Low occurrence of colistin-resistant \textit{Escherichia coli} in faecal content of pigs in French commercial herds

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SUMMARY

We tested the susceptibility to colistin of \textit{Escherichia coli} indicator strains isolated from the faecal content of pigs of various ages housed in commercial French farrow-to-finish herds. This antimicrobial had been administered to piglets during the lactating period and more frequently after weaning in most of the selected farms (13 out of 16). Twenty-three strains (22 from young pigs) out of the 4333 tested were classified as resistant by disk diffusion method and confirmed by MIC determination (4 to 16 µg/ml). Our results indicate that disk diffusion method can be a valuable method for the assessment of colistin resistance and illustrate the infrequent occurrence of resistance to this antimicrobial.

Keywords: Colistin, \textit{Escherichia coli}, antibiotic, resistance, pig.

INTRODUCTION

Occurrence of antimicrobial resistant bacterial strains is a key issue for therapeutic outcome both in human and veterinary medicine. Moreover, when considering food animals the risk of their contribution to antibiotic resistance in humans has to be taken into account. Consequently surveys are needed to describe evolution of drug sensitivity in pathogens and indicator bacteria obtained from these animals. Several types of studies can be carried out that document resistance either in pathogenic or in indicator bacterial strains. In addition to regular surveys, \textit{ad hoc} studies can be designed to answer specific questions about an antimicrobial compound.

Colistin (polymyxin E) is an old antimicrobial frequently used in swine production in France for treatment and prevention of colibacillosis mainly in suckling piglets and weaners and less frequently in finishers [4]. This antimicrobial has also been shown to be the most frequently administered to pigs in Spain [3]. Colistin is very poorly absorbed when administered by oral route and is considered as almost always effective against \textit{Escherichia coli} [14]. Nevertheless, colistin-resistant \textit{E. coli} strains have been reported in pigs after diarrhoea outbreaks [15]. However, to our knowledge, the relationship between colistin use and occurrence of resistant strains has not been investigated.

The objective of this study was to describe the occurrence of \textit{E. coli} resistant strains in faecal content of pigs housed in conventional farrow-to-finish herds whose antimicrobial use was monitored.

MATERIALS AND METHODS

ISOLATION OF BACTERIA

\textit{Escherichia coli} strains were obtained from faeces of pigs housed in sixteen commercial farrow to finish herds selected on their antimicrobial use (including both high and low level users). Faeces were collected at different moments through the animals’ life. Sows were collected four times during one production cycle: before farrowing and then at 7 and 30 days of lactating period. The last sample was obtained during pregnant stage (2 months after farrowing). Young pigs were sampled at 7, 30, 60 and 150 days of age. Antimicrobial treatments administered to animals during the study period were recorded. One gram of faeces was suspended in 1ml of sodium chloride (0.9%) and serial decimal dilutions were plated onto selective Levine agar (Difco Laboratories, Detroit, MI, USA) and incubated at 37°C overnight. For each faecal sample four colonies with \textit{E. coli} phenotypic aspect \textit{(i.e. metallic sheen colonies)} were randomly selected for further study.
SUSCEPTIBILITY TESTING

Susceptibility to colistin was tested using standard disk diffusion method according to the CA-SFM guidelines. Strains were classified as sensitive or resistant if the diameter of inhibition zone was ≥ 15 mm or ≤ 14 mm respectively with a disk containing 50 µg of colistin sulphate (Bio-Rad, Marnes la Coquette, France). When resistant strains were obtained, colistin susceptibility was further studied by MIC determination using liquid method. Colistin sulphate powder was obtained from Sigma Chemical Co. (St Louis, Mo). Susceptibility testing by broth microdilution using Mueller-Hinton broth was carried out in accordance with NCCLS guidelines [20]. Colistin concentrations from 0.0625 to 64 µg/ml were tested and *Escherichia coli* ATCC 25922 strain was used as a quality control. The MIC was defined as the lowest concentration with which no growth was visible after overnight incubation at 37°C. A ≥ 4 µg/ml colistin concentration was used to designate resistant isolates [9].

Results

Only collective treatments administered to sows and young pigs were taken into account and they were either systematic (such as in-feed supplementation) or occasional following a pathologic event. The number of collective antimicrobial administrations was highly variable among herds depending on the sanitary status and ranged from no treatment to seven ones during one production cycle.

The most frequently used antimicrobial was colistin which was administered to young pigs in 13 out of the 16 tested farms. The number of colistin treatments was one (8 herds), two (4 herds) or four (1 herd). This antibiotic was administered to control digestive disorders in piglets either during the lactating period (1 herd) in food after weaning (10 herds) or via drinking water to growers (5 herds) or finishers (3 herds). Administrations of oral paste or in drinking water occurred after a diarrheoa outbreak whereas in-feed medications were systematically applied. Daily doses were 100000 UI per kg bw during either 3 days for occasional treatments or during 10 to 15 days in case of in-feed administration.

A total of 4333 *E. coli* isolates were tested for susceptibility to colistin among which 1145 had been isolated from sows and 3188 from young pigs. Inhibition zone diameters exhibited a unimodal distribution and ranged from 12 to 28 mm in sows (figure 1) and from 11 to 34 mm in young pigs (figure 2).

