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Methicillin-Resistant *Staphylococcus aureus* in Northeastern Scotland in 2003 to 2007: Evolving Strain Distribution and Resistance Patterns▽

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This study explored strain distribution and resistance patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) over a 5-year period in northeastern Scotland. We noted a shift in the relative rates of epidemic strains and an increase in community-associated strains. Use of oral antibiotics to eradicate throat carriage may have contributed to trimethoprim resistance, which was observed to increase 10-fold.

In the decades following its first description, methicillin-resistant *Staphylococcus aureus* (MRSA) comprised a relatively minor component of the total *S. aureus* population, before dramatically increasing as a cause of invasive *S. aureus* infections during the 1990s (12). As methicillin resistance became more widespread, MRSA became an additional burden of disease on top of that caused by methicillin-sensitive *S. aureus* (MSSA) (9). A number of epidemic MRSA strains have been characterized (1, 3, 13, 18), of which two in particular have become dominant in hospital environments in the United Kingdom. The aim of this study was to look at MRSA strain distribution and resistance patterns in northeastern Scotland as it was felt that these were evolving from what had previously been seen in the region.

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Clinical isolates. Isolates were obtained from hospital and general practitioner specimens submitted to Aberdeen Royal Infirmary (ARI) from 2003 to 2007. NHS Grampian is the health board responsible for northeastern Scotland (population, 500,000). ARI has the only microbiology laboratory in this health board and receives all samples from the region, making it representative of the northeastern region of Scotland. Isolates were confirmed as MRSA by isolation on either ORSAB or Brilliance chromogenic agar (Oxoid) and agglutination using the Prolex-Blue Staph latex kit (Pro-Lab). All new isolates of MRSA were referred to the Scottish MRSA Reference Laboratory (SMRSARL), Stobhill Hospital, Glasgow, for typing by pulsed-field gel electrophoresis (PFGE) and susceptibility testing. For the purpose of this study, we defined community-associated (CA) strains as those carrying SCC*mec* type IV, with the exception of the health care-associated (HA) epidemic strain EMRSA15.

Isolate selection criteria. Multiple MRSA isolates from individual patients were included in this study according to the following criteria. (i) If a single specimen yielded more than one strain (as determined by PFGE), all strains were included in the study. (ii) Where the same strain was recovered from an individual patient over any time period and where the antibiograms differed by ≤2 agents, only the first isolate recovered was included in the study. (iii) If the same strain was isolated from more than one specimen type on the same date, the isolate representing the most invasive infection was kept in the study.

PFGE. Pulsed-field gel electrophoresis (PFGE) typing was performed by a modification of a previously described method (2). Briefly, *S. aureus* colonies from overnight cultures were incorporated into agarose plugs. After lysis, genomic DNA was digested with SmaI (Invitrogen). PFGE was performed by clamped homogeneous electric field (CHEF) electrophoresis with a CHEF-mapper system (Bio-Rad). Fragments were separated with a linear ramped pulse time of 6.8 to 63.8 s over 23 h at 14°C. Gels were analyzed with GelCompar II version 5.1 (Applied Maths), using the Dice correlation coefficient. A dendrogram was generated by the unweighted-pair group method using average linkages (UPGMA) with a tolerance of 1.5%.

Assignment of isolates to MLST CCs. Isolates were typed by the methods routinely used at the time they were received. Methods varied slightly, but nearly all isolates had PFGE, extended antibiogram, and urease testing. Provisionally, most isolates could be assigned phenotypically to a known group, and this was confirmed by the PFGE pattern having fewer than three band differences from a known isolate of this group. A subset of isolates, including representatives of all groups and all with unusual phenotypes or PFGE patterns, have been typed by additional methods (multilocus sequence typing [MLST] or *spa* typing) to assign the groups to MLST clonal complexes (CCs). More frequent *spa* typing performed since 2007 has supported the reliability of this method of assignment. Confirmatory MLST was performed on a small number of additional isolates from this study, and the results were consistent with the previously inferred CC assignments.

SCC*mec* typing. SCC*mec* typing was performed according to previously described methods (16).

Susceptibility testing. Antibiotic susceptibility testing was performed with a Vitek instrument with custom-made *Staphylococcus* cards—GPS-528 (bioMérieux).

