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Emergent and evolving antimicrobial resistance cassettes in community-associated fusidic acid- and meticillin-resistant *Staphylococcus aureus*

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ABSTRACT

Fusidic acid is a topical and systemic antimicrobial used for the treatment of staphylococcal infections in hospitals and the community. Sales of fusidic acid and resistance rates among meticillin-resistant *Staphylococcus aureus* (MRSA) doubled between 1990 and 2001. For the following decade, fusidic acid resistance rates among isolates from Addenbrooke's Hospital (Cambridge, UK) were compared with national resistance rates from MRSA bacteraemia surveillance data and with antimicrobial sales data. Sales of fusidic acid remained relatively constant between 2002 and 2012, whilst fusidic acid resistance increased three- and four-fold in MRSA bacteraemias nationally and in MRSA isolates from Cambridge, respectively. A subgroup of MRSA resistant only to fusidic acid increased after 2006 by 7-fold amongst bacteraemias nationally and 17-fold (to 7.7% in 2012) amongst Cambridge MRSA isolates. All of the available local isolates from 2011 to 2012 ($n = 23$) were acquired in the community, were not related epidemiologically and belonged to multilocus sequence typing (MLST) groups ST1, 5, 8, 45 or 149 as revealed from analysis of whole-genome sequence data. All harboured the *fusC* gene on one of six distinct staphylococcal cassette chromosome (SCC) elements, four of which were dual-resistance chimeras that encoded β-lactam and fusidic acid resistance. In summary, fusidic acid-resistant MRSA increased in prevalence during the 2000s with notable rises after 2006. The development of chimeric cassettes that confer dual resistance to β-lactams and fusidic acid demonstrates that the genetics underpinning resistance in community-associated MRSA are evolving.

1. Introduction

The emergence and dissemination of community-associated meticillin-resistant *Staphylococcus aureus* (CA-MRSA) lineages in the community [1] and hospital settings [2] pose a significant clinical problem due to the lack of response to first-line empirical antimicrobials (β-lactams). Alternative agents that can be easily administered in either setting are required, particularly in light of the fact that skin and soft-tissue infections (SSTIs) are now the predominant cause of MRSA bacteraemia in the UK (R. Hope, unpublished data). One option is fusidic acid, which is licensed in Western Europe, Canada, Australia, New Zealand and numerous countries in Asia (but not in the USA) [3] for the topical treatment of superficial staphylococcal infections affecting the skin and eye as well as for systemic administration for deep-seated infections including those of the bones and joints.

The utility of fusidic acid depends on the rate of resistance, which differs between countries [4,5]. For example, the rate of resistance among *S. aureus* in 2007–2008 in the USA was only 0.3%

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compared with 7% in Canada and Australia and 11.8% in the UK [4,5]. Fusidic acid inhibits protein synthesis by preventing the turnover of elongation factor G (EF-G) from the ribosome, and resistance usually emerges through point mutation(s) in the chromosomal gene encoding EF-G (*fusA*), which typically confers high-level resistance [6]. More rarely, lower-level resistance can arise via the acquisition of factors that protect the translational machinery (encoded by plasmid-borne genes *fusB* or *fusC*) [5,7].

Fusidic acid resistance among MRSA causing bacteraemia in the UK increased from 1.8% in 1990 to 5.5% in 2001, which accompanied the increased use of this drug from 1.2 t to 3 t annually during the same period [8]. In this study, fusidic acid usage was correlated with fusidic acid resistance rates in bacteraemia isolates in the decade from 2002 in England. Fusidic acid resistance in all MRSA (bacteraemia and non-bacteraemia) was investigated by examining data from patients admitted to Addenbrooke's Hospital in Cambridge (UK). This was complemented with a detailed genomic investigation of the resistance mechanisms of MRSA isolates that were resistant to fusidic acid but susceptible to the panel of other drugs commonly tested. Specifically, the hypothesis that fusidic acid resistance had emerged and was disseminating via a mobile genetic element in one or more CA-MRSA lineages was examined.

