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Polyclonal multiply antibiotic-resistant methicillin-resistant *Staphylococcus aureus* with Panton–Valentine leucocidin in England

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Objectives: Community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) including those encoding Panton-Valentine leucocidin (PVL) are often described as more susceptible to a range of antibiotics than their hospital-associated counterparts. Recent scattered reports of the emergence of multiresistant PVL-MRSA have highlighted the potential for resistance to emerge. Here we detail polyclonal multiply antibiotic-resistant PVL-MRSA occurring in England.

Methods: PVL-MRSA from community-based and hospitalized patients located across England were identified by PCR. Isolates were characterized via MIC determinations, toxin gene profiling, PFGE, SCC*mec*, *spa* and *agr* typing. Multilocus sequence typing (MLST) was performed on selected isolates. Patient demographic and available disease data were retained for analysis.

Results: Seventy-six PVL-MRSA isolates resistant to three further classes of antibiotic were identified between 2005 and 2008 from centres in each of the Health Protection Agency's geographic regions in England. Patient demographics were typical for PVL-MRSA, and some travel associations were identified along with clonal spread. One instance of familial transmission in the community was detected. PVL-MRSA belonging to MLST clonal complex (CC) 1 (sequence type 772) were consistently highly resistant; multiply antibiotic-resistant representatives of CCs 5, 8, 22, 59 and 80 were also identified. Ciprofloxacin resistance was common amongst the study isolates (51 of 76 isolates).

Conclusions: Genetically diverse multiply antibiotic-resistant PVL-MRSA were identified, and included representatives of a recently emerged multiresistant clone (dubbed the Bengal Bay clone). Risk factors and disease presentations were typical for PVL-MRSA infections. This work highlights the diminishing utility of ciprofloxacin susceptibility for putative identification of PVL-MRSA.

Keywords: PVL, community, MRSA

Introduction

Worldwide, healthcare-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) are commonly resistant to multiple classes of antibiotic with pandemic lineages including the New York–Japan clone [sequence type (ST)5-MRSA-SCC*mec*II] and the Brazilian clone (ST239-SCC*mec*III).[1] Recently emerged community-associated strains of MRSA (CA-MRSA) with enhanced transmissibility and/or virulence have become increasingly recognized worldwide. CA-MRSA are associated with a significant burden of disease, most often skin and soft tissue infections in young adults (18–40 years), but also severe diseases such as necrotizing pneumonia associated with high mortality rates.[2]

CA-MRSA are often more genetically diverse within a locale, and less antibiotic resistant than HA-MRSA,[3] with many being susceptible to most antimicrobials excepting β-lactams, although this situation is evolving.[4] In North America the predominant CA-MRSA clone, USA300, which encodes Panton–Valentine leucocidin (PVL), has caused an epidemic of infections in community settings and has now infiltrated and become endemic in some US healthcare establishments.[5] Recently, a multiply antibiotic-resistant variant of USA300 has emerged in San Francisco with resistance to mupirocin, erythromycin and clindamycin, and variably to ciprofloxacin and tetracycline.[4] Additionally, multiply antibiotic-resistant representatives of the South-East Asian clone (ST59-MRSA-SCC*mec*VII/IV) with PVL

have also been reported.[6] Nevertheless, multiply antibiotic-resistant PVL-MRSA remain unusual, for the time being at least.

The Health Protection Agency's national *Staphylococcus* Reference Unit has reported 11 lineages of PVL-MRSA in England. Herein we report the widespread geographic distribution of genetically diverse ciprofloxacin and multiply drug-resistant PVL-MRSA, and the detection of a newly emerged multiply antibiotic-resistant clone of PVL-MRSA in England.

Methods

Bacterial isolates and susceptibility testing

MICs for *S. aureus* isolates from suspected PVL-related disease and close contact screenings, submitted to the national *Staphylococcus* reference laboratory for England, between 2005 and 2008 that tested positive for the *mecA* and *lukSF*-PV (PVL) genes were determined by Etest (AB BIODISK, Solna, Sweden), or agar dilution using Iso-Sensitest agar (Oxoid, Basingstoke, UK) according to the BSAC method. The antimicrobials tested were: penicillin, oxacillin, ciprofloxacin, tetracycline, doxycycline, minocycline, tigecycline, erythromycin, gentamicin, neomycin, fusidic acid, clindamycin, rifampicin, quinupristin/dalfopristin, teicoplanin, vancomycin, daptomycin, mupirocin and linezolid. Inducible resistance to clindamycin was tested by the BSAC D-disc test. Seventy-six PVL-MRSA resistant to three or more classes of commonly used antimicrobials [fluoroquinolones, aminoglycosides, macrolides/lincosamides (erythromycin and clindamycin), tetracyclines, fusidic acid and mupirocin] were retained for study. Wherever possible, patient demographic and clinical data were collected from referral forms, returned clinical questionnaires and telephone follow-up.

