes in the distribution of SCCmec types I, II, III and V in MRSA isolates between 2016 and 2017 (Table 2). MRSA isolates carrying type VI increased (p ≤ 0.05) from 7% in 2016 to 11.2% in 2017, while those carrying SCCmec type IV decreased (p ≤ 0.05) from 50% in 2016 to 44.5% in 2017 (Table 2).

Table 2 Distribution of SCCmec types among MRSA isolates in 2016–2017

| SCCmec Types | 2016 | 2017 | Total | p-value |
|--------------|------|------|-------|---------|
|              | No.  | %    | No.   | %       |
| I            | 1    | 0.04 | 3     | 0.1     | 0.08    |
| II           | 14   | 0.6  | 10    | 0.4     | 0.5     |
| III          | 291  | 13   | 282   | 11.7    | 573     | 12.1    |
| IV           | 1148 | 50   | 1077  | 44.5    | 2225    | 47.0    | ≤ 0.05 |
| V            | 701  | 30   | 708   | 29      | 1409    | 29.8    |
| VI           | 170  | 7    | 273   | 11.2    | 443     | 9.3     | ≤ 0.05 |
| IV + V       | 12   | 0.5  | 22    | 1       | 34      | 0.7     |
| ND           | –    | –    | 46    | 2       | 46      | 0.9     |

Prevalence of spa types among MRSA isolates in 2016–2017

In total, 382 spa types were identified among the MRSA isolates obtained in 2016–2017. The distribution of the common spa types among the isolates obtained in 2016 and 2017 is shown in Table 3. Spa type t688 (23%) was the dominant spa type detected in both years. This was followed by t304, t860, t127, t044, t311, t002 and t223 (Table 3). In addition, 354 spa types were detected in less than 10 isolates. Spa types could not be assigned for 118 isolates in both years.

A comparison of the distribution of spa types of MRSA isolates obtained in 2016–2017 presented in Table 3 revealed that the prevalence of spa type t688 increased (p ≤ 0.05) from 10.4% in 2016 to 12.6% in 2017, while the proportions of t002 and t044 were significantly decreased during the 2 years. No significant changes were observed among the other major spa types. Some sporadic spa types were observed in isolates obtained either in 2016 or 2017 as shown in the supplementary Table S1. The association of spa types with specific genotypes is presented in Table S1.

Distribution of MRSA clones in 2016–2017

The clonal complexes (CCs) of 3004 MRSA isolates obtained in 2016 (N = 1327) and 2017 (N = 1677), selected on the basis of spa types was determined using DNA microarray. The selection included all clinical samples from different hospitals with the same spa type.

Twenty-six clonal complexes (CCs) were obtained in both years. The clonal complexes (CCs) were CC1, CC5, CC6, CC7, CC8, CC9/ST384, CC15, CC22, CC30, CC45, CC49, CC80, CC88, CC96, CC97, CC121, CC152, CC361, CC398, CC509, CC779, CC913, CC1153, CC2198, CC2250/2277 and CC2596. In addition, three sequence types, ST59, ST72 and ST2867 were identified. The distribution of the MRSA clones is shown in supplementary Table (Table S1). The dominant clonal complexes identified in both years were CC5 (833 isolates), CC22 (421 isolates), CC8 (292 isolates), CC1 (248 isolates), CC6 (241 isolates), CC30 (177 isolates), CC80 (177 isolates) and CC97 (171 isolates) (Table S1). The other clonal complexes including CC7, CC9/ST384, CC15, CC45, CC49, CC88, CC96, CC121, CC152, CC361, CC398, CC509, CC779, CC913, CC1153, CC2198, CC2250/2277, CC2596 and the three sequence types ST59, ST72 and ST2867 were less frequently detected among MRSA isolates during 2016 and 2017 (Table S1). The composition of the major clonal complexes is presented below.

Clonal Complex 1 (CC1)

CC1 consisted of 16 MRSA genotypes. The most common CC1 genotype was CC1-MRSA-V + SCCfus [PVL †] (78 isolates) which occurred in 35 isolates in 2016 and...
in 43 isolates in 2017. Of the 16 spa types identified among the CC1-MRSA-V + SCCfus [PVL+] isolates, the t127 was the most common spa type in 2016 and 2017. Spa types t3896, t6693, t948, t1252, t693, t591 were only detected in 2016, while t114, t1589, t177, t2207, t398, t605, t17556, t2658 were only detected in 2017. Other common genotypes of CC1 were CC1-MRSA-V + SCCfus (50 isolates) and CC1-MRSA-IV, WA MRSA-1/57.

