Unique Activity Spectrum of Colicin FY: All 110 Characterized Yersinia enterocolitica Isolates Were Colicin FY Susceptible

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Abstract

Colicin FY is a plasmid encoded toxin that recognizes a yersinia-specific outer membrane protein (YiuR) as a receptor molecule. We have previously shown that the activity spectrum of colicin FY comprises strains of the genus Yersinia. In this study, we analyzed the activity of colicin FY against 110 Yersinia enterocolitica isolates differing in geographical origin and source. All isolates were characterized through analysis of 16S rRNA genes, serotyping, biotyping, restriction profiling of genomic DNA, detection of virulence markers and susceptibility to antibiotics. This confirmed the broad variability of the collection, in which all 110 Y. enterocolitica isolates, representing 77 various strains, were inhibited by colicin FY. Although isolates showed variable levels of susceptibility to colicin FY, it was not associated with any strain characteristic. The universal susceptibility of Y. enterocolitica strains to colicin FY together with the absence of activity towards strains outside the Yersinia genus suggests potential therapeutic applications for colicin FY.

Introduction

Based on both genetic and phenotypic features, Yersinia enterocolitica is considered to be a heterogeneous species [1–3]. Y. enterocolitica is often found in aquatic environments and in various animal reservoirs, with swine being a major reservoir of human pathogenic strains. The most frequently isolated human strains belong to bioserotypes 1B/O:8, 2/O:5,27, 2/O:9, and 4/O:3, with 4/O:3 being the most common and typical for Europe [4–9].

Infections caused by Y. enterocolitica are the third most common bacterial alimentary infections of humans in the European Union [9]. Yersiniosis ranges from self-limited enteritis to life-threatening systemic infections. The most frequent manifestation is diarrhea, mainly affecting children [4,10–14]. Although antibiotic treatment is recommended for serious cases, the benefits of antibiotic therapy in uncomplicated cases is not well established [5,15–17]. Instead, rehydration and use of probiotics are often suggested for simple diarrheal cases.

Production of bacteriocins has been described in many genera of enteric bacteria including Escherichia, Shigella, Citrobacter, Salmonella, and Yersinia [18]. Although antibacterial activity of various species of genus Yersinia has been previously documented [19–24], only three yersinia-produced bacteriocins have been intensively studied and characterized on the molecular level. These include the bacteriocin of Y. pestis (pestici I; [25–30]), a phage tail-like bacteriocin produced by Y. enterocolitica (enterocoliticiin; [31,32]), and a bacteriocin from Y. frederiksenii Y27601 (colicin FY; [33]). Colicin FY is produced by an environmental isolate of Y. frederiksenii, which contains the colicinogenic plasmid pYP27601 (5,574 bp) harboring the colicin FY activity (cfyA) and immunity (cfyI) gene. Colicin FY (54 kDa) recognizes a yersinia-specific outer membrane protein (YiuR) as a receptor molecule in susceptible bacterial strains. YiuR protein is encoded by many yersiniae (e.g. Y. pestis, Y. pseudotuberculosis, Y. enterocolitica, and Y. frederiksenii). YiuR belongs to the family of TonB-dependent proteins with putative outer membrane function. Colicin FY uses the TonB system for translocation, similar to colicins B, D, Ia, and Ib. Lethal activity of colicin FY is exerted by formation of voltage-gated pore in the cytoplasmic membrane. The lethal effect of colicin FY is directed against several nonpathogenic and opportunistic yersiniae (i.e. Y. frederiksenii, Y. aldovae, Y. kristensenii, and Y. intermedia) and also against pathogenic strains of Y. enterocolitica. Previously published data [33] suggested that Y. enterocolitica is widely susceptible to colicin FY; however, the strain collection was limited to only 31 Y. enterocolitica isolates originated in the Czech Republic that were not characterized in detail.

