

Short communication

## Virulence-associated gene pattern of porcine and human *Yersinia enterocolitica* biotype 4 isolates

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### Abstract

*Yersinia enterocolitica* 4/O:3 is the most important human pathogenic bioserotype in Europe and the predominant pathogenic bioserotype in slaughter pigs. Although many studies on the virulence of *Y. enterocolitica* strains have showed a broad spectrum of detectable factors in pigs and humans, an analysis based on a strict comparative approach and serving to verify the virulence capability of porcine *Y. enterocolitica* as a source for human yersiniosis is lacking. Therefore, in the present study, strains of biotype (BT) 4 isolated from Swiss slaughter pig tonsils and feces and isolates from human clinical cases were compared in terms of their spectrum of virulence-associated genes (*yadA*, *virF*, *ail*, *inv*, *rovA*, *ymoA*, *ystA*, *ystB* and *myfA*). An analysis of the associated antimicrobial susceptibility pattern completed the characterization. All analyzed BT 4 strains showed a nearly similar pattern, comprising the known fundamental virulence-associated genes *yadA*, *virF*, *ail*, *inv*, *rovA*, *ymoA*, *ystA* and *myfA*. Only *ystB* was not detectable among all analyzed isolates. Importantly, neither the source of the isolates (porcine tonsils and feces, humans) nor the serotype (ST) had any influence on the gene pattern. From these findings, it can be concluded that the presence of the full complement of virulence genes necessary for human infection is common among porcine BT 4 strains. Swiss porcine BT 4 strains not only showed antimicrobial susceptibility to chloramphenicol, cefotaxime, ceftazidime, ciprofloxacin, colistin, florfenicol, gentamicin, kanamycin, nalidixic acid, sulfamethoxazole, streptomycin, tetracycline and trimethoprim but also showed 100% antibiotic resistance to ampicillin. The human BT 4 strains revealed comparable results. However, in addition to 100% antibiotic resistance to ampicillin, 2 strains were resistant to chloramphenicol and nalidixic acid. Additionally, 1 of these strains was resistant to sulfamethoxazole.

The results demonstrated that *Y. enterocolitica* BT 4 isolates from porcine tonsils, as well as from feces, show the same virulence-associated gene pattern and antibiotic resistance properties as human isolates from clinical cases, consistent with the etiological role of porcine BT 4 in human yersiniosis. Thus, cross-contamination of carcasses and organs at slaughter with porcine *Y. enterocolitica* BT 4 strains, either from tonsils or feces, must be prevented to reduce human yersiniosis.

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**Keywords:** Swine; Pig; Switzerland; Yersiniosis; Antibiotic resistance

## 1 Introduction

Based on biochemical characteristics, *Yersinia enterocolitica* is divided into several distinct biotypes (BTs) (BT 1A, 1B, 2, 3, 4 and 5), of which the pathogenic BTs (1B, 2, 3, 4 and 5) are responsible for the fourth most common foodborne zoonosis in Europe (EFSA, 2013). The most common human pathogenic *Y. enterocolitica* in the European Union and Switzerland are of bioserotype 4/O:3 (Fredriksson-Ahomaa et al., 2012; EFSA, 2013). Pigs have been characterized as the main reservoir for pathogenic *Y. enterocolitica* BT 4 and are supposed to be the most important infection hazard for humans (EFSA, 2013), but other mammals, e.g., sheep, cattle, cats and dogs, are also discussed as sources of human BT 4 infection (Fearnley et al., 2005; Stamm et al., 2013). BT 4 strains are detected in carcasses and in pig feces at slaughter, as well as in tonsils, where they are present at an up to 6-fold higher detection rate than in feces (Fredriksson-Ahomaa et al., 2001; Nesbakken et al., 2003). However, the route of infection and the impact of different sources (tonsils versus feces) have not been clarified.

Although previous studies on molecular typing have demonstrated the genetic relatedness of porcine and human *Y. enterocolitica* (Kuehni-Boghenbor et al., 2006; Laukkanen et al., 2009), the characterization of virulence capability by screening for virulence-associated genes is required to clarify the role of porcine BT 4 in human disease. All pathogenic *Y. enterocolitica* isolates host the 70-kb virulence plasmid (pYV), which is indispensable for full virulence (Cornelis et al.,