### Table 3

| Spa types | 2016 No. | % | 2017 No. | % | Total No. | % | p-value |
|-----------|---------|---|----------|---|-----------|---|---------|
| t688      | 240     | 10.4 | 307      | 12.6 | 547       | 23 | < 0.05 |
| t304      | 211     | 9.1  | 217      | 8.9  | 428       | 18 |         |
| t860      | 190     | 8.2  | 204      | 8.4  | 394       | 16.6|         |
| t127      | 151     | 6.5  | 155      | 6.4  | 306       | 12.9|         |
| t044      | 132     | 5.7  | 98       | 4    | 230       | 9.7 |         |
| t311      | 124     | 5.3  | 119      | 4.9  | 243       | 10.2|         |
| t002      | 103     | 4.4  | 78       | 3.2  | 181       | 7.6 | < 0.05 |
| t223      | 94      | 4    | 90       | 3.7  | 184       | 7.7 |         |
| t267      | 65      | 2.8  | 68       | 2.8  | 133       | 5.6 |         |
| t019      | 58      | 2.5  | 58       | 2.3  | 116       | 4.8 |         |
| t3841     | 54      | 1.6  | 55       | 2.2  | 99        | 3.8 |         |

Spa types detected in < 10 isolates: t024, t463, t1020, t1211, t5708, t026, t7011, t11659, t13697, t17282, t1985, t4407, t12849, t1045, t5634, t1339, t2518, t680, t6068, t17399, t10795, t14235, t11649, t5562, t4545, t310, t112219, t790, t16360, t12720, t17200, t1086, t729, t10888, t1427, t1186, t5608, t4557, t1375, t1247, t19228, t16681, t1279, t1348, t088, t1342, t1120, t14392, t1830, t5673, t4565, t3379, t1309, t19867, t117330, t1037, t8731, t091, t17358, t11288, t144838, t1186, t570, t14687, t5652, t1332, t1012, t1752, t3325, t1878, t1094, t17466, t1114, t15181, t12164, t15704, t4892, t13782, t2526, t1062, t17556, t1334, t1902, t10028, t247, t11174, t15435, t1217, t1578, t14955, t3825, t14019, t10002, t1777, t1345, t1934, t10094, t774, t1118, t16360, t12717, t15582, t19981, t13697, t1442, t10892, t189, t14018, t954, t11034, t177, t11836, t16470, t12335, t1605, t15045, t1398, t14859, t1975, t203, t1425, t9606, t10306, t8369, t121, t16606, t2413, t6071, t5146, t1416, t10602, t9042, t1317, t17117, t258, t11034, t853, t122, t116877, t1242, t1525, t1422, t17281, t12907, t1681, t11725, t12658, t110347, t18657, t12413, t16945, t12467, t6584, t537, t14336, t12790, t19448, t18645, t115729, t2627, t101935, t18962, t12743, t116946, t12529, t6675, t5414, t1437, t41022, t903, t13158, t107084, t12571, t6769, t547, t4403, t1046, t1154, t11498, t1215, t138, t138165, t18395, t2601, t3364, t1362, t4326, t8154, t1010, t1014, t10208, t11863, t12352, t1131, t14228, t14700, t1548, t16182, t16186, t16202, t11977, t1211, t1393, t13012, t13243, t13387, t1355, t13896, t4045, t14724, t521, t5485, t1579, t6369, t18221, t845, t18506, t19673, t1004, t1016, t1034, t1050, t1067, t11016, t11018, t11039, t110405, t11081, t110836, t111113, t11206, t11462, t1147, t11715, t11901, t12068, t12236, t12398, t12537, t13429, t13699, t14090, t114, t1476, t1504, t15069, t15224, t1556, t15778, t157801, t15858, t1593, t16181, t16356, t161361, t16373, t16468, t16486, t16549, t16578, t16579, t16604, t16605, t16901, t16903, t16904, t16905, t1836, t1855, t190, t1965, t1991, t214, t2251, t128, t228, t253, t2622, t2734, t2770, t2802, t2933, t3010, t3029, t1916, t221, t242, t1325, t330, t13949, t16351, t2422, t14224, t14359, t1450, t504, t1504, t1517, t1593, t15772, t1586, t15994, t16258, t16670, t16693, t16627, t711, t1713, t1743, t17604, t17623, t17665, t17685, t1779, t18009, t18166, t18168, t18348, t18934, t1899, t19231, t19434, t1948, t19638
(34 isolates). Nineteen CC1-MRSA-V + SCCfus isolates were detected in 2016. This increased to 31 isolates in 2017. They consisted of eight spa types with spa types t1252 detected only in 2016 and t2720, t948, t177, t217, t2720 detected only in 2017 respectively while spa types t127 and t693 were found in both years. Similarly, strains of CC1-MRSA-IV, WA MRSA-1/57 increased from nine isolates in 2016 to 25 isolates in 2017 and were associated with nine spa types with three spa types t3494, t086 and t688 detected only in 2016 and t1589, t17649, t5388 detected only in 2017. Four novel variants of CC1-MRSA were identified. These were CC1-MRSA-[V/VT + fus] (PVL’), CC1-MRSA-PseudoSCCmec [classB+fus + ccrAB1], CC1-MRSA-[V/VT + fus] and CC1-MRSA-[V/VT + fus + ccrAB1] (PVL’). While eight isolates of CC1-MRSA-[V/VT + fus] (PVL’) were detected in 2016, only four of the isolates were detected in 2017. The other genotypes, CC1-MRSA-PseudoSCCmec [classB+fus + ccrAB1], CC1-MRSA-[V/VT + fus] and CC1-MRSA-[V/VT + fus + ccrAB1] (PVL’) were detected only in 2016. The remaining CC1 MRSA strains are presented in Table S1.