Genetic characterization of isolates

In addition to multiplex PCR, which was used to detect *mecA*, *lukSF*-PV and 13 other toxin genes, isolate relatedness was analysed by PFGE of total DNA restricted with SmaI, as described previously.[7] Banding patterns were analysed using BioNumerics software (Applied Maths, Ghent, Belgium). Isolates with banding profiles >80% similar were considered closely related. As described previously,[7] PCR to determine *agr*, SCC*mec* and *ccr* type, in addition to *spa* sequence typing, was used, and for selected isolates, multilocus sequence typing (MLST) was performed to determine the ST and clonal complex (CC).

Results

Seventy-six multiresistant PVL-MRSA belonging to six distinct genetic lineages were identified amongst isolates referred to the national reference laboratory, and comprised 3 in 2005, 9 in 2006, 27 in 2007 and 37 in 2008. The isolates were referred from each of the Health Protection Agency's nine regions in England, with no more than three highly resistant PVL-MRSA of the same lineage referred by each centre, indicating geographic dissemination (Table 1). Patient demographics were typical for individuals with PVL-MRSA: the male/female ratio was almost equal and patients had a mean age of 32 years (median 31 and mode of 27 years; data from 75 individuals were available). Available clinical data (from 62 individuals) indicated that the majority (53/76) presented with skin and soft tissue infections, four individuals were asymptomatic (identified as a result of outbreak control screening) and five patients had invasive PVL-associated disease (two of whom subsequently died). The isolates from the five patients with invasive disease represented four distinct lineages. Direct strain transmission between two community-based family members was detected. Various antibiotic susceptibility profiles were identified within each of five genetic lineages of highly resistant PVL-MRSA detected (see below). Heterogeneous oxacillin MICs (range: 1–128 mg/L) were observed, and ciprofloxacin resistance was detected in 49 of 76 isolates, representing MLST CCs 8 (ST8), 22 (ST22), 80 (ST80), 1 (ST772) and 5 (ST866). All isolates tested remained susceptible to rifampicin, quinupristin/dalfopristin, linezolid and vancomycin. The characteristics of the multiresistant PVL-MRSA identified in England are summarized below.

Table 1. Regional distribution of multiply antibiotic-resistant PVL-MRSA detected in England between 2005 and 2008

	MLST CC						
HPA regions[a]	CC8	CC22	CC59	CC80	CC1 (ST772-like)	CC5 (ST866-like)	Total
East of England	1	1	2	1	2		7
East Midlands		2		1		3	6
London	3	1		3	5		12
North East		2	2	1	1		6
North West		2	2		4	1	9
South East	1		4		8	1	14
South West		2		2	3		7
West Midlands		2		1	4	1	8
Yorkshire and Humber	2			2	2	1	7
Total	7	12	10	11	29	7	76

[a]See: http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/Page/1169747331532.

CC1 (ST772-like) PVL-MRSA

Thirty multiply antibiotic-resistant isolates, referred from 23 different centres were related to known ST772 isolates (CC1; single locus variant of ST1) by *spa* and SCC*mec* typing and other genetic characteristics (Table 2). Seventeen of the 30 cases had evidence of a travel or familial link with the Bengal Bay area or the Indian subcontinent. All 30 isolates were ciprofloxacin resistant and a further 'core' resistance pattern of erythromycin, gentamicin and trimethoprim resistance was observed in 26/30 isolates; tetracycline resistance was also noted in two isolates.

CC5 (ST866-like) isolates

Six isolates were genetically similar and grouped by PFGE with isolates known to be ST866 (CC5; double locus variant of ST5) (Table 2). The six isolates had highly variable antibiotypes: four were resistant to gentamicin and three were resistant to erythromycin (clindamycin inducible). Resistance to tetracycline, mupirocin and ciprofloxacin was variable.