In this study, 110 Y. enterocolitica isolates with different geographical origins and sources were characterized in detail to exclude any potential clonal character of the isolates. Colicin FY inhibited growth of all tested isolates indicating that the vast majority of Y. enterocolitica strains are susceptible to colicin FY.
Materials and Methods

Bacterial strains and growth conditions

Colicin Fv producer, Yersinia frederikseni strain Y27601, was obtained from the National Reference Laboratory for Salmonella, The National Institute of Public Health (NIPH), Prague. The recombinant strain producing colicin Fv (Escherichia coli TOP10FpDNA1068) was constructed in our laboratory [33]. Y. enterocolitica isolates were obtained from several institutions including the National Reference Laboratory for Salmonella, NIPH, Prague; the Department of Clinical Microbiology, University Hospital Brno (UHB), Brno; and the Max von Pettenkofer-Institute (MVPI), Ludwig Maximilian University of Munich, Munich. Detailed information for the isolates of Y. enterocolitica used in this work is presented in Table S1. We used a set of 118 Escherichia isolates containing E. coli (39 isolates), E. fergusonii (10 isolates), E. hermanii (42 isolates), and E. vulneris (27 isolates). An additional 18 isolates of enterobacterial species included Budovia aquatuita (24510; from E. Aldová), Citrobacter youngae (42/57; NIPH), C. braakii (B718; UHB), C. freundii (B607; UHB), Enterobacter aerogenes (1832; NIPH), E. cloacae (B604; UHB), Klebella pneumoniae (B615; UHB), K. oxytoca (B632; UHB), Klyuyvera ascorbata (B792; UHB), Lecellaria adorcleopsis (2665; from L. Sledáček), Morganella morganii (B619; UHB), Pseudomonas galls (4613; from E. Aldová), Proteus vulgaris (B633; UHB), Serratia ficaria (B753; UHB), Shigella flexneri (strain 4; [66]), S. sonnei (strain 17; from laboratory stock), and S. boydii (U1; from V. Horáček).

Trypton-yeast (TY) broth consisting of 8 g/l tryptone (Hi-Media, Mumbai, India), 5 g/l yeast extract (Hi-Media), and 5 g/l sodium chloride in water was used throughout the study. For cultivation on solid media, TY broth was supplemented with agar powder (1.5%, w/v; Hi-Media). Mueller-Hinton agar (38 g/l; Hi-Media) was used for analysis of antibiotic susceptibility. TY agar plates supplemented with L-(-)-arabinose (0.2 g/l; Sigma-Aldrich, St. Louis, USA) were used to induce expression of colicin Fv in recombinant E. coli TOP10FpDNA1068.

16S rRNA analysis

To analyze the 16S rRNA in Y. enterocolitica isolates, a part of the 16S rRNA gene, consisting of 524 bp, was amplified from a single bacterial colony resuspended in 100 μl of deionized water. The yersinia DNA was amplified using Tak polymerase (New England Biolabs, Beverly, USA) and a pair of 16S rDNA-specific primers (16SRNAF: 5′-AGTTTGGATATGGGCTGAG-3′ and 16SRNAR: 5′-TTACGGCGGGCTGGTGGA-3′) [34]. Colony PCR started with denaturation at 94°C for 5 min, followed by 40 cycles at 95°C for 30 s, 50°C for 30 s, 72°C for 1 min, and extension at 72°C for 10 min. PCR products were sequenced using Tak DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, USA). The Lasergene program package (DNASTAR, Madison, USA) was used for assembly of the sequencing reads and further data analyses. Isolates were classified to subspecies on the basis of polymorphisms in the 30 bp region of the 16S rDNA [35].

Biovar classification

Y. enterocolitica isolates were serotyped and biotyped using previously described methods [1,2]. Isolates from the Czech Republic were serotyped using diagnostic agglutination antiserum O:3, O:5, O:8 and O:9 (IEST Test PLUS, Hradec Králové, Czech Republic) and biotyped on the basis of esculin hydrolysis, indole production, xylode and/or trehalose utilization. Biovar types of 50 isolates from outside the Czech Republic were provided with isolates. For verification of the provided biovar type characterization, a random subgroup (n = 15) of these isolates was also biovar typed.