A total of 49 isolates of ST772-MRSA-V [PVL’], Bengal Bay Clone were identified in 2016 (27 isolates) and 2017 (22 isolates). They consisted of seven spa types. Spa types t10795, t345, t5414, t657 were detected in 2016 and 2017 while two spa types, t1839 and t3387 were detected only in 2016 while t17441 was detected in 2017. Other ST772-MRSA genotypes identified only in 2017 consisted of ST772-MRSA-V, ST772-MRSA-V [PVL’], Bengal Bay Clone/WA MRSA-60 [ccr mutation/deletion] and ST772-MRSA-[mec V + fus] (PVL’). The ST772-MRSA-[mec V + fus] (PVL’) strain was identified as a new variant of ST772 in 2017 and belonged to spa type t20638.

Clonal Complex 5 (CC5)
CC5 consisted of 19 MRSA genotypes with CC5-MRSA-IV + SCCfus (337 isolates) as the dominant genotype. The CC5-MRSA-IV + SCCfus genotype was detected in 87 isolates in 2016 and 250 isolates in 2017 which represented a significant increase. It was associated with 11 spa types with t2235, t535, t688, t954 found in both years. (Table S1). The other common genotype was CC5-MRSA-V + SCCfus, WA MRSA-14/109 which slightly increased in prevalence from 79 in 2016 to 89 in 2017. CC5-MRSA-IV, [PVL’] and CC5-MRSA-V [sed/j/r’], WA MRSA-11/34/35/90/108 strains increased from 40 and 26 isolates in 2016 to 55 and 42 isolates in 2017 respectively. Four CC5 strains detected only in 2017 were CC5-MRSA-V [PVL’], CC5-MRSA-II [ACME’], WA MRSA-125 and the new variant strains CC5-MRSA-[V/VT + fus] and CC5-MRSA-[II + ccrAB4]. In addition, a new CC5 variant, CC5-MRSA-[V/VT + fus] (PVL’) was detected in 2016.

Clonal Complex 6 (CC6)
CC6 consisted of three MRSA genotypes. One strain was identified as MSSA by DNA microarray although phenotypically resistant to cefoxitin which could be due to failure in the hybridization of meca gene to the probe. The CC6-MRSA-IV, WA MRSA-51 genotype increased slightly from 100 isolates in 2016 to 136 isolates in 2017. They were associated with 27 spa types detected either in 2016 or 2017 (Table S1). Spa types t10888, t11288, t2849, t304, t701, t6845, t4403, t711, t190 were observed in CC6-MRSA-IV, WA MRSA-51 strains detected both in 2016 and 2017. Two isolates belonging to CC6-MRSA-V were detected only in 2017, while a new variant, CC6-MRSA-[IV + fus + ccrC], was only detected in 2016.

