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Patterns of antimicrobial resistance in *Streptococcus suis* isolates from pigs with or without streptococcal disease in England between 2009 and 2014

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ABSTRACT

Antimicrobial resistance in *Streptococcus suis*, a global zoonotic pathogen of pigs, has been mostly studied only in diseased animals using surveys that have not evaluated changes over time.

We compared patterns of resistance between *S. suis* isolates from clinical cases of disease (CC) and non-clinical case (NCC) pigs in England, collected over two discrete periods, 2009–2011 and 2013–2014. Minimum inhibitory concentrations (MIC) of 17 antimicrobials (nine classes) were determined on 405 *S. suis* isolates categorised by sampling period and disease association to assess changes in resistance over time and association with disease. First, isolates were characterized as resistant or susceptible using published clinical breakpoints. Second, epidemiological cut-offs (ECOFF) were derived from MIC values, and isolates classified as wild type (WT) below the ECOFF and non-wild type (NWT) above the ECOFF. Finally, isolate subsets were analysed for shifts in MIC distribution.

NCC isolates were more resistant than CC isolates to cephalosporins, penams, pleuromutilins, potentiated sulphonamides and tetracyclines in both study periods. Resistance levels among CC isolates increased in 2013–2014 relative to 2009–2011 for antimicrobials including aminoglycosides, cephalosporins, fluoroquinolones, pleuromutilins, potentiated sulphonamides and tetracyclines. The prevalence of isolates categorised as NWT for five or more classes of antimicrobials was greater among NCC than CC isolates for both time periods, and increased with time. This study used standardised methods to identify significant shifts in antimicrobial resistance phenotypes of *S. suis* isolated from pigs in England, not only over time but also between isolates from known clinical cases or disease-free pigs.

1. Introduction

Streptococcus suis (*S. suis*) is a global pig pathogen which has a major impact on productivity, antimicrobial use and pig welfare (Gottschalk, 2012). Human disease due to *S. suis* was first described in Europe in the 1950s (Wertheim et al., 2009). In Great Britain, *S. suis* is one of the most common causes of systemic disease in post-weaned pigs to be reported by diagnostic laboratories in recent years, resulting in septicemia, meningitis, pneumonia and arthritis.

There is marked and large diversity among *S. suis* strains, with 33 serotypes based on capsular polysaccharides (Gottschalk, 2012), and many non-serotypable strains exist, but most clinical cases are caused by a small number of serotypes. Disease associated strains are characterized by an ensembles of a diverse group of virulence related genes, which may vary geographically, and other genomic features but other strains with apparently low pathogenic potential can be isolated widely

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as part of the microbiota in the respiratory tract and tonsils of pigs without streptococcal disease (Weinert et al., 2015).

Over the past decade, an increasing level of antimicrobial resistance has been noted in food-borne and other pathogens (Palmieri et al., 2011). This has been recognized as a global problem for public health and the worldwide emergence of multidrug-resistant phenotypes is causing increasing concern (O'Neill, 2016). Antimicrobial resistance profiles, and genetic determinants regulating resistance mechanisms, have been studied in isolates of *S. suis* from pigs and, to a lesser extent, from human cases (Palmieri et al., 2011). Penicillin resistance in *S. suis* was first reported in the UK from a serotype 2 isolate from a human in 1980 (Shneerson et al., 1980) and has emerged in *S. suis* isolates from pigs worldwide (Zhang et al., 2008; Callens et al., 2013). More recently, resistance to third-generation cephalosporins was reported in China and Europe (Hu et al., 2011; Zhang et al., 2015; van Hout et al., 2016). Extensive resistance has been reported against aminoglycosides (Holden et al., 2009; Hu et al., 2011; Palmieri et al., 2011), β-lactams, trimethoprim and amphenicols (Wisselink et al., 2006; Holden et al., 2009; Hu et al., 2011; Ge et al., 2012).

Resistance mechanisms in *S. suis* include new gene acquisition and gene expression modifications, as described for tetracyclines, macrolides, lincomycin, streptogramin B (Palmieri et al., 2011; Chen et al., 2013) and fluoroquinolones (Escudero et al., 2011). Other mechanisms based on gene mutations have been described for tiamulin, quinolones and penicillin (Martel et al., 2001; Gurung et al., 2015). However, other reasons underlying ineffective responses to antimicrobial treatment of *S. suis* disease might include biofilm formation and the production of persistent cells (Seitz et al., 2016). Although reports from different parts of the world indicate widespread clinical resistance in *S. suis* to diverse antimicrobials (Aarestrup et al., 1998; Callens et al., 2013; Varela et al., 2013; de Jong et al., 2014; Zhang et al., 2015; van Hout et al., 2016), there have been no systematic comparisons of antimicrobial susceptibility for *S. suis* isolates collected from pig populations in the same geographic area at different time points using standardised methodology.