Porcine	Tonsil	O <sub>3</sub>	16	+	+	+		+	+	+	+	+
			2	+		+		+	+	+		+
			1	+	+	+		+	+	+		+
		O <sub>3</sub> , O <sub>27</sub>	15	+	+	+		+	+	+	+	+
		O <sub>8</sub> , O <sub>27</sub>	3	+	+	+		+	+	+	+	+
	O <sub>3</sub> , O <sub>8</sub> , O <sub>27</sub>	1	+	+	+		+	+	+	+	+	
	Feces	O <sub>3</sub>	4	+	+	+		+	+	+	+	+
	O <sub>3</sub> , O <sub>27</sub>	5	+	+	+		+	+	+	+	+	
	O <sub>8</sub> , O <sub>27</sub>	4	+	+	+		+	+	+	+	+	
Human	Feces	O <sub>3</sub>	17	+	+	+		+	+	+	+	+
		O <sub>3</sub> , O <sub>27</sub>	16	+	+	+		+	+	+	+	+
		O <sub>27</sub>	1	+	+	+		+	+	+	+	+
		O <sub>8</sub>	1	+	+	+		+	+	+	+	+
		O <sub>27</sub> , O <sub>9</sub>	1	+	+	+		+	+	+	+	+

ST serotype.

### 2.3 Antimicrobial testing

The minimal inhibitory concentration (MIC) of the antibiotics was determined by broth microdilution in cation-adjusted Mueller-Hinton broth using the Sensititre susceptibility plate EUMVS2 (Sensititre, TREK Diagnostic System, Cleveland, Ohio, USA; TREK Diagnostic System, East Grinstead, West Sussex, England). The antibiotics and the concentration ranges are presented in Tables 2 and 3. The plates were incubated for 18–24 h at 30 ± 1 °C. Isolates were classified as susceptible or resistant according to clinical breakpoints for *Enterobacteriaceae* issued by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines version 4.0 (www.eucast.org). If EUCAST breakpoints were unavailable, human clinical breakpoints for *Enterobacteriaceae* from Clinical and Laboratory Standards Institute (CLSI) documents M100-S22 (Clinical and Laboratory Standards Institute, 2013) were used. For florfenicol and streptomycin, no breakpoints from EUCAST or CLSI were available. Therefore, MIC50 and MIC90 were calculated. *Escherichia coli* ATCC 25922 was used as the control strain with 37 ± 1 °C as the incubation temperature. Measured MICs were in the appropriate range for this strain.

**Table 2** Minimal inhibitor concentration (MIC) from of porcine tonsil *Y. enterocolitica* biotype 4 isolates.

Antimicrobial agent	No. of strains with MIC (mg/L)																			
	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	
Ampicillin <sup>a</sup>														34	4					
Cefotaxime <sup>a</sup>				34	2	2														
Ceftazidime <sup>a</sup>					37	1														
Chloramphenicol <sup>a</sup>									3	12	23									
Ciprofloxacin <sup>a</sup>	18	20																		
Colistin <sup>a</sup>									38											
Florfenicol <sup>b</sup>									11	26	1									
Gentamicin <sup>a</sup>						15	23													
Kanamycin <sup>b</sup>										38										
Nalidixic acid <sup>b</sup>										38										
Streptomycin <sup>a</sup>										6	26	6								
Sulfamethoxazole <sup>b</sup>											36	2								
Tetracycline <sup>b</sup>							3	22	13											
Trimethoprim <sup>a</sup>							2	22	13	1										

Numbers indicate the number of isolates with corresponding MIC value. White areas indicate the range of dilutions tested for each antimicrobial agent; values above or below this range denote MIC values greater than the highest concentration tested and MIC values smaller than or equal to the lowest concentration tested, respectively. Vertical lines indicate clinical breakpoints, when two vertical lines exist, the lower breakpoint indicate susceptibility and the higher breakpoint

resistance with an intermediate range in between. <sup>a</sup>Clinical breakpoint is available according to EUCAST guidelines (clinical breakpoint for version 4.0, 2014 *Enterobacteriaceae*). <sup>b</sup>Clinical breakpoint is available according to CLSI (M100-S22, 2012 human clinical breakpoint for *Enterobacteriaceae*). <sup>c</sup>No EUCAST or CLSI clinical breakpoint available.