Clonal Complex 8 (CC8)
Sixteen MRSA genotypes consisting of ST8 (N = 74) and ST239 (N = 218) were identified as CC8. The distribution of all CC8 strains is presented in Table S1. The most common CC8 genotypes were CC8-MRSA-IV [tst1+] (15 isolates), CC8-MRSA-IV, UK-EMRSA-14/WA MRSA-5 (13 isolates), and ST8-MRSA-IV [PVL’/ACME’], USA300 (11 isolates). The CC8-MRSA-IV [tst1+] genotype was detected in seven isolates in 2016 and in eight isolates in 2017, while CC8-MRSA-IV, UK-EMRSA-14/WA MRSA-5 genotype was detected in four isolates in 2016 and nine isolates in 2017. The USA300 genotype was found in two isolates in 2016 and nine isolates in 2017. The CC8 strains found only in 2016 include CC8-MRSA-IV [sea-N315’], CC8-MRSA-IV, [PVL’, sed/j/k/q/r’], WA MRSA-62 and CC8-MRSA-[V/VT + fus] (a new variant of CC8), whereas CC8-MRSA-VI + SCCfus was identified once in 2017. Other CC8 strains were found in a small number in 2016 and 2017 (Table S1).

A total of 180 isolates were identified as ST239-MRSA-III + SCCmer, Vienna/Hungarian/Brazilian clone. This genotype was found in 101 isolates in 2016 and 79 isolates in 2017. Nine spa types, t1247, t1339, t15224, t6258, t713, t16187, t421, t860, t945 were associated with the ST239-MRSA-III + SCCmer, Vienna/Hungarian/Brazilian Clone in 2016, while six spa types, t16187, t421, t860, t945, 1037, t680 were found in 2017. The distribution of the other ST239 strains is shown in Table S1.

Clonal Complex 22 (CC22)
CC22 consisted of 12 MRSA genotypes with CC22-MRSA-IV [tst1’], UK-EMRSA-15/Middle Eastern variant detected in 202 isolates as the most prevalent genotype of CC22. The UK-EMRSA-15/Middle Eastern variant was detected in 89 isolates in 2016 and in 113 isolates in 2017. Another common CC22 genotype was the PVL-positive CC22-MRSA-IV (98 isolates) which was detected in 56 isolates in 2016 and in 42 isolates in
2017. The CC22-MRSA-V [fusC+] strain was identified once in 2017. The new variants, CC22-MRSA-IV (tst1/PVLP+) and CC22-MRSA-[VI + fus] detected in 2016–2017 were found in 25 and four isolates respectively. Another new variant, identified as CC22-MRSA-[IV + fus + ccrAB4], appeared for the first time in 2017.

**Clonal Complex 30 (CC30)**

CC30 consisted of seven ST30 and one ST36 MRSA isolates. The most common genotype was the CC30-MRSA-IV [PVLP+], Southwest Pacific Clone (130 isolates) which was detected in 68 isolates in 2016 and in 62 isolates in 2017. The most common spa type identified with this genotype was t019. Three genotypes carrying SCCmecVI with fusC were identified as variants of CC30. Twelve and three isolates were identified as CC30-MRSA-[VI + fus] (PVLP+) in 2016 and 2017 respectively. Four isolates of CC30-MRSA-[VI + fus] (PVLP+/tst1) were detected only in 2017.

**Clonal Complex 80 (CC80)**

PVLP-positive and PVLP-negative variants of CC80-IV-MRSA were identified in 177 isolates in 2016–2017. The CC80-MRSA-IV [PVLP+], European CA-MRSA Clone (138 isolates) was prevalent among MRSA isolates in 2016 (62 isolates) and 2017 (76 isolates). Spa types t044, t042, t005, t11863, t16186, t3196, t376, t10892, t1200, t15435, t416, t1247, t131, t203, t639 were identified in the PVLP-positive CC80-IV-MRSA isolates with t044 (95 isolates) as the most common spa type in 2016 and 2017. The PVLP-negative CC80-MRSA-IV variant (36 isolates) was found in 13 isolates in 2016 and in 23 isolates in 2017. The CC80-MRSA-(truncated/typical SCCmec) was identified in two PVLP-positive isolates in 2016 and in one PVLP-negative isolate in 2017.