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TABLE 1. MRSA isolates by strain

Strain	No. (%) of isolates					
	2003	2004	2005	2006	2007	2003–2007
CC22-MRSA-IV	504 (51.2)	751 (58.8)	1,212 (75.0)	1,411 (77.6)	1,518 (81.6)	5,396 (71.4)
CC36-MRSA-II	403 (40.9)	325 (25.4)	174 (10.8)	157 (8.6)	82 (4.4)	1,141 (15.1)
CC45-MRSA-II	21 (2.1)	114 (8.9)	144 (8.9)	100 (5.5)	75 (4.0)	454 (6.0)
CC45-MRSA-IV	4 (0.4)	7 (0.5)	5 (0.3)	5 (0.3)	1 (0.1)	22 (0.3)
CC5-MRSA-IV	6 (0.6)	48 (3.8)	43 (2.7)	90 (4.9)	101 (5.4)	288 (3.8)
CC5-MRSA-II	20 (2.0)	8 (0.6)	15 (0.9)	10 (0.5)	6 (0.3)	59 (0.8)
CC8-MRSA-IV	2 (0.2)	2 (0.2)	2 (0.1)	12 (0.7)	19 (1.0)	37 (0.5)
CC80-MRSA-IV	2 (0.2)	4 (0.3)	3 (0.2)	4 (0.2)	3 (0.2)	16 (0.2)
CC1-MRSA-IV	0 (0)	0 (0)	1 (0.1)	1 (0.1)	8 (0.4)	10 (0.1)
CC59-MRSA-IV	0 (0)	0 (0)	2 (0.1)	3 (0.2)	4 (0.2)	9 (0.1)
CC30-MRSA-IV	1 (0.1)	0 (0)	0 (0)	2 (0.1)	4 (0.2)	7 (0.1)
Other	22 (2.2)	19 (1.5)	13 (0.8)	24 (1.3)	39 (2.1)	119 (1.6)
Total	985	1,278	1,616	1,819	1,860	7,558

Statistal analysis. Data were compared by using Fisher's exact test. Statistical analysis was performed with GraphPad.

The number of isolates sent to the SMRSARL increased from 985 in 2003 to 1,860 in 2007 (Table 1). The number of clinical isolates increased slightly, reaching a plateau of around 1,000 in 2006. The number of screening samples rose dramatically due to increased admission screening; equaling the number of clinical isolates by 2007. The majority (85 to 90%) of isolates were of either of the two United Kingdom epidemic strains: EMRSA15 (CC22-MRSA-IV) and EMRSA16 (CC36-MRSA-II). Although the overall proportion of these strains remained consistent, there was a substantial shift in their relative rates, with an increase in EMRSA15 from 51% to 82% and a corresponding decrease in EMRSA16. We also noted an increase in CA strains from 3.0% to 7.5%, due largely to increases in CC5-MRSA-IV and CC8-MRSA-IV.

Analysis of the distribution of each strain in different specimen types showed that most strains were uniformly found in different specimens (Table 2). However, there were some exceptions. CC45-MRSA-II/IV comprised 6.3% of isolates (5.6% of clinical isolates) but was found in 12.1% of bacteremia cases ($P < 0.001$ compared to both total and other clinical isolates). EMRSA16 comprised 15.1% of isolates (14.7% of clinical isolates) but was found in 29.3% of respiratory specimens ($P < 0.001$). EMRSA15 was underrepresented in bacteremia (71.4% overall [70.9% of clinical isolates] compared with 60.3% of blood culture isolates; $P < 0.001$). CC8-MRSA-IV was never found from blood culture in this study, but this was not statistically significant.

For the majority of antibiotics, the levels of resistance remained fairly constant (Table 3). However, resistance to trimethoprim and fusidic acid increased from 7.8% and 4.4% to 71.6% and 10.6%, respectively. Trimethoprim resistance increased across all strains and was not due strictly to the increase in numbers of any particular strain (data not shown). In contrast, the increase in fusidic acid resistance appears to be linked to CC5-MRSA-IV (data not shown). Resistance to clindamycin, kanamycin, neomycin, and tobramycin decreased from ~43% to ~10%, and ciprofloxacin resistance was seen to decrease slightly. No difference in antibiotic resistance was observed between clinical and screening isolates (Table 3).