Table 2. Characteristics of multiresistant PVL-MRSA

Isolates (*n*)	MLST CC	ST	Antibiotic resistances	*spa* type	*spa* repeat succession	SCC*mec* type	*ccr*	*agr*	Toxin gene profile
24	1	772	OXA, (CHL), CIP, ERY, GEN, TMP	t657 t345	r26r23r13r21r17r34r33r34 r26r23r13r21r17r34r34r33r34	VII	C2	2	*sea*, (*sec*), *seg*, *sei*, *lukSF*-PV
3	1		OXA, (CHL), CIP, ERY, (GEN), TET, TMP	t657 t345	r26r23r13r21r17r34r33r34 r26r23r13r21r17r34r34r33r34	VII	C2	2	*sea*, *seg*, *sei*, *lukSF*-PV
3	1		OXA, (CHL), CIP, GEN, (TET), (TMP)	t657	r26r23r13r21r17r34r33r34	VII	C2	2	*sea*, (*sec*), *seg*, *sei*, *lukSF*-PV
6	5	866	OXA, ERY, CLI (I), TET, DOX, (GEN), (TMP), (CIP)	t002	r26r23r17r34r17r20r17r12r17r16	IV	2	2	*(sea)*, *(sec)*, *seg*, *sei*, *tst*, *lukSF*-PV
10	59	59	OXA, (CIP), ERY, CLI, NEO, TET, DOX	t437 t3590	r04r20r17r20r17r25r34 r04r02r17r20r17r25r34	VII	C2	1	*(seb)*, *lukSF*-PV
4	80	80	OXA, NEO, TET, FUS,	t044	r07r23r12r34r34r33r34	IV	2	3	*seh*, (*etd*), *lukSF*-PV
4	80		OXA, ERY, CLI (I), (NEO), TET, DOX, FUS	t044 t131	r07r23r12r34r34r33r34 r07r23r12r34r33r34	IV	2	3	*seh*, (*etd*), *lukSF*-PV
3	80		OXA, (CIP), (GEN), TET, (TMP), FUS	t044	r07r23r12r34r34r33r34	IV	2	3	*seh*, (*etd*), *lukSF*-PV
3	22	22	OXA, CIP, ERY, GEN, TMP	t852	r07r23r13r23r31r05r17r25r17r25r16r28	IV	2	1	*seg*, *sei*, *lukSF*-PV
4	22		OXA, CIP, GEN, TMP	t005 t2816 t1516	r26r23r13r23r31r05r17r25r17r25r16r28 r26r23r13r23r31r31r05r17r25r17r25r16r28 r26r25r17r25r16r28	IV	2	1	*seg*, *sei*, *lukSF*-PV
4	22		OXA, ERY, CLI (I), GEN, TMP, (MUP)	t005	r26r23r13r23r31r05r17r25r17r25r16r28	IV	2	1	*seg*, *sei*, *lukSF*-PV
1	22		OXA, CIP, ERY, CLI (I), FUS	t1790	r26r23r23r29r17r25r17r25r28	IV	2	1	*sec*, *seg*, *sei*, *lukSF*-PV
3	8	8	OXA, CIP, ERY, CLI (I), TET	t008 t121	r11r19r12r21r17r34r24r34r22r25 r11r19 . . . r21r17r34r24r34r22r25	IV	2	1	*lukSF*-PV
2	8		OXA, CIP, ERY, CLI (I), FUS, (MUP)	t008	r11r19r12r21r17r34r24r34r22r25	IV	2	1	*lukSF*-PV
2	8		OXA, CIP, ERY, CLI (I), GEN, (MUP)	t008	r11r19r12r21r17r34r24r34r22r25	IV	2	1	*lukSF*-PV

OXA, oxacillin; CHL, chloramphenicol; CIP, ciprofloxacin; ERY, erythromycin; GEN, gentamicin, TMP, trimethoprim; TET, tetracycline; CLI (I), clindamycin (inducible); DOX, doxycycline; NEO, neomycin; FUS, fusidic acid; MUP, mupirocin.
All isolates tested for toxin genes *sea-e*, *seg-j*, *eta*, *etb*, *etd*, *tst* and *lukSF*-PV.
Bracketed data denote variable findings.
All isolates tested were susceptible to quinupristin/dalfopristin, minocycline, daptomycin, tigecycline, vancomycin, teicoplanin and linezolid.

CC8 isolates

Seven isolates, from four geographically distinct centres, included six ACME-*arcA*-positive USA300-like isolates (Table 2). All seven isolates were resistant to erythromycin and ciprofloxacin. Resistance to gentamicin, tetracycline, fusidic acid and mupirocin was variable. The two most resistant isolates were resistant to four antibiotic classes including fluoroquinolones, macrolides and mupirocin with variable aminoglycoside or fusidic acid resistance.