**Clonal Complex 97 (CC97)**

A total of 171 isolates were identified as CC97 with 140 isolates recognized as CC97-MRSA-V [fusC+]. The other genotypes of CC97, CC97-MRSA-IV, WA MRSA-54/63 and CC97-MRSA-V were found in 11 and 15 isolates respectively in 2016–2017.

**Discussion**

This study investigated antibiotic resistance and clonal composition of MRSA isolates obtained from patients in Kuwait public hospitals in 2016–2017. The results revealed some changes in the prevalence of resistance to some antibiotics over the 2 years. Significantly, the prevalence of high-level mupirocin resistance decreased from 3.7% in 2016 to 2.4% in 2017. This is consistent with previous report of low prevalence of high-level mupirocin resistance in Kuwait hospitals in recent years [9]. This is reassuring because it indicates that mupirocin can still be used successfully to control MRSA infections. On the other hand, the prevalence of fusidic acid resistance increased from 51% in 2016 to 55% in 2017 which was consistent with previous reports of the high prevalence of fusidic acid resistance in Kuwait hospitals [9]. High prevalence of fusidic acid resistance in MRSA isolated in Kuwait has been suggested to be due to several factors including the emergence or importation of many MRSA genotypes that are resistant to fusidic acid in Kuwait hospitals [10], and the extensive use of topical fusidic acid creams that are readily available over the counter without prescription in Kuwait.

Most of the MRSA isolates in this study carried SCCmec IV, V and VI indicating that the majority of the isolates belonged to the community-associated MRSA genotypes. The significant increase in the proportion of isolates carrying SCCmec VI from 2016 to 2017 could be explained by the increase in the number of CC5 isolates with SCCmec VI.

The majority of the isolates obtained in the 2 years belonged to spa types t688, t304, t860, t127, t044, t311, t223 and t002 that were also dominant previously in Kuwait hospitals [5]. Most of the CC5 isolates detected in this study were of spa type t688. In contrast, t002 was the dominant spa type of CC5-MRSA obtained in New Zealand [11], Switzerland [12] and Canada [13]. However, similar to the findings of this study, t688 is also a common spa type among MRSA isolates reported in Egypt [14], a country with a large population of expatriates in Kuwait.

The established eight dominant MRSA clones consisting of CC1, CC5, CC6, CC8/ST239, CC22, CC30, CC80 and CC97 [15–19] were also the dominant clones in Kuwait hospitals in 2016 and 2017 and have been present in Kuwait since the early 2000’s [5] indicating that these clones are now well established in Kuwait hospitals. CC5 is one of the dominant and widely spread MRSA clones reported worldwide [15–19]. In this study, the CC5-MRSA isolates significantly increased (p ≤ 0.05) from 294 isolates in 2016 to 539 isolates in 2017 which could be due to the introduction of new CC5 strains in 2017.

We detected CC5-MRSA-II [ACME+] genotype for the first time in Kuwait in this study. The strain is similar to the pandemic ST5-MRSA-II clone that was reported previously in China and USA [19]. In addition, other CC5 genotypes, including CC5-MRSA-IV + SCCfus, Maltese Clone, CC5-MRSA-IV, [PVLP+/edlnA+], WA MRSA-121, and CC5-MRSA-V + SCCfus, WA MRSA-14/109, reported in this study were also reported in Saudi Arabia [20–22] suggesting that these clones maybe common in the Arabian Peninsula.
The study revealed a significant reduction in the proportion of CC8 and CC30 isolates in Kuwait hospitals during the study period. CC8 (ST239-MRSA-III) was the predominant clone among MRSA isolates obtained in Kuwait hospitals in the 90’s but its prevalence has since reduced [5]. The low prevalence of ST39-MRSA-III isolates observed in both 2016 and 2017 confirms its decline as a major contributor to MRSA infections in Kuwait hospitals although it is still a major cause of health-associated infections elsewhere [23].