Only 23 strains (22 from young pigs and one from a sow) were classified as resistant by disk diffusion method. When tested using MIC determination method these strains exhibited MIC values ranging from 4 to 16 µg/ml. The resistant strains originated from several herds all of them belonging to the colistin user category with two or four colistin treatments during young pigs’ rearing period. These isolates have been obtained from (i) piglets aged 7 days (6/22) or 30 days (16/22) and (ii) a sow sampled one week after farrowing whose piglets had been treated with colistin during the lactating period. MIC values of the reference strain were ≤ 0.25 µg/ml.

Discussion

Herds included in the study were randomly selected by veterinary practitioners among farms exhibiting various levels (low to high) of antimicrobial use. Although the use of colistin was not taken into account for the selection, this antimicrobial was administered to young pigs in most of the selected herds. This is consistent with the fact that this drug is among the most extensively administered to pigs in France as reported by MOULIN and ROUX [18].

Our results obtained on a high number of isolates in a selective context indicate that colistin treatment is seldom followed by the emergence of resistant strains in faecal flora of pigs. Few data are available concerning acquired resistance to colistin either in human or in veterinary medicine. Attempts to select *in vitro* polymyxin-resistant *E. coli* strains have suggested that these antimicrobials exhibit a low endogenous resistance potential probably due to the fact that their target is the bacterial membrane. Indeed, multiple outer membrane protein and LPS alterations consecutive to several mutations are needed to express a resistant phenotype [17]. In human medicine resistance has been reported in *Pseudomonas aeruginosa* from patients with cystic fibrosis receiving colistin daily by inhalation [14]. However, several studies have reported that the majority of isolates remain susceptible.
despite an extensive use of this drug in the hospital [19, 8]. The occurrence of colistin resistance seems to be less rare than reported here when assessed on pathogenic isolates, although these results have to be interpreted with caution since the number of tested isolates is usually low in such studies. MATEU and MARTIN [15] examined 86 porcine E. coli strains from diarrhoea outbreaks and found seven ones with MIC values >8 µg/ml. A similar study on bovine showed that nine out of 97 isolates from calves exhibited a MIC value ≥ 4 µg/ml [10]. The antimicrobials administered to animals from which strains had been obtained were not documented in these two studies neither were reported clinical parameters and outcome of infections. In the context of the survey of antimicrobial resistance in bacteria from food animals in Denmark, 208 E. coli strains were collected from pigs at slaughter and 0.5% of them exhibited a MIC value of 8 µg/ml for colistin [6]. Additionally, colistin resistance was more prevalent in pig isolates obtained from sick animals (42/118) [11] than from healthy ones (3/358) [13]. In our study, resistant strains did not possess virulence factors specific for porcine E. coli pathogenic strains when tested using specific antisera. A reason for this apparent higher resistance in pathogenic E. coli could be that resistance is a selective advantage for pathogenic isolates keeping in mind the frequent use of colistin for the treatment of diarrhoea in swine or bovine production. In some case resistance is associated with a biological cost for bacteria leading to their counter-selection in the absence of treatment. Nevertheless, to our knowledge, this cost has not been documented for colistin resistance in E. coli. ENNE et al [7] demonstrated that the fitness cost associated with (i) mutation on chromosomal genes and (ii) carriage of several antibiotic resistance elements was low to non-existent in E. coli. These variable results depending on the context of studies emphasize the interest in surveys based on commensal bacteria as complementary to description of pathogenic isolates in order to monitor temporal trends in evolution of susceptibility to antimicrobials.

The predictive value of disk diffusion method for the evaluation of colistin resistance appeared quite good since all strains that had been classified as resistant were confirmed by MIC determination. However, other investigators reported discrepant results with no primarily identification of resistant isolates by disk diffusion for some non enterobacteriaceae [1, 8, 22]. On the contrary MATEU and MARTIN found false positive resistance results [15]. The poor agar diffusion characteristics of polymyxins make the disk diffusion test dependent on lab conditions such as inoculum’s density. In our study the antimicrobial activity of colistin was evaluated in a single laboratory using standardized conditions which may lead to such reliable results. Such reproducibility is more difficult to achieve when results are collected from laboratory networks for resistance surveys.

The MIC values observed were similar to those reported in previous studies either for reference strain E. coli ATCC 29255 [12] or for resistant isolates [10] whereas MATEU and MARTIN [15] found porcine strains exhibiting MIC values as high as 64 µg/ml. From a clinical point of view, interpretative criteria are not homogeneous between working groups with susceptibility breakpoint of ≤ 2 µg/ml [5, 16] or ≤ 4 µg/ml [1] leading to differences in interpretation of amount of resistance according to studies.

Currently the consequence of colistin use in terms of resistance is not a matter of concern in veterinary medicine because unsensitive target bacteria are not frequently reported. Moreover, up to now, colistin had not been widely used in human medicine because of its toxicity. However, the interest in its administration to humans has increased recently due to the emergence of multiple-drug-resistant (MDR) gram negative bacteria such as Pseudomonas aeruginosa or Acinetobacter baumannii which are responsible for nosocomial infections [2, 20]. This recent change in therapeutic schemes in human medicine makes necessary the collection of data on colistin resistance. Our results suggest that despite a wide use of colistin in pigs occurrence of resistant strains among the E. coli sentinel population in the gut is a rare event.

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