**Table 3** Minimal inhibitor concentration (MIC) of ~~from~~ humane feces *Y. enterocolitica* biotype 4 isolates.

Antimicrobial agent	No. of strains with MIC (mg/L)																			
	0.008	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024	2048	
Ampicillin <sup>a</sup>														30	6					
Cefotaxime <sup>a</sup>				31	5															
Ceftazidime <sup>a</sup>					36															
Chloramphenicol <sup>a</sup>								3	30	1					2					
Ciprofloxacin <sup>a</sup>	15	19			2															
Colistin <sup>a</sup>									36											
Florfenicol <sup>a</sup>								26	10											
Gentamicin <sup>a</sup>						1	35													
Kanamycin <sup>b</sup>										36										
Nalidixic acid <sup>b</sup>									34						2					
Streptomycin <sup>c</sup>									2	29	1					4				
Sulfamethoxazole <sup>b</sup>											31	2	1		1					1
Tetracycline <sup>b</sup>								5	28	3										
Trimethoprim <sup>a</sup>								23	13											

Numbers indicate the number of isolates with corresponding MIC value. White areas indicate range of dilutions tested for each antimicrobial agent; values above or below this range denote MIC values greater than the highest concentration tested and MIC values smaller than or equal to the lowest concentration tested, respectively. Vertical lines indicate clinical breakpoints, when two vertical lines exist, the lower breakpoint indicate susceptibility and the higher breakpoint resistance with an intermediate range in between. <sup>a</sup>Clinical breakpoint is available according to EUCAST guidelines (version 4.0, 2014 *Enterobacteriaceae*). <sup>b</sup>Clinical breakpoint is available according to CLSI (M100-S22, 2012 *Enterobacteriaceae*). <sup>c</sup>No EUCAST or CLSI clinical breakpoint available.

### 3 Results and Discussion

#### 3.1 Detection of virulence-associated genes

Pigs are the focus of interest as the primary reservoir for human pathogenic *Y. enterocolitica*, especially for BT 4, which is predominantly found in porcine tonsils and also represents the principal BT in human isolates (EFSA, 2013). Therefore, the current study focused on BT 4. The virulence genes chosen (*yadA*, *virF*, *ail*, *inv*, *rovA*, *ymoA*, *ystA*, *ystB* and *myfA*) covered those involved in important steps during host infection (Fàbrega and Vila, 2012). In total, 87 porcine and human isolates were tested for virulence-associated gene patterns. Eighty-four of the strains (< 96%) possessed the *yadA*, *virF*, *ail*, *inv*, *rovA*, *ymoA*, *ystA* and *myfA* genes, whereas none of them possessed the *ystB* gene, demonstrating the high potential of Swiss porcine BT 4 isolates to be pathogenic for humans. Only 1 strain isolated from porcine tonsils (1/38) lacked *yadA*, and another 2 isolates from porcine tonsils (2/38) were *yadA*- and *inv*-negative, whereas isolates from porcine feces showed no difference in their virulence-associated gene patterns in comparison to the human isolates (Table 1). In part, these results contradict the findings of previous studies. Bonardi et al. (2013) detected 12.5% Italian porcine BT 4 *ail* negative strains, and Zheng et al. (2008) also detected *ystA*-negative and *ystB*-positive human BT 4 strains in China, in contrast to our data. The detection rate in the current study, with 92.1% of *yadA*-positive porcine tonsil strains, was still higher than that in recent studies on porcine or human BT 4 strains (54–83%) (Zheng et al., 2008; Stephan et al., 2013; Bonardi et al., 2013). As we tested 2 plasmid-borne genes and the strains still carry the *virF* gene, it is unlikely that the lack of the *yadA* gene is a consequence of a plasmid loss during the cultivation process. Two tested BT 4 strains from porcine tonsils lacked the *inv* gene. It has been reported that *inv* negative strains still have the ability to invade the host cells (Pepe and Miller, 1993). Therefore it is possible that the 2 porcine isolates lacking *inv* are still pathogenic. In addition to the abovementioned virulence-associated genes, the *ymoA* and *rovA* genes were tested. Both of these genes are involved in the regulation of *inv* expression. Moreover, *ymoA* is also relevant for the modulation of the expression of the *virF* and *yst* genes (Revell and Miller, 2000) (Ellison et al., 2003). Our results showed that, independent of the source, all tested isolates possessed both effector and regulator genes. To the authors' knowledge these findings are not demonstrated up to now elsewhere.

#### 3.2 Serotype distribution

The tested isolates comprised a broad spectrum of known STs (O:3; O:8; O:27; O:3,27; O:3,8,27; O:8,27 and O:9,27). The most frequent STs were O:3 and O:3,27, followed by O:8,27, except for human isolates, which did not show ST O:8,27 (Table 1). This finding is in contrast to the results of other studies that determined almost 100% bioserotype 4/O:3 for both porcine and human strains. This discrepancy can be explained by methodological differences of serotyping. In other studies, only antiserum O:3 (Laukkanen et al., 2009) or a different panel of antisera was used (Bonardi et al., 2013). In the present study, the virulence-associated gene pattern was independently detected from STs for both porcine and human isolates. Thus, determination of STs is of limited value for the characterization of pathogenic *Y. enterocolitica* isolates.