Unlike the rest of the United Kingdom, in northeastern

TABLE 2. Number of strains per specimen type

Strain	No. (%) of isolates								
	Blood	Swab[a]	Respiratory	Urine	Genital	Screen[b]	All clinical	Not stated	Total
CC22-MRSA-IV	140 (60.3)	1,579 (68.7)	329 (58.3)	768 (85.2)	185 (78.7)	2,369 (72.1)	3,001 (70.9)	26 (61.9)	5,396 (71.4)
CC36-MRSA-II	45 (19.4)	326 (14.2)	165 (29.3)	64 (7.1)	16 (6.8)	517 (15.7)	615 (14.7)	9 (21.4)	1,141 (15.1)
CC45-MRSA-II	27 (11.6)	102 (4.4)	37 (6.6)	45 (5.0)	9 (3.8)	229 (7.0)	221 (5.2)	4 (9.5)	454 (6.0)
CC45-MRSA-IV	1 (0.4)	14 (0.6)	1 (0.2)	0 (0)	0 (0)	6 (0.2)	16 (0.4)	0 (0)	22 (0.3)
CC5-MRSA-IV	8 (3.4)	135 (5.9)	18 (3.2)	13 (1.4)	17 (7.2)	94 (2.9)	191 (4.6)	3 (7.1)	288 (3.8)
CC5-MRSA-II	2 (0.9)	39 (1.7)	3 (0.5)	2 (0.2)	3 (1.3)	10 (0.3)	49 (1.1)	0 (0)	59 (0.8)
CC8-MRSA-IV	0 (0)	31 (1.3)	1 (0.2)	0 (0)	1 (0.4)	4 (0.1)	33 (0.8)	0 (0)	37 (0.5)
CC80-MRSA-IV	0 (0)	12 (0.5)	0 (0)	0 (0)	1 (0.4)	3 (0.1)	13 (0.3)	0 (0)	16 (0.2)
CC1-MRSA-IV	0 (0)	4 (0.2)	0 (0)	0 (0)	0 (0)	6 (0.2)	4 (0.1)	0 (0)	10 (0.1)
CC59-MRSA-IV	0 (0)	4 (0.2)	1 (0.2)	1 (0.1)	0 (0)	3 (0.1)	6 (0.1)	0 (0)	9 (0.1)
CC30-MRSA-IV	0 (0)	3 (0.1)	0 (0)	0 (0)	0 (0)	4 (0.1)	3 (0.1)	0 (0)	7 (0.1)
Other	9 (3.9)	50 (2.2)	9 (1.6)	8 (0.9)	3 (1.3)	40 (1.2)	79 (1.8)	0 (0)	119 (1.6)
Total	232	2,299	564	901	235	3,285	4,231	42	7,558

[a] Wound and surface swabs (excluding those taken for MRSA screens).
[b] MRSA screening samples consisting of nasal, throat, axilla, and groin swabs (and wounds if present).

TABLE 3. Antibiotic resistance of the isolates in this study

Antibiotic	No. (%) of isolates							
	2003	2004	2005	2006	2007	2003–2007		
						Total	Clinical	Screening
Methicillin	985 (100.0)	1,278 (100.0)	1,616 (100.0)	1,819 (100.0)	1,860 (100.0)	7,558 (100.0)	4,231 (100.0)	3,285 (100.0)
Penicillin	985 (100.0)	1,278 (100.0)	1,616 (100.0)	1,819 (100.0)	1,860 (100.0)	7,558 (100.0)	4,231 (100.0)	3,285 (100.0)
Cefoxitin	928 (94.2)	1,256 (98.3)	1,616 (100.0)	1,818 (99.9)	1,853 (99.6)	7,471 (98.8)	4,157 (98.3)	3,274 (99.7)
Ciprofloxacin	949 (96.3)	1,210 (94.7)	1,542 (95.4)	1,678 (92.2)	1,709 (91.9)	7,088 (93.8)	3,906 (92.3)	3,184 (95.6)
Erythromycin	820 (83.2)	1,031 (80.7)	1,283 (79.4)	1,428 (78.5)	1,376 (74.0)	5,938 (78.6)	3,256 (77.0)	2,644 (80.5)
Trimethoprim	77 (7.8)	341 (26.7)	886 (54.8)	1,173 (64.5)	1,331 (71.6)	3,808 (50.4)	2,067 (48.9)	1,726 (52.5)
Clindamycin	426 (43.2)	437 (34.2)	380 (23.5)	305 (16.8)	209 (11.2)	1,757 (23.2)	926 (21.9)	818 (24.9)
Kanamycin	424 (43.0)	430 (33.6)	318 (19.7)	276 (15.2)	183 (9.8)	1,631 (21.6)	873 (20.6)	745 (22.7)
Fusidic acid	43 (4.4)	78 (6.1)	112 (6.9)	177 (9.7)	197 (10.6)	607 (8.0)	396 (9.4)	207 (6.3)
Neomycin	423 (42.9)	431 (33.7)	317 (19.6)	265 (14.6)	178 (9.6)	1,614 (21.4)	847 (20.0)	754 (23.0)
Tobramycin	420 (42.6)	426 (33.3)	313 (19.4)	259 (14.2)	172 (9.2)	1,590 (21.0)	837 (19.8)	740 (22.5)
Tetracycline	33 (3.4)	109 (8.5)	178 (11.0)	131 (7.2)	136 (7.3)	587 (7.8)	365 (8.6)	221 (6.7)
Mupirocin	38 (3.9)	39 (3.1)	26 (1.6)	35 (1.9)	38 (2.0)	176 (2.3)	99 (2.3)	76 (2.3)
Sulfamethoxazole	26 (2.6)	124 (9.7)	146 (9.0)	103 (5.7)	83 (4.5)	482 (6.4)	227 (5.4)	250 (7.6)
Gentamicin	3 (0.3)	7 (0.5)	9 (0.6)	21 (1.2)	18 (1.0)	58 (0.8)	35 (0.8)	23 (0.7)
Rifampin	21 (2.1)	26 (2.0)	17 (1.1)	21 (1.2)	16 (0.9)	101 (1.3)	56 (1.3)	45 (1.4)
Chloramphenicol	0 (0)	20 (1.6)	13 (0.8)	11 (0.6)	8 (0.4)	52 (0.7)	13 (0.3)	39 (1.2)
Total	985	1,278	1,616	1,819	1,860	7,558	4,231	3,285