Current efforts to improve provision of surveillance data to allow monitoring and international comparisons of antimicrobial resistance for *S. suis* are hampered by differences in testing methodologies and interpretation criteria that are subjective. Standardized methods and cut-offs have been proposed by the Clinical and Laboratorial Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), but the need remains for better harmonization and normalization of results (Kronvall, 2010; Kahlmeter, 2015). Furthermore, clinical breakpoints are not defined for most of the antimicrobials; the literature reports of antimicrobial resistance in *S. suis* apply different clinical breakpoints, which further complicates comparisons of results from different studies. Given these limitations, antimicrobial resistance phenotypes for bacteria have also been studied by determining minimum inhibitory concentration (MIC) values and by categorizing isolates according to epidemiological cut-off (ECOFF) values for each antimicrobial.

This study describes the comparative phenotypic antimicrobial resistance characteristics of 405 isolates of *S. suis* from commercial slaughter pigs in England, representing carefully catalogued isolates of known disease-associated or non-disease associated provenance, from two time periods (2009–2011 and 2013–2014).

2. Materials and methods

2.1. Sample collection

A total of 405 isolates of *S. suis* were obtained covering two periods 2009–2011 and 2013–2014. These were further split into two classes: disease associated clinical cases (CC) and non-disease associated non-clinical cases (NCC). Disease-associated CC isolates from both 2009–2011 (N = 93, from 83 different laboratory submissions) and 2013–2014 (N = 117, from 113 different laboratory submissions) were cultured from lung, meninges, or other systemic sites of pigs between weaning and slaughter age (1–5 months) with clinical signs and/or gross pathology consistent with *S. suis* infection (including meningitis, septicaemia, arthritis, pneumonia) submitted from pig farms from different geographic locations in England to Animal and Plant Health Agency (APHA) veterinary investigation centres (VICs).

Non-disease associated NCC isolates from 2009 to 2011 (N = 66 from 44 different laboratory submissions) were cultured from tonsils or tracheobronchial swabs of pigs between weaning and slaughter age from different geographic locations in England submitted to the APHA VICS for post-mortem examination in which *S. suis* disease was not diagnosed. None of the clinical histories of these cases reported streptococcal disease at the time of submission. NCC isolates from 2013 to 2014 (N = 129 from 113 pigs) originated from nine breeding sources in the East of England which reported no *S. suis* related clinical signs at the time; these isolates were obtained from 250 tonsils scrapes, 125 from 5 week old pigs and 125 from 20 week old pigs, and submitted to the Scottish Agricultural College (SAC) veterinary laboratories for isolation of *S. suis*. Antimicrobial treatments prior to sample collection were not considered in this study.

The NCC isolates from 2009 to 2011 were isolated by inoculating the samples from pigs onto Columbia agar containing 5% (v/v) sheep blood (TCS biosciences Ltd., Bucks, UK) and incubating at 37 °C in aerobic conditions for up to 48 h. Up to three suspect *S. suis* colonies were selected from each plate based on α-haemolysis and colony morphology, then sub-cultured and tested in pure culture with a biochemical profiling kit (API 32-Strep, Bio-Mérieux, Mercy-l'Étoile, France).

For the NCC samples collected in 2013–2014, three colonies were selected per inoculated plate; API biochemical profile was done and *S. suis* colonies from the same plate presenting the same biochemical profile were considered the same strain so just one of them was selected for the final collection and stored at −80 °C until testing. NCC isolates collected in 2013–2014 were epidemiologically related as they came from the same production pyramid, some of them came from the same farm, and some tonsillar scrapes yielded more than one isolate, which reduces this collection representativeness. In contrast, most of the NCC samples collected in 2009–2011, and the CC samples in both periods, represented cases submitted from pig producers located in different geographic areas in England.

2.2. Antimicrobial susceptibility testing

MIC were determined using the micro-broth dilution method, at Quotient Bioresearch, Fordham, UK in accordance the CLSI Approved standard M100-S25 (2015), VET01-A4 (2013b) and VET01-S2 (2013a) as recently described (de Jong et al., 2014; van Hout et al., 2016). Seventeen different antimicrobial compounds, representing nine antimicrobial classes, were tested across a range of two-fold step dilutions (Table 1). Quality controls were included according to CLSI recommendations VET01-A4 (2013b) and VET01-S2 (2013a); reference strains of *Enterococcus fecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 29213), and *Streptococcus pneumoniae* (ATCC 49619) were used for this purpose.

2.3. Data analysis

MIC distributions for CC and NCC isolates were analysed separately for 2009–2011 and 2013–2014, using the following methods.

2.3.1. MIC value distribution and epidemiological cut-off values (ECOFF)

MIC distributions were evaluated for the presence of one or more clusters. Distributions were classed as unimodal where MIC values were spread surrounding a central value, or median, in one "bell-shaped" cluster and multimodal when two or more clusters represented multiple