

SHORT REPORT

First report of identification of livestock-associated MRSA ST9 in retail meat in England

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SUMMARY

Sixty percent of all meat consumed in the UK is imported from European countries where there have been increasing reports of methicillin-resistant *Staphylococcus aureus* (MRSA) identified in food-producing animals, but rarely from such animals in the UK. Thirty samples each of raw chicken, pork and beef, sourced in England, were collected from retail outlets in Greater Manchester. MRSA was recovered from three chicken samples and one each of pork and beef, all from prepackaged supermarket meat. Four isolates were identified as representatives of the most common human healthcare-associated MRSA clone in the UK [EMRSA-15, *spa* type t032, belonging to multilocus sequence type clonal complex 22 (MLST-CC22)], suggesting contamination from human source(s) during meat processing. The fifth isolate (from chicken) was multiply-resistant (including oxacillin, ciprofloxacin, erythromycin, clindamycin and tetracycline), identified as ST9-SCC*mecIV*, *spa* type t1939 and lacked the immune evasion cluster, a characteristic of livestock-associated strains. This lineage has been identified previously from animals and meat products in Asia and mainland Europe but not the UK.

Key words: livestock, meat, MRSA, *Staphylococcus aureus*.

Staphylococcus aureus is a major pathogen of humans, causing a wide range of diseases from minor skin infections to more severe illnesses such as food poisoning, toxic shock, septicaemia and pneumonia. Antibiotic-resistant strains of *S. aureus*, in particular methicillin-resistant *S. aureus* (MRSA) are a public health and therapeutic concern. MRSA were first reported in humans in the 1960s and, subsequently, have emerged as an important nosocomial pathogen with multiple healthcare-associated MRSA

(HA-MRSA) clones having disseminated internationally. More recently, distinct lineages of MRSA have been identified in the community and also from livestock [known as community-associated MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA), respectively], highlighting the adaptation of the species to diverse ecological niches [1].

Worldwide, various clones of MRSA have been reported in domestic pets, livestock, wild birds, and other animals [1]. One LA-MRSA lineage has emerged of particular note: multilocus sequence type clonal complex 398 (MLST CC398). Following its detection in animals and in-contact humans, most notably pigs and pig farmers, CC398 LA-MRSA is now recognized as an important cause of zoonotic

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Table 1. Breakdown of raw meat samples and sources

Type of raw meat and source	No. of MRSA-positive samples			Date of purchase
	Beef	Pork	Chicken	
Pre-packaged				
Supermarket 1	1/3	1/3	3/3	6 March 2011
Supermarket 2	0/3	0/3	0/3	7 March 2011
Supermarket 3	0/3	0/3	0/3	10 March 2011
Supermarket 4	0/3	0/3	0/3	12 March 2011
Supermarket 5	0/3	0/3	0/3	14 March 2011
Supermarket 6	0/3	0/3	0/3	15 March 2011
Unpackaged				
Butcher's shop 1	0/3	0/3	0/3	9 March 2011
Butcher's shop 2	0/3	0/3	0/3	9 March 2011
Butcher's shop 3	0/3	0/3	0/3	11 March 2011
Butcher's shop 4	0/3	0/3	0/3	11 March 2011

infections in several countries throughout Europe, Asia, USA and has recently been identified in a pig in Northern Ireland [2].

MRSA has been identified in retail meat (including beef, veal, lamb, pork, poultry) with varying frequencies worldwide. In sporadic surveys of raw meat conducted in the European Union, MRSA rates of up to 37.2% have been reported [3], with CC398 LA-MRSA predominating. Of further concern, rates in excess of 60% have been observed in turkey meat [4].

Although food is not currently considered to be a relevant source of MRSA for infection or colonization of humans, the monitoring of MRSA in various food products in several countries in Europe indicates that MRSA can be detected quite frequently in different types of raw meat [4]. The identification of MRSA in food in the UK could be of public health concern. We therefore undertook a study to determine the prevalence of MRSA in raw meat. Herein, we describe the first reported isolation of MRSA from raw chicken, beef and pork, including a LA-MRSA strain not reported previously in England.

Thirty samples each of raw beef, pork and chicken were collected from six supermarkets and four butcher's shops chosen at random within a 2 mile radius in Greater Manchester, during March 2011. The meat purchased from supermarkets was pre-packaged in 200–500 g weights; the meat from butchers was purchased in 25–35 g aliquots. All samples were stored overnight at 5 °C prior to testing.

Using a Stomacher blender, 25 g of each meat sample was homogenized with 9 ml peptone water (Oxoid, UK) for 2 min. A 1 ml aliquot was added to 9 ml Mueller–Hinton broth containing 6.5% NaCl

(MHB, Oxoid) and incubated at 37 °C for 16–24 h. After enrichment, 1 ml was added to 9 ml Phenol Red mannitol broth containing 5 µg/ml ceftizoxime plus 75 µg/ml aztreonam (PHMB, Media Products BV, The Netherlands) and incubated at 37 °C for 16–24 h. One loopful (10 µl) of PHMB was subcultured onto Brilliance™ MRSA agar (Oxoid) and incubated at 37 °C for 24–48 h. Presumptive MRSA (denim-blue colonies) were subcultured onto tryptone soya agar (TSA) for further characterization.