The USA300 (ST8-MRSA-IV [PVL+/ACME+]) clone, the dominant CA-MRSA clone in North America [24] was detected for the first time in Kuwait in 2010 [5]. Although the number of USA300 isolates detected in 2017 (N = 9) remained remarkably low, it represents a significant increase on the single isolate obtained in 2010. As the USA300 is an important cause of infections, it is important to monitor its prevalence among patients in Kuwait hospitals.

The CC22-MRSA-IV is a well-known epidemic MRSA clone that emerged in the United Kingdom in the early 1990s [25] and soon became prevalent in other European countries [26–28]. Although CC22-MRSA was the second most common clonal complex in this study (421 isolates) similar to results of a previous study in Kuwait [5], their genotypes have increased from three genotypes identified in 2005 and 2010 [29] to 11 different genotypes in 2016 and 2017 with the CC22-MRSA-IV [tst1+], UK-EMRSA-15/Middle Eastern variant still the most common genotype. The tst-positive ST22-MRSA-IV, Middle Eastern variant is also common in Saudi Arabia [21], Gaza strip [30] and Jordan [31]. In contrast, there were no remarkable changes in the distribution of the other CC22 MRSA variants, CC22-MRSA-IV [tst1/PVL+], CC22-MRSA-[VI + fus], CC22-MRSA-[IV + fus + ccrAB4] in 2016–2017.

The proportion of isolates belonging to CC80-MRSA-IV and CC97-MRSA-V remained stable in 2016 and 2017. However, new spa types were seen for the first time in 2017 associated with these strains suggesting changes in their genetic composition. Furthermore, 45 isolates belonging to CC1, CC5, CC8, CC22, CC45, CC88, CC9/ST834 and ST72 carried spa types in 2016 that were different from those obtained in 2017 (Table S1). For example, the three isolates belonging to CC1-MRSA-V [PVL+] obtained in 2016 were associated with t127, t321, t386 while one isolate obtained in 2017 was associated with t2720. The CC9/ST834-MRSA-IV, WA MRSA-13 isolate obtained in 2016 was associated with t1379, while in 2017 it was associated with t1830. CC88-MRSA-V isolate obtained in 2016 was associated with t3153, while in 2017 it was associated with t6769. Also, ST72-MRSA-V, WA MRSA-91 was associated with t3092 in 2016 but with t537 in 2017. These observations suggest that the isolates obtained in both years were different although they belonged to the same clonal complex. This may impact proper management of infection caused by these isolates. Similar observation was seen in isolates belonging to CC5-MRSA-IV, Pediatric clone, CC8-MRSA-IV [sea*], Lyon Clone/UK-EMRSA-2, CC8-MRSA-V, WA MRSA-115/–132, ST8-MRSA-IV [PVL+/ACME–], CC22-MRSA-[VI + fus], CC22-MRSA-IV + V [PVL+], CC45/agrIV-MRSA-IV, WA MRSA-23, and CC88-MRSA-[IV + fus] (Table S1).

**Conclusion**

The study revealed the diversity in the genetic backgrounds of MRSA isolates and the stable maintenance of the dominant MRSA clones in Kuwait hospitals in 2016–2017 suggesting an on-going transmission of these clones. It also demonstrated the emergence of new variants of known genotypes in Kuwait hospitals in 2016 and 2017. Novel and creative infection prevention and control measures are required to curtail further transmission. It is still not clear why some MRSA clones are able to persist while others fail to survive in the healthcare environment. This warrants further investigations to identify the factors that contribute to the spread and maintenance of the successful MRSA clones.

**Methods**

**Sample collection**

In total, 4726 single patients, MRSA isolates were obtained from different clinical samples submitted to the clinical Microbiology diagnostic laboratory in 11 Public hospitals in Kuwait in 2016 (N = 2305) and 2017 (N = 2421). The isolates were identified using biochemical tests and tube coagulase at the diagnostic microbiology laboratory. Once it was identified as MRSA in the diagnostic laboratories, the isolates were sent to the MRSA Reference Laboratory located in the Department of Microbiology, Faculty of Medicine, Kuwait University for molecular typing where they were retested and confirmed as MRSA. The isolates were sub-cultured twice on brain-heart infusion agar (BHIA) plates to obtain pure colonies and incubated at 35 °C for 18 h. Pure cultures were preserved in beads and stored at −20 °C and −80 °C. They were recovered on brain-heart infusion agar (BHIA) and incubated at 35 °C prior to further testing.