### 3.3 Antimicrobial testing

In general, the majority of all strains of this study were susceptible to almost all the tested antibiotics. In contrast, 100% antibiotic resistance to ampicillin was found (Tables 2 and 3). The high rate of resistance to  $\beta$ -lactam antibiotics has been well described (Fàbrega and Vila, 2012), and these findings also agree with previous studies of the antimicrobial antibiotic resistance of porcine BT 4 strains from Switzerland (Fredriksson-Ahomaa et al., 2007; Baumgartner et al., 2007). Although human BT 4 isolates shared the overall antibiotic susceptibility phenotype with porcine isolates, 2 human BT 4 isolates possessed antibiotic resistance to chloramphenicol and nalidixic acid. One strain was also resistant to sulfamethoxazole. *Y. enterocolitica* antibiotic resistance to chloramphenicol is rarely described in the literature, but Bonardi et al. (2014) recently found BT 4 strains resistant to chloramphenicol. Additionally, Sihvonen et al. (2011b) detected antibiotic resistance to chloramphenicol in human *Y. enterocolitica* strains. For streptomycin, the human and the porcine tonsil strains showed an MIC<sub>50</sub> of 8 mg/L and an MIC<sub>90</sub> of 16 mg/L. In contrast, the 2 chloramphenicol-resistant strains showed an elevated MIC of >128 mg/L, which indicated that they are resistant to streptomycin as well. The origin of this antibiotic resistance pattern (chloramphenicol, streptomycin and sulfonamide) is most likely a conjugative plasmid, as Sihvonen et al. (2011b) have indicated. The 2 chloramphenicol-resistant strains also possessed antibiotic resistance to nalidixic acid. Antibiotic resistance of *Y. enterocolitica* to nalidixic acid has been described in Switzerland (Fredriksson-Ahomaa et al., 2012), and a substantial increase in nalidixic acid resistance in human clinical isolates has been detected in Spain between 1995 and 2002 (Capilla et al., 2004). MIC<sub>50</sub> and the MIC<sub>90</sub> of florfenicol were 4 mg/L each for isolates from porcine tonsils and from porcine feces. Only 1 porcine isolate exhibited an elevated MIC to florfenicol (MIC = 8 mg/L). For human isolates MIC<sub>50</sub> of florfenicol was 2 mg/L and the MIC<sub>90</sub> = 4 mg/L, respectively.

## 4 Conclusions

The virulence-associated gene pattern detected in the human strains from clinical cases is supposed to be sufficient for human infection. The same virulence-associated gene pattern was found in the strains isolated from porcine tonsils as well as porcine feces, demonstrating the potential to cause human infection for all of the Swiss porcine *Y. enterocolitica* BT 4 strains tested. Therefore, measures must be taken at slaughter to avoid *Y. enterocolitica* cross-contamination from tonsils as well as fecal contamination of the carcasses.

Recent studies in Europe have reported increasing antibiotic resistance of both human and porcine *Y. enterocolitica* isolates (Sihvonen et al., 2011b; Bonardi et al., 2014). Low level of antibiotic resistance found in Swiss human strains from 2001 to 2003 underline the increasing tendency in human strains, whereas the rate of acquired antibiotic resistance in recently isolated predominant Swiss porcine *Y. enterocolitica* BT 4 strains is currently very low. However, the use of antibiotics in Swiss livestock is still considerable. Therefore, the prudent use of antimicrobials in veterinary medicine is important for the control of antibiotic resistance phenomenon in Swiss *Y. enterocolitica* isolates.

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### Highlights

- The same virulence-associated gene pattern were found in *Y. enterocolitica* BT 4 strains isolated from porcine tonsils and as well as from porcine feces are equal
- Porcine *Y. enterocolitica* BT 4 strains are capable to infect humans despite the source of isolation and serotype
- The rate of acquired resistance in predominant Swiss porcine *Y. enterocolitica* BT 4 strains is currently low

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## Queries and Answers

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**Answer:** The first highlight was shortened. The second and third highlight were not shortened as they consist of 112 and 108 characters, respectively.

### Query:

The citation "Paixão et al., 2013" has been changed to match the author name/date in the reference list. Please check here and in subsequent occurrences, and correct if necessary.

**Answer:** The citations are correct.

**Query:**

The citation "Paixão et al. 2013" has been changed to match the author name/date in the reference list. Please check here and in subsequent occurrences, and correct if necessary.

**Answer:** The citations are correct.

**Query:**

Please provide the volume number and page range for the bibliography in 'Stephan et al., 2013'.

**Answer:** The volume number and page range is provided.