Pulsed field gel electrophoresis (PFGE)

Overnight TY cultures were centrifuged, diluted in suspension buffer (100 mM Tris (Sigma-Aldrich), 100 mM EDTA (Sigma-Aldrich), pH = 8) to OD600 = 1.4 and mixed with equal volume of 1.6% Pulsed Field Certified Agarose (Bio-Rad Laboratories, Hercules, USA) containing 1% SDS, 500 μg proteinase K, pH = 8) and incubated at 54°C for 2 hours. The plugs were then washed in deionized water at 54°C. (2×10 min) followed by washing (4×10 min) with TE buffer (10 mM Tris, 1 mM EDTA, pH = 8). After bacterial lysis, the plugs were digested with 50 U of MboI enzyme (New England Biolabs) at 37°C for 3 hours and were loaded into a 1% Pulsed Field Certified Agarose gel. Electrophoresis was performed using a CHEF-DR II system (Bio-Rad Laboratories) in 0.5% TBE (50 mM Tris, 50 mM boric acid, 1.5 mM EDTA) at 14°C, 6 V/cm and a ramping time of 2.5 to 25 s over 24 hours. Gels were stained with ethidium bromide (1 μg/ml) and visualized under UV light. Genomic DNA from Salmonella enterica, serotype Braenderup H9812, digested using XbaI enzyme (New England Biolabs), was used as a molecular weight standard.

BioNumerics v6.1 (Applied Maths, Sint Martens Latem, Belgium) was used to analyze restriction profiles (bands from ~50 kbp to ~500 kbp). Based on the PFGE data, dendrograms were constructed using Dice’s coefficient of similarity and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering at 0.5% tolerance.

Detection of virulence factors

Genomic DNA was isolated from overnight cultures using DNAzol® Reagent (Invitrogen, Carlsbad, USA), according to the manufacturer’s instructions. Isolated DNA (1 μl) was used as a template for multiplex PCR [36]. Three different virulence markers – ystA (gene encoding yersinia stable toxin, 134 bp), vinF (gene encoding transcriptional activator of yop genes, 231 bp) and ail (attachment and invasion locus, 356 bp), were amplified using 0.5 U Tag polymerase (New England Biolabs) and specific primer pairs (YstA: 5’-GTCCTTAGTTGAGGATTGCCG-3’ and 3’-AACACTAGTACGTTGCGCCCA-3’), VinF: 5’-GCTTTGCGTCTGAGGTTTGC-3’ and 3’-AGAATTCTCGTCTCCTTTGC-3’ and Ail: 5’-TTGCCTTTTGACACTGATACTG-3’ and 3’-GCTTTTGTGCAGAGGTTGCT-3’.

PCR started with denaturation at 94°C for 5 min, followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and extension at 72°C for 10 min. When multiplex PCR results were negative, virulence markers were analyzed separately under the same PCR conditions.

Antibiotic susceptibility assay

Yersiniae were tested for susceptibility to 14 antibiotics using the disc diffusion method and the National Committee for Clinical Laboratory Standards guidelines [37]. Susceptibility assays with antibiotic disks (Oxoid, Basingstoke, UK) were performed on Mueller–Hinton agar at 37°C. The following antimicrobial drugs and quantities were used: ampicillin (AMP; 10 μg), cephalothin (KF; 30 μg), doxycycline (DO; 30 μg), cefuroxime (CXM; 30 μg), ciprofloxacin (CIP; 5 μg), sulfamethoxazole-trimethoprim
Colicin activity assay

Detection of colicin activity was performed as described previously [38]. Briefly, the agar plates were inoculated with a stab from the bacterial culture (E. frederiksenii Y27601, E. coli TOP10F' carrying pBAD-A), incubated at 37°C for 48 hours and the resulting macrocolonies were killed with chloroform vapors. Each plate was overlaid with a 0.75% TY agar (3 ml) containing 1 x 10^8 cells of a tested isolate. Simultaneously, the level of bacterial susceptibility to colicin F_Y was detected by spotting of serial dilutions (diluting factor 0.25) of purified colicin F_Y [33] on the same agar plates. The plates were then incubated at 25°C overnight and zones of growth inhibition were read. The reciprocal value of the highest dilution of the purified colicin causing complete and partial growth inhibition were considered to be the colicin titer (in arbitrary units, dilution of the purified colicin causing complete and partial growth inhibition were read. The reciprocal value of the highest dilution of the purified colicin causing complete and partial growth inhibition were considered to be the colicin titer (in arbitrary units, dU).

Detection of colicin F_Y immunity gene

To analyze for the presence of the colicin F_Y immunity gene in Y. enterocolitica isolates, a part of the cdy gene, consisting of 162 bp, was amplified from a single bacterial colony resuspended in 100 µL of deionized water. Bacterial suspensions (1 bd) were inoculated and used as a DNA templates, which were amplified using Taq polymerase (New England Biolabs) and specific primers (immFy-F: 5'-GGACGTTAACCCCGCTACGG-3' and immFy-R: 5'-AC- CCTGAAAGCGCAACGAC-3'). Colony PCR started with denaturation at 94°C for 5 min, which was followed by 40 cycles at 95°C for 30 s, 58°C for 30 s, 72°C for 1 min, and finished by extension at 72°C for 10 min.