CC22 isolates

Twelve genetically similar isolates, from 11 geographically distinct centres, were grouped with known ST22 (CC22) PVL-MRSA based upon PFGE banding profiles. Trimethoprim and gentamicin resistance was observed in 11/12 CC22-PVL-MRSA. Eight isolates were ciprofloxacin resistant (Table 2).

CC59 isolates

Ten multiresistant CC59 isolates harbouring either SCC*mec*IV or VII referred from 10 different centres were otherwise genetically similar (Table 2). All 10 isolates were erythromycin, clindamycin, neomycin and tetracycline resistant; a single isolate was also resistant to ciprofloxacin.

CC80 isolates

Eleven genetically similar multiply antibiotic-resistant isolates (each from different centres) similar to known European clone CC80 PVL-MRSA were detected. All 11 isolates were resistant to tetracycline and fusidic acid in addition to one or more of erythromycin (with inducible clindamycin resistance), gentamicin or trimethoprim. Ciprofloxacin resistance was detected in one multiply antibiotic-resistant CC80 PVL-MRSA isolate.

Discussion

Community-associated (including PVL-positive) MRSA are broadly considered less resistant than their HA-MRSA counterparts, but do appear to be evolving resistance.[4] Using the criterion of MRSA resistant to three or more further classes of antibiotic (including fluoroquinolones, aminoglycosides, macrolides, tetracyclines, fusidic acid, mupirocin), we report increasing numbers of multiply antibiotic-resistant PVL-MRSA identified amongst isolates referred to the national *Staphylococcus* reference laboratory.

Globally disseminated PVL-MRSA clones were represented amongst the six lineages of multiply antibiotic-resistant PVL-MRSA identified; no single lineage was more associated with invasive disease than another. Notwithstanding reference laboratory bias, the geographic distribution and the partial travel association of the cases suggest that these multiply antibiotic-resistant PVL-MRSA were disseminated across England, albeit at a low level, during the study period; however, their numbers did build annually across the four study years. Previously, ST772 *S. aureus* with PVL have been detected in India,[8] Bangladesh,[9] as well as Malaysia where common travel to/from Bangladesh was noted.[10] The ST772 PVL-MRSA cases detected in England in this study were often linked with India and Bangladesh ($n=17/30$). The consistently high level of antimicrobial resistance, clonality and geographic dissemination of the isolates detected emphasizes the potential transmissibility of this lineage (herein dubbed the 'Bengal Bay clone'). Multiply antibiotic-resistant representatives of other international CA-MRSA clones included USA300, but in contrast to the situation in the USA,[4] mupirocin, erythromycin/clindamycin, ciprofloxacin and tetracycline resistance did not occur in the same UK USA300 isolate in this study. The convergence of ciprofloxacin resistance in multiple PVL-MRSA lineages highlights the diminishing utility of the identification of ciprofloxacin susceptibility in MRSA as a putative marker for PVL-MRSA. This, alongside reference laboratory bias/non-systematic collection of isolates, suggests a current under-ascertainment of the totality of multiply antibiotic-resistant PVL-MRSA and, by inference, multiply antibiotic-resistant CA-MRSA in the UK, and underlines the need for continued and enhanced surveillance of antibiotic susceptibility amongst them.

Guidance for the treatment of PVL *S. aureus*-related infections in England[11] recommends agents such as linezolid, clindamycin and rifampicin for the treatment of serious disease and mupirocin for decolonization purposes. Whilst resistance to linezolid and rifampicin was not detected amongst the isolates tested, resistance to clindamycin (constitutive or inducible) and mupirocin was detected in multiple lineages of PVL-MRSA. Similarly, doxycycline is one of the agents recommended for treatment of skin and soft tissue infections, and here we identified tetracycline resistance (with a concomitant decrease in doxycycline susceptibility), in multiple lineages of multiply antibiotic-resistant PVL-MRSA suggesting these compounds to be of diminishing efficacy against some PVL-MRSA. Together, these data highlight the need for (i) improved ongoing surveillance via prospective studies, (ii) the choice of therapeutic regimen to be backed up by susceptibility data and (iii) the referral of isolates to reference laboratories for PVL testing to be based on clinical suspicion rather than susceptibility data.

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Transparency declarations

None to declare.

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