The Staphaurex Plus® kit (Thermo Fisher Scientific, UK) was used to confirm the isolates as *S. aureus*. Minimum inhibitory concentrations (MICs) of antibiotics were determined by agar dilution using the British Society for Antimicrobial Chemotherapy method [5]. The following antimicrobials were tested: vancomycin, mupirocin, gentamicin, rifampicin, daptomycin, teicoplanin, linezolid, erythromycin, tetracycline, clindamycin, fucidin and ciprofloxacin.

All *S. aureus* were examined by real-time multiplex polymerase chain reaction [6] to detect the thermonuclease gene (*nuc*), methicillin resistance genes (*mecA* and *mecC*), and the Pantón–Valentine leucocidin gene (*luk-PV*). MRSA were further characterized by *spa* typing and pulsed-field gel electrophoresis as described previously [7]. One MRSA with an unrecognized pulsotype was analysed further by MLST [7] and DNA microarray analysis using the StaphyType kit [8] in accordance with manufacturer's instructions (Alere Technologies GmbH, Germany).

MRSA was recovered from 5/90 (5.6%) meat samples examined (Table 1), all of which were pre-packaged meat collected from the same supermarket; MRSA was not isolated from the unpackaged meat

tested from individual butcher's shops. The highest prevalence of MRSA was identified in chicken (3/30, 10% samples); MRSA was also recovered from one each of 30 beef and 30 pork samples.

Molecular analyses showed all five MRSA isolates were *mecA* positive and *luk-PV* negative. Four (two from chicken and one each from beef and pork) were identified as *spa* type t032, which belongs to epidemic MRSA-15 (EMRSA-15), the predominant HA-MRSA lineage identified in humans in the UK (MLST CC22). All four had an antibiogram typical for this clone (resistant to oxacillin, ciprofloxacin and erythromycin) and belonged to the most common pulsotype of EMRSA-15 seen in humans in England (A. M. Kearns, unpublished data).

The remaining MRSA (from chicken) identified as *spa* type t1939, by MLST was ST9 and was resistant to penicillin, oxacillin, ciprofloxacin, erythromycin, clindamycin and tetracycline. By microarray, a range of resistance genes was detected (*blaZ*, *mecA*, *ermB*, *aadD*, *fosB* and a *tet* efflux marker); the enterotoxin gene cluster which encodes multiple enterotoxin genes (G, I, M, N, O, U) was also present. In addition, the isolate encoded staphylococcal cassette chromosome *mec* (SCC*mec*) type IV (*ccrA-2*, *ccrB-2* plus class B *mec* gene complex). The human innate immunomodulatory genes carried by Sa3 prophages (*sak*, *chp*, *scn*) were absent, a finding consistent with LA-MRSA.

This is the first study in the UK to report the recovery of MRSA from raw chicken, beef and pork. There is no standardized method for the isolation of MRSA from raw meat, and no quantitative studies were undertaken, so the MRSA bioburden in each of the five MRSA-positive samples is unknown.

In this study, four of the MRSA belonged to the dominant MRSA lineage identified in humans in the UK (CC22; EMRSA-15) suggesting contamination of meat from human sources (e.g. farm/meat industry workers, food handlers, etc.). This echoes previously published reports of the recovery of human-associated MRSA from various types of raw meat and carcasses in continental Europe, North America and Asia [9, 10]. This may present a risk to human health and precautions need to be implemented to prevent these strains from getting into the meat from process workers.

The isolate identified as LA-MRSA (ST9-SCC*mec*IV, *spa* type t1939) was recovered from a sample of 'British' retail chicken meat and represents the first report of LA-MRSA in retail meat purchased in the UK. To our knowledge, there are no reports of CC9 LA-MRSA from animals or humans in the UK. By contrast, CC9

LA-MRSA has been reported occasionally in continental Europe and, more frequently, in Asia where it is the dominant LA-MRSA in pigs, pork meat and farm workers in China, Malaysia and Thailand [11]. Of the CC9 LA-MRSA from Asia, various *spa* types (t889, t337, t4358) and SCC*mec* types (III, V, IX) have been identified. In a survey conducted in Germany in 2011, two isolates of ST9-SCC*mec*IV were identified from chicken products (one originated from Germany, the other from The Netherlands), but with a different *spa* type (t1430) from the isolate from England reported herein [3]. Collectively, the data suggest CC9 LA-MRSA has evolved on multiple, independent occasions in different geographical locations and, as the isolate reported here, may be multiply-resistant [3].

The risk these findings pose is somewhat unclear. From a human public health perspective, providing meat is stored appropriately, handled hygienically and cooked thoroughly, any bacteria that may be present will be destroyed. Nevertheless, the presence of a multiply-resistant, enterotoxigenic strain (as identified in this study), emphasizes the need for further studies to assess possible health hazards for consumers. MRSA has rarely been implicated in cases of food poisoning but a report from the USA identified a colonized food handler as the likely source of a food poisoning outbreak caused by MRSA [12].

The extent and diversity of the MRSA reservoir in meat in the UK remains speculative because this was a relatively small-scale study and only a single colony per MRSA-positive sample was analysed. Future surveillance with a broader farm-to-fork approach is warranted to (i) gain further insights into the prevalence of HA- and LA-MRSA in meat and food-producing animal species in the UK, (ii) promote awareness, (iii) evaluate occupational risk factors, and (iv) assess public health impact.

DECLARATION OF INTEREST

None.

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