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Association of resistance to ampicillin and other antibiotic agents as evidence of co-selection in *E.coli* isolates from diarrheic nursery pigs



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Introduction

Ampicillin resistance in *E. coli* isolated from human bacteraemia is occurring frequently and concerns about a porcine reservoir for ampicillin resistant *E. coli* which may be transferred to humans, has been raised. Direct selection of resistant *E. coli* by ampicillin treatment of pigs and co-selection of ampicillin resistance by tetracycline and other antibiotic classes has been suggested. Statistical association between resistances to different antibiotic agents is a strong indicator of co-selection.

Objectives

The objective of this study was to evaluate the association between resistances to commonly used antibiotic agents such as tetracycline and resistance to ampicillin in *E. coli* isolates from diarrheic nursery pigs

Conclusions

- Evidence of co-selection of resistance to ampicillin and other antibiotic agents in *E. coli* isolates from diarrheic nursery pigs
- Tetracycline was found to have a lower co-selecting capacity for ampicillin resistance than other antimicrobial agents
- Co-selection seems to be related to ETEC status of isolate

Table 1: Antibiotic concentration ranges and resistance breakpoints used for susceptibility testing of *E. coli* (n = 380) isolated from faecal samples from weaned pigs and pen floors

| Antimicrobial agent | Concentration used (µg/ml) | Clinical breakpoint (µg/ml) |
|---------------------|----------------------------|-----------------------------|
| Ampicillin | 1 - 32 | ≥32 ^a |
| Tetracycline | 2 - 32 | ≥16 ^a |
| Sulphamethoxazole | 64 - 1024 | ≥512 ^a |
| Trimethoprim | 1 - 32 | ≥16 ^b |
| Spectinomycin | 16 - 256 | ≥128 ^b |
| Streptomycin | 8 - 128 | ≥32 ^b |

^aCLSI-approved breakpoints based on human data. ^bBreakpoints routinely used by the Laboratory of Swine diseases, Kjellerup, Denmark and by the Danish Veterinary Institute, Frederiksberg, Denmark.

Results

In 89 ETEC isolates, tetracycline and streptomycin resistance was statistically associated to ampicillin resistance (p<0.001, 0.002). For tetracycline resistant isolates a lower level of ampicillin resistance (OR = 0.09) was observed compared to tetracycline sensitive whereas streptomycin resistant isolates had a higher level of ampicillin resistance (OR = 5.57) compared to streptomycin sensitive isolates.

In 291 Non-ETEC isolates all tested antibiotic agents except spectinomycin was positively associated to ampicillin resistance.

Table 2: Association between resistance of five antimicrobial agents and ampicillin stratified by type of isolate (ETEC/NON-ETEC)

| | Isolate type | Resistance status | Ampicillin resistant | Ampicillin sensitive | P-value | Odds ratio |
|--------------------|--------------|-------------------|----------------------|----------------------|---------|------------|
| Tetracycline | ETEC | + | 14 | 28 | <0.001 | 0.09 |
| | | - | 40 | 7 | | |
| | NON-ETEC | + | 52 | 43 | 0.001 | 2.33 |
| | | - | 67 | 129 | | |
| Sulpha-methoxazole | ETEC | + | 34 | 28 | 0.141 | 0.43 |
| | | - | 20 | 7 | | |
| | NON-ETEC | + | 104 | 25 | 0.000 | 40.77 |
| | | - | 15 | 147 | | |
| Trimethoprim | ETEC | + | 34 | 28 | 0.141 | 0.43 |
| | | - | 20 | 7 | | |
| | NON-ETEC | + | 76 | 16 | <0.001 | 17.23 |
| | | - | 43 | 156 | | |
| Spectinomycin | ETEC | + | 13 | 3 | 0.115 | 3.38 |
| | | - | 41 | 32 | | |
| | NON-ETEC | + | 27 | 35 | 0.739 | 1.15 |
| | | - | 92 | 137 | | |
| Streptomycin | ETEC | + | 26 | 5 | 0.002 | 5.57 |
| | | - | 28 | 30 | | |
| | NON-ETEC | + | 101 | 29 | <0.001 | 27.67 |
| | | - | 18 | 143 | | |

Materials and Methods

- Rectal samples from diarrhoeic pigs two-four week post weaning and faecal pen floor samples were collected from three nursery facilities in Denmark
- 380 *E. coli* isolated after culture on blood agar and analysed by PCR for adhesion factor-, and enterotoxin-genes to determine ETEC status
- Minimum inhibitory concentrations determined by the broth micro dilution method
- Isolates classified as resistant based on clinical breakpoints
- Association of resistance was tested by Chi2-test

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