**Antibiotic susceptibility testing**

Susceptibility to penicillin G, gentamicin, kanamycin, erythromycin, clindamycin, tetracycline, fusidic acid, trimethoprim, mupirocin, ciprofloxacin, chloramphenicol, rifampicin, cefoxitin, linezolid, vancomycin and teicoplanin were tested using the disc diffusion method according to the Clinical Laboratory Standards Institute.
(CLSI) [32]. Susceptibility to cefoxitin, vancomycin, teicoplanin, and mupirocin were confirmed by minimum inhibitory concentration (MIC) determination with Etest strips (BioMérieux, Marcy l’Étoile, France) according to the manufacturer’s instructions. S. aureus strain ATCC25923 and ATCC29213 were used as quality control strains for the disc diffusion and MIC determination, respectively. Susceptibility to fusidic acid was interpreted according to the British Society to Anti-microbial Chemotherapy (BSAC) [33].

Staphylococcal cassette chromosome mec (SCCmec) typing
SCCmec typing was performed using PCR for all MRSA isolates. Six types of SCCmec was determined by multiplex PCR using primers and protocols published previously [34]. Five μl of the PCR product was analyzed by 1.5% agarose gel electrophoresis to confirm amplification. Five S. aureus strains represented by COL (SCCmec I), XU642 (SCCmec II), WBG 525 (SCCmec III), WBG 9465 (SCCmec IV), WBG 8318 (SCCmec V) were used as quality control for each SCCmec type. The SCCmec types of the isolates were also derived from DNA microarray analysis.

Staphylococcal protein a (spa) typing
All MRSA isolates were investigated by spa typing. Amplification of spa gene was performed using synthetic primers previously published [35]. The PCR protocol consisted of an initial denaturation at 94 °C for 4 min, followed by 25 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, and extension for 3 min at 72 °C, and a final cycle with a single extension for 5 min at 72 °C. Five μl of the PCR product was analyzed by 1.5% agarose gel electrophoresis to confirm amplification. The amplified PCR product was purified using MicroElute Cycle-Pure Spin kit (Omega Bio-tek, Inc. USA) and the purified DNA was then used for sequencing PCR. The sequencing PCR product was then purified using Ultra-Sep Dye Terminator Removal kit (Omega Bio-tek, Inc. USA). The purified DNA was sequenced in an automated 3130 × 1 genetic analyzer (Applied Biosystem, USA). The sequence of spa gene was analyzed using the Ridom Staph Type software (Ridom GmbH, Wurzburg, Germany). The software detected the spa repeat and assigned each isolate with spa type.

DNA microarray
Based on spa typing, representative MRSA isolates obtained in 2016 and 2017 were subjected to DNA microarray to determine their clonal complex (CC) using the S. aureus Genotyping kit 2.0 (Alere, GmbH, Germany) with a protocol provided by the manufacturer [36].

Statistical analysis
To determine if the difference in the distribution of the isolates obtained in 2016 and 2017 is statistically significant, 2-tailed Chi square and Fisher exact was performed using Graphpad (https://www.graphpad.com/quickcalcscatMenu/). Also, the significance was calculated by comparing the proportions between two sample sizes using Epicalc Version 1.02 (J & Myatt M, Brixton Books, Brixton, UK). P ≤ 0.05 was considered to be statistically significant.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12866-020-02099-w.

Additional file 1: Table S1. Distribution of clonal complexes (CCs) among MRSA isolates 2016–2017.

Abbreviations
MRSA: Methicillin-resistant Staphylococcus aureus; HA-MRSA: Healthcare-associated or healthcare-acquired MRSA; CA-MRSA: Community-associated or community-acquired MRSA; spa: Staphylococcal protein A; MLST: Multilocus sequence typing; PFGE: Pulsed-field gel electrophoresis; CC: Clonal Complex; MIC: Minimum inhibitory concentration

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Availability of data and materials
All relevant data are available in the manuscript and supplementary file.

Ethics approval and consent to participate
This study did not involve human participants, human data or human tissue; thus ethical approval was not required. All the MRSA isolates were obtained as part of routine diagnostic microbiology investigations.

Consent for publication
Not Applicable.

Competing interests
The authors declare that they have no competing interests.

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