Sequencing of yiuR and tonB genes

The yiuR gene from nine Y. enterocolitica isolates (YE48, YE61, YE66, YE73 YE81, YE84, YE85, YE93, and YE97) was amplified from a single colony using Taq polymerase (New England Biolabs) and specific primers (YE1461SD-F: 5'-ACCGAATAATGGCTAAGGCTTATTTG-3' and YE1461-R: 5'-TATAAGCCTGTAAGGCGCCG-3'). Colony PCR started with denaturation at 94°C for 5 min, followed by 40 cycles at 95°C for 30 s, 50°C for 2 min, 72°C for 1 min, and extension at 72°C for 10 min. Additionally, the tonB gene was amplified using specific primers (tonB_YE2222-F: 5'-ATGGACGCTAAATTTTTTTCTTTTG G-3' and tonB_YE2222-R: 5'-TTAGTCCATTCTGCTGTG-3'). All PCR products were sequenced using a Taq DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems). The Lasergene program package (DNASTAR) was used for assembly of the sequencing reads and further data analyses.

Results

Sequencing of 16S rRNA genes, serological and biochemical characterization of isolates

Sequencing analysis of the 16S rRNA coding DNA confirmed that all 110 tested isolates belonged to the Y. enterocolitica species (data not shown). Moreover, sequencing results were used to classify isolates into subspecies; 108 isolates were identified as Y. enterocolitica subsp. palaearctica and 2 isolates as Y. enterocolitica subsp. enteritidis (Fig. S1). Seven different serotypes and all six different biotypes (1A, 1B, 2, 3, 4, and 5) were present within the isolates (Fig. S1). Common human pathogenic serotypes, (i.e. O:3, O:5,27, O:6, and O:9) and several atypical serotypes (i.e. O:5, O:6,30 and O:36) were present in our collection. Y. enterocolitica bioserotype 4/O:3 was the most frequent (77%).

PFGE analysis

To determine the genetic variability of Y. enterocolitica isolates, restriction profiles of all isolates were determined (Fig. S1). Altogether, 41 various pulsotypes (at the 85% similarity level) were identified (Fig. 1). Moreover, 24 different pulsotypes were found within the most abundant bioserotype 4/O:3 subgroup.

Identification of virulence markers

Two chromosome- and one plasmid-encoded virulence markers (ail, yiaH, and virF, respectively) were detected using PCR (Fig. S1). In contrast to the chromosomal genes, which were detected in more than 90% of isolates [91% and 96% for ail and yiaH, respectively], the virF gene was found in only 30% of Y. enterocolitica isolates. All three determinants were identified in 52 isolates (47%) and only four isolates were negative for all tested markers. Taken together, most of the collected isolates could be considered to be potentially pathogenic Y. enterocolitica.

Susceptibility to antibiotics

All tested yersinia were susceptible to ciprofloxacin, ceftoxime, cefazidime, aztreonam, and colistin sulphate. About 20% of isolates showed an intermediate susceptibility to cefuroxime and amoxicillin with clavulanic acid. In addition, more than 90% of isolates were susceptible to doxycycline, sulfamethoxazole-trimethoprim, oxolinic acid, gentamicin, and chloramphenicol. Less than 10% of yersinia were susceptible to ampicillin and cephalothin. Altogether, 20 different antibiograms were identified (Table S2), with the most frequent antibiogram, A1, identified in 46% of isolates (Fig. S1). Isolates with antibiogram A1 were susceptible to all tested antibiotics with the exception of ampicillin and cephalothin.

