Extended spectrum β-lactamase producing *Escherichia coli* and methicillin resistant *Staphylococcus aureus* in pig production: possible zoonotic implications.

Espectro de β-lactamases produzindo *Escherichia coli* e *Staphylococcus aureus* resistente à meticilina em suinocultura: possíveis implicações zoonóticas.

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**INTRODUCTION**

β-lactamase (ESBL)-producing *Enterobacteriaceae* and Methicillin Resistant *Staphylococcus aureus* (MRSA) are increasing clinical and economical problem in human medicine [14,16]. ESBLs are normally encoded by plasmid-borne genes and confer resistance to penicillins, cephalosporins, and aztreonam [4]. They are often multi-drug resistant due to concurrent presence of other classes of antibiotics, and they have been associated with hospital outbreaks [14]. MRSA are resistant to most antibiotics available for treatment of staphylococcal infections and are associated with prolonged hospitalization [16]. Human infections with this bacterium are notifiable in some EU Member States such as Denmark, Sweden and the United Kingdom (bacteraemia).

ESBL-producing *Enterobacteriaceae* can be isolated from food-producing animals [13], suggesting that food animals and food of animal origin may be a factor in the spread of such bacteria. Similarly, reports have documented cases of MRSA colonization or infection in animals [10]. In relation to pig production, MRSA clone ST398 is of particular concern, since it seems to be wide spread in animal populations [7]. This clone may cause severe infection in humans, including osteomyelitis, endocarditis and sepsis [6]. This presentation presents results from investigation of the presence of ESBL and MRSA clone ST398 in pig production and the carrier status of persons in close contact with positive animals.

**I - RESULTS**

1 - ESBL in pigs

The possible impact of ceftiofur, a third-generation cephalosporin approved for therapeutic use in swine and cattle, on the occurrence of cephalosporin-resistant and ESBL-producing *Escherichia coli* in pigs was investigated in Danish farms [9]. Ten farms using ceftiofur and 10 control farms without a history of ceftiofur usage were selected. Faecal samples were taken from 10 piglets and 10 slaughter pigs at each farm. *E. coli* with reduced susceptibility to cefotaxime were obtained from 69 animals at five farms using ceftiofur and from 3 animals at one control farm. ESBL production was demonstrated in 19 *E. coli* isolates originating from two farms. These isolates were resistant to cefotaxime and ceftiofur and susceptible to cefazidime and cefoxitin. PCR and sequence analyses demonstrated CTX-M-1 in all isolates and TEM-1B in five isolates. The resistant isolates each had distinct PFGE types and antimicrobial resistance patterns, which suggest that CTX-M-1 was present in different *E. coli* strains rather than clonal spread within farms. The remaining isolates had lower resistance to ceftiofur (MIC = 8–16 mg/L). Analysis of eight randomly selected isolates showed mutations in the promoter region, which is associated with up-regulation of AmpC production.
2 - MRSA in pigs

One hundred slaughter pigs from 3 farms located in distinct regions in Denmark were screened for presence of erythro-mycin and MRSA by nasal swabs [2]. MRSA were not detected using three different methods, however, one erythro-mycin resistant isolate was typed as t034 by spa typing. This type has been reported as one of the spa-types related to MRSA in pigs (ST398) [19]. This prompted a reinvestigation of selected isolates by standard methods for MRSA detection (cefoxitin disk agar diffusion, meCA PCR, and PBP2 latex agglutination test) [7]. One isolate was found to be a MRSA according to both phenotypic and genotypic tests, and additional isolates were found to be part of the ST398 clonal complex. Porcine ST398 isolates were generally susceptible to most antimicrobials tested and no variability was observed in their resistance patterns except for two strains resistant to streptomy-cin, including the MRSA strain.

3 - Carrier of MRSA in exposed humans

MRSA can colonize the skin and the mucosa of animals, and has been reported in pet owners when the animals are positive and from people in contact with patients, such as hospital employees [3,15]. Transmission can occur in both directions, and studies have recently demonstrated transmission of MRSA between animals and individuals in daily contact with animals such as veterinarians [12] and pig farmers [18]. Here we report the carrier status of veterinarians compared to controls of humans not exposed to animals [A. Moodly, personal communication]. High risk for nasal carriage of MRSA among Danish veterinary practitioners. In preparation for publication). Nasal swabs were obtained from participants (n=702) at five conferences organized by national veterinary and farmer associations and students at a business school in Denmark (study performed with necessary legal permission). In addition, information on animal exposure and known MRSA risk factors were obtained. Samples were screened by microbiological techniques for MRSA detection and isolates were characterized as above. MRSA carriage was significantly (p <0.02) higher in veterinary practitioners (3.9%) than in people not professionally exposed to animals (0.7%). Typing was performed on isolates obtained from veterinary practitioners. Six of nine strains belonged to clonal complexes previously associated with horses, small animals and pigs. Four of the nine positive veterinarians carried the clonal complex associated with pigs (ST398), but only exposure to small animals, cattle or horses, but not to pigs, was found to be a significant risk factor.

II - DISCUSSION

In Denmark, ceftiofur is only registered to pigs for treatment of respiratory diseases. Data from the Danish programme for surveillance of antimicrobial resistance (DANMAP) however show a considerable use in sows/piglets [5]. This indicates that off-label use is widespread, since bacterial respiratory diseases are relatively uncommon in sows and piglets. A statistical significant association between the use of ceftiofur and reduced susceptibility to cefotaxime was reported in pig farms [9]. ESBL was due to CTX-M-1 positive E. coli, and such bacteria were only detected in farms using ceftiofur. This strongly indicated that the use of cephalosporins was the reason for the selection of ESBL in farmed pigs. The true prevalence of CTX-M-positive isolates may have higher than reported, since only a single isolate was isolated from each animal. Other reports have linked the use of cephalosporin with selection for E. coli-producing CTX-M-1 [1,17] supporting the conclusion. The human risks associated with the presence of ESBL in pigs cannot be truly assessed by the current knowledge, and further investigations are required to understand the magnitude transmission via the food chain and by contact with animals.

Thirty two human cases of MRSA ST398 have been reported in Denmark, and based on a case-control study, ST398 cases were significantly associated with pig farming [11]. Thirteen (62%) cases, but no controls, had been exposed to pigs prior to MRSA infection. A survey conducted on one third of total veterinarians in Denmark revealed a carriage rate of 4%, with MRSA ST398 being the most prevalent strain [A. Moodley, personal communication]. MRSA was not detected in the control group of people professionally not exposed to animals, indicating an occupational health hazard in the veterinary profession. Independent studies in North America [8] and Holland [19] showed that veterinary personnel have a high risk of nasal MRSA carriage.

To the knowledge of the authors, no reports on ST398 as cause of disease in pigs have been published. Thus the presence of MRSA ST398 in pig production foremost represents a zoonotic problem. Low prevalence countries, such as Denmark and Netherlands have issued “Search and Destroy” policy for MRSA among humans.
This strategy may be severely damaged by the presence of MRSA in animal populations. MRSA ST398 was detected in two out of three Danish pig farms tested [2]. This suggests that this clone could be widespread in the pig population in Denmark. Detection of ST398 is cumbersome, and there is a need to develop a sensitive and rapid method for detection of both methicillin resistant and susceptible variants of ST398 to allow surveillance of ST398 in animals and food products.

REFERENCES