Scotland, EMRSA16 had previously been present at a higher rate than EMRSA15. This pattern has changed dramatically over the last decade; during 1997 to 2000, EMRSA15 and EMRSA16 comprised 15.4% and 80% of all MRSA isolates, respectively (15). At a similar time, the proportions of United Kingdom bacteremia isolates identified as either EMRASA15 or EMRSA16 were 60.2% and 35.4%, respectively (11). During our study, the proportions of these two strains completely reversed, bringing the pattern into line with the rest of the United Kingdom (5).

The increase in CA strains mirrors the increase seen across the United Kingdom (6, 7), although different strains appear to be more prevalent in different regions (7, 17). Although we observed an increase in CC8-MRSA-IV, the number of isolates remains relatively low. The absence of this strain from blood culture in our study is therefore unlikely to be significant, and it has been identified from blood culture in other United Kingdom studies (4, 7, 17). The related North American strain (USA300) is the predominant U.S. CA strain. In recent years, it has become a highly prevalent cause of skin and soft tissue infections (8) and has been reported to be the causative isolate in 26% of MRSA bloodstream infections (14).

For the majority of antibiotics, resistance levels were comparable to the rest of the United Kingdom (10). Averaged out over the study period, trimethoprim resistance in bacteremia isolates was 38.4%, nearly double the level reported by the British Society for Antimicrobial Chemotherapy (BSAC) (10). In 2007, 73.8% of MRSA bacteremia isolates in northeastern Scotland were resistant to trimethoprim. During our study period, trimethoprim resistance increased 10-fold, and in 2007, it was 42 times higher than the 1.7% reported in the same region in 1997 to 2000 (15). In 2003, a policy was introduced by Grampian's NHS Board, the University Hospitals Trust and the Primary Care Trust, to ensure prompt identification and effective management of MRSA-positive individuals. Part of this policy, perhaps unusually for the United Kingdom, recommended that patients with throat carriage should be decolonized with oral antibiotics. Recommended first-line antibiotics were trimethoprim and fusidic acid together, and in the event of intolerance or resistance to fusidic acid, rifampin was used in its place. It may be likely that this use of trimethoprim has driven the observed resistance or has selected for a high prevalence of a resistant clone, as this level of resistance appears to be unique to the region. We have no explanation for why there does not appear to have been the same effect on fusidic acid or rifampin resistance. We believe that the initial high levels of and subsequent decrease in resistance to clindamycin, kanamycin, neomycin, and tobramycin are related to EMRSA16. The increase in ciprofloxacin susceptibility can most likely be explained by the increase in CA strains.

A variety of different intervention strategies have been proposed or implemented in order to reduce the number of HA MRSA infections. As this study has shown, our particular aggressive approach to eradication may have unfavorable outcomes on local resistance patterns without clear benefits in the control of MRSA.

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