Susceptibility to colicin F_Y

Colicin F_Y producers, Y. frederiksenii Y27601 and a recombinant strain of E. coli carrying pDS1068 [33], inhibited growth of all tested Y. enterocolitica isolates. All Y. enterocolitica isolates were also susceptible to purified colicin F_Y. Degree of susceptibility to purified colicin F_Y (shown as a colicin titer in A.U.) varied from 64 to 1024 and from 256 to 65536 for clear and turbid zones of growth inhibition, respectively. The susceptibility of individual isolates of Y. enterocolitica to colicin F_Y is shown in Fig. S1 and the distribution of colicin F_Y susceptibility among Y. enterocolitica is shown in Fig. 2. The susceptibility of Y. enterocolitica isolates to colicin F_Y showed a nonrandom distribution (chi-squared goodness of fit test; p<0.001), where the majority of isolates were susceptible to 1024 A.U. for bacterial inhibition. Moreover, no single variable position of YiuR or TonB was associated with susceptibility to colicin F_Y (Fig. 3). All 110 isolates did not encode a colicin F_Y immunity protein, which should affect the susceptibility to colicin F_Y.

In contrast to the universal susceptibility of Y. enterocolitica strains to colicin F_Y, no colicin F_Y-susceptible strains outside the Yersinia genus were identified when 136 isolates belonging to the 13 other enterobacterial genera (i.e. Bordetella, Citrobacter, Enterobacter, Esche-
**Discussion**

The aim of this study was to evaluate susceptibility of *Y. enterocolitica* to colicin F$_Y$ using a set of characterized isolates. The 110 isolates were collected from different geographical areas with the majority of isolates originating in Europe. Most of *Y. enterocolitica* isolates originated from human clinical material, but veterinary and environmental isolates were also present. To exclude the possibility that the collection of *Y. enterocolitica* contains multiple isolates of identical strains, further characterization of these isolates was performed including analysis of the 16S rRNA genes, serotyping, biotyping, restriction profiling of genomic DNA, detection of virulence markers and susceptibility to antibiotics. Using these typing techniques, 77 different *Y. enterocolitica* strains were identified in our collection, from which 59 strains were represented by a single isolate, while the other 18 strains contained more than one isolate with identical characteristics. High genetic variability was shown for nonhuman isolates, while more subtle variability was found within the 4/O:3 subgroup. Almost all tested isolates can be considered to be pathogenic or potentially pathogenic. With the exception of two beta-lactam antibiotics, isolates of *Y. enterocolitica* were susceptible to all tested antibiotics. As results of the characterization assays are in agreement with other studies on *Y. enterocolitica* [5,9,39–48], our set of *Y. enterocolitica* isolates represents a group of isolates with typical features.

None of the 110 isolates of *Y. enterocolitica* encoded the colicin FY immunity protein (data not shown) and all were susceptible to colicin FY; however they differed in the degree of susceptibility to it. No obvious association between colicin F$_Y$ susceptibility and other described strain parameters was found, indicating that it is independent of other strains characteristics (Fig. S1). Moreover, no amino acid replacements in the YiuR and TonB proteins were associated with differences in colicin F$_Y$ susceptibility. Others features (e.g. cell wall composition) seem to affect susceptibility to colicin F$_Y$.

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**Figure 1. Identified pulsotypes among *Y. enterocolitica* isolates.** Similarities (%) between restriction patterns were calculated using the Dice’s index and are shown as the numbers close to nodes. The data were sorted using the UPGMA method. For construction of this dendrogram, 41 *Y. enterocolitica* pulsotypes with similarity lower than 85% were selected from a dendrogram containing all 110 isolates (Fig. S1). Susceptibility to colicin F$_Y$ is shown in the right panel, followed by additional strain characteristics. Colicin F$_Y$ titers are shown as the reciprocal exponent of the highest four-fold dilution causing clear (light grey) and turbid (dark grey) zones of inhibition. Subspecies: *P* = *palearctica*, *E* = *enterocolitica*. *Serotypes O:1 and O:2 have been combined to O:3 serotype according to [1].

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A) Distribution of colicin F_Y susceptibility levels. Colicin F_Y susceptibility ranged from 256 to 65536 A.U. Half of the detectable growth inhibition (last turbid zone). The susceptibility to colicin F_Y is shown as colicin titers representing the highest dilution causing growth inhibition.