2. Methods

2.1. Antimicrobial usage data, and local and national surveillance data

National antimicrobial prescription data collected by IMS Health (London, UK) between 2002 and 2012 were supplied and analysed for changes in the amounts and proportions of the drug that was sold. National surveillance data for MRSA bacteraemias submitted by National Health Service (NHS) trusts to Public Health England via the voluntary reporting system [9] between 2002 and 2013 was accessed. From 2002 to 2013, the overall proportion of MRSA reported with associated fusidic acid susceptibility data was 80.4% (annual median rate 80.8%, range 71.8–89.0%), whilst the overall proportion with susceptibility data for fusidic acid, a β-lactam drug, erythromycin, ciprofloxacin, gentamicin and tetracycline was 47.5% (annual median proportion 52.1%, range 27.6–65.2%) (Supplementary Fig. S1). To examine local trends, the microbiology laboratory database at Cambridge University Hospitals NHS Foundation Trust (CUH) was accessed to identify all patients who were MRSA-positive between 2002 and 2013 and it was found that >90% of first isolates of MRSA had been tested for fusidic acid plus a β-lactam drug, erythromycin, ciprofloxacin, gentamicin and tetracycline between 2002 and 2013 (Supplementary Fig. S1). Data were collected on the first MRSA-positive sample from each case and included year of isolation, patient location at the time of sampling and sample type. Cases with an MRSA isolate that was resistant to fusidic acid but otherwise susceptible to other routinely tested drugs were identified and were expressed as a proportion of the MRSA tested for fusidic acid per year (see Supplementary Fig. S1).

2.2. Local clinical setting and microbiology

CUH is a 1000-bed secondary and tertiary referral hospital. The on-site Clinical Microbiology and Public Health Laboratory provides diagnostic microbiology services to CUH, two additional NHS trusts and three primary care trusts in the area. For CUH, ca. 600 000 clinical specimens are processed per year including screening samples for MRSA, which since 2009 have been taken for all emergency and elective admissions. Antimicrobial susceptibility testing was performed by the diagnostic laboratory at CUH for cefoxitin, erythromycin, ciprofloxacin, gentamicin, tetracycline, rifampicin, fusidic acid and mupirocin using the disk diffusion method as defined by the British Society for Antimicrobial Chemotherapy [10]. Additional testing was performed using a VITEK® 2 instrument (bioMérieux, Marcy-l'Étoile, France) to determine susceptibility to oxacillin, trimethoprim/sulfamethoxazole, linezolid and tigecycline as well as the minimum inhibitory concentration (MIC) to fusidic acid.

2.3. Bacterial genome sequencing and sequence analysis

DNA was extracted from *S. aureus*, sequencing libraries were prepared and whole-genome sequencing (WGS) was performed on an Illumina MiSeq instrument (Illumina Inc., San Diego, CA) as previously described [11]. The genome data have been deposited in the European Nucleotide Archive (see Supplementary Table S1). Multilocus sequence typing (MLST) types were assigned from the sequence data [11]. Having established the sequence type (ST), sequence reads were mapped to the relevant reference genome representing four STs (ST1, ST5, ST8 and ST45; accession nos. BX571857, BA000018, CP000255 and BX571856, respectively) using SMALT (https://www.sanger.ac.uk/resources/software/smalt/) [12]. Single nucleotide polymorphisms (SNPs) were identified using a standard approach by removing SNPs with low quality scores and by filtering for SNPs that were present in ≥75% of the mapped reads [11]. Mobile genetic elements were excluded from the resulting whole-genome alignments [13]. Genes encoding antimicrobial resistance were detected by mapping a pseudomolecule that included the known acquired fusidic acid resistance genes in *S. aureus* (Supplementary Table S2) against de novo genome assemblies using SMALT. This allowed the same gene to map multiple times to the assembly using 90% nucleotide identity as the cut-off for detection as described previously [11]. Sequence reads were also mapped against the susceptible variant of the *fusA* gene (encoding the EF-G) to detect mutations conferring resistance towards fusidic acid. Staphylococcal cassette chromosome (SCC) regions were visualised (including sequence coverage and SNP variations) using Artemis [14] and were compared using the Artemis Comparison Tool. The *fusC*-encoding region in the ST8 isolate (MRSA18) was highly fragmented and was not analysed further. Maximum likelihood phylogenies of the SCCs were estimated using RAxML [13].

3. Results

3.1. Trends in fusidic acid sales and MRSA fusidic acid resistance

The amount of fusidic acid prescribed annually in the UK was relatively constant at ca. 3 t between 2002 and 2009, with a decrease to 2.5 t in 2012 (Fig. 1A). The majority (78–85%) of fusidic acid was prescribed in the community, of which between 82% and 90% was for topical use (Fig. 1A). By contrast, topical preparations accounted for 9–13% of fusidic acid sales in the hospital (data not shown). Contrary to the modest decline in overall sales of fusidic acid, the percentage of fusidic acid resistance amongst MRSA bacteraemia isolates in England approximately doubled from 8.4% to between 15 and 18% over the same time period (Fig. 1B). Notably, MRSA bacteraemia isolates that were only resistant to fusidic acid but were otherwise susceptible were not detected in 2002 but steadily increased in number to reach to 2.4% of MRSA bacteraemia isolates by 2012 and 3.9% in 2013 (Fig. 1B). These increases occurred in the context of a six-fold decrease in national MRSA bacteraemias, from a peak of 5522 cases in 2003 to 887 cases in 2013 (Fig. 1B).

To investigate whether these bacteraemia surveillance data were representative for MRSA isolated from other sample sites, the

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