Bacterial resistance to bacteriocins is considered to be a successful strategy in antimicrobial competition [49–51]. It has been shown that around 75% of E. coli strains isolated from different source populations are resistant to one or more bacteriocin types [52,53]. Resistance to bacteriocin-like substances produced by yersiniae has been found among Y. enterocolitica strains [21]. In addition, enterocoliticin, a characterized phage tail-like bacteriocin, has been shown to inhibit pathogenic Y. enterocolitica serotypes O:3, O:5,27, and O:9, but not serotype O:8 and various nonpathogenic isolates (biotype 1A) [31]. In contrast, colicin F_Y appears to inhibit all Y. enterocolitica isolates. A similar finding has been published with respect to an uncharacterized bacteriocin from Y. kristensenii and 35 tested strains of Y. enterocolitica [24,54,55]; however, it is not known whether this uncharacterized bacteriocin could, in fact, be colicin F_Y.

Although the reason for the universal susceptibility of Y. enterocolitica to colicin F_Y is unknown, it can be speculated that this could be a result of the clonal character of isolates and/or the essential character of the YiuR receptor for yersiniae. The clonal character of Y. enterocolitica was obvious only in pathogenic isolates (e.g. serotype O:3), while nonpathogenic isolates of biotype 1A were more heterogeneous [56,57]. This explanation therefore appears unlikely. Similarly, the essential character of the YiuR receptor also appears unlikely, since yersiniae possess several iron uptake systems (e.g. Y. pestis harbors several systems and Yiu-mediated transport is not first in the iron uptake hierarchy [58]).

Another possible explanation for universal activity of colicin F_Y against Y. enterocolitica involves a close relationship between Y. enterocolitica and colicin F_Y producers, with little or no contact between them. A phylogenetic analysis showed that Y. enterocolitica was clearly related to other environmental enterocolitica-like species including Y. frederiksenii [59,60]. In addition, contact between the two bacterial species appears to be quite rare despite the sporadic co-occurrence of both species [61–63]. The separation of Y. enterocolitica strains from environmental colicin F_Y-producers could explain the absence of colicin F_Y resistant mutants among Y. enterocolitica strains. In fact, colicin F_Y resistant colonies of Y. enterocolitica were induced in the presence of colicin F_Y in laboratory conditions (data not shown). Moreover, the environmental yersiniae (e.g. Y. frederiksenii and Y. kristensenii) that live in the same environment as colicin producers contain both resistant and susceptible strains to colicin F_Y [33].

There is increasing interest in nonpathogenic microorganisms and their antimicrobial substances that naturally antagonize pathogenic agents. To date, several nonpathogenic Escherichia coli strains have been used as probiotics (e.g. E. coli Nissle 1917; [64]). Although the role of bacteriocin synthesis in probiotic bacteria is not known, production of bacteriocins is a common feature of many of them [65–67]. It is therefore tempting to speculate that synthesis of colicin F_Y could represent an important feature of recombinant probiotic E. coli strains used in cases of diarrhea caused by yersiniae. However, the effect of colicin F_Y synthesis should be tested using in vivo experiments to see whether colicin F_Y has therapeutic potential relative to intestinal yersiniosis.

**Supporting Information**

**Figure S1** Dendrogram of Y. enterocolitica isolates.

Similarities (%) between restriction patterns were calculated using the Dice’s index and are shown as the numbers close to nodes. The data were sorted using the UPGMA method. Susceptibility to colicin F_Y is shown in the right panel, followed by additional strain characteristics. Colicin F_Y titers are shown as the reciprocal exponent of the highest four-fold dilution causing clear (light grey) and turbid (dark grey) zones of inhibition. *Serotypes O:1 and O:2 have been combined to O:3 serotype according to [1]. The lines on the right side show isolates with the same characteristics (i.e. considered to be identical strains).

(TIF)

Table S1 Y. enterocolitica isolates used in this study.

(DOCX)

Table S2 Antibiotic susceptibility of Y. enterocolitica isolates.

(DOCX)

**Author Contributions**

Conceived and designed the experiments: JB DS. Performed the experiments: JB LM MV DG. Analyzed the data: JB LM DS. Contributed reagents/materials/analysis tools: MV AS DD DG. Wrote the paper: JB DS.
Figure 3. Sequence variability between highly and less susceptible Y. enterocolitica isolates. A) Sequence analysis of YiuR protein from various isolates identified variability in 26 amino acid positions. A consensus sequence is shown at the top of the figure. The numbers correspond to amino acid position in the YiuR and TonB proteins, respectively. B) Sequence analysis of TonB from various isolates identified variability in 18 amino acid positions. # doi:10.1371/journal.pone.0081829.g003

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