Brief communication

Antimicrobial resistance and plasmid replicons in Yersinia enterocolitica strains isolated in Brazil in 30 years

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ABSTRACT

Some studies evaluated the resistance profile of the Y. enterocolitica strains isolated in diverse countries. However, in Brazil the isolation and the study of Y. enterocolitica are not common and therefore information about the antimicrobial resistance profile of this species in this country is scarce. Therefore, the aim of this study was to evaluate the antimicrobial resistance of Y. enterocolitica of biotypes 1A, 2 and 4 isolated from clinical and non-clinical sources between 1979 and 2012, in Brazil. This study showed that some Yersinia enterocolitica of different biotypes remain susceptible to antimicrobials used for gastroenteritis treatment. Moreover, neither acquired resistance genes nor diversity of plasmids replicons were found; however, variation in the in vitro intrinsic resistant pattern was detected, except the non-resistance to cefoxitin in all strains. Notwithstanding, due to epidemiological link between Y. enterocolitica and the pork production chain, monitoring plasmid acquired resistance in Y. enterocolitica could also be considered for antimicrobial resistance control purposes and food safety measures.

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Yersinia enterocolitica is the most prevalent Yersinia species that causes illness in humans and animals. Y. enterocolitica strains can be classified into six biotypes, being biotypes 1B, 2, 3, 4, and 5 associated with illness in humans and animals, while biotype 1A comprises strains that are considered to be primarily nonpathogenic.1

Y. enterocolitica intrinsic resistance to ampicillin, ticarcillin, amoxicillin-clavulanate, cefazolin, and cephalothin has been assigned by the Clinical & Laboratory Standards Institute (CLSI).2 In addition, intrinsic resistance to cefamandole and cefoxitin has also been recognized by EUCAST.3 Y. enterocolitica have shown susceptibility in vitro to aminoglycosides, tetracycline, chloramphenicol, extended-spectrum cephalosporins, and trimethoprim-sulfamethoxazole. Resistance to fluoroquinolones has been observed in some countries due to chromosomal mutation mechanism.4 In Brazil, the isolation and the study of Y. enterocolitica are not common and thus information about the antimicrobial resistance of isolates of this species in this country is scarce.5-8

Therefore, the aims of this study were to determine the antimicrobial susceptibility profile and assess the intrinsic resistance pattern, to search for plasmid acquired resistance genes, and to investigate plasmid replicons in Y. enterocolitica isolated in Brazil.

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A total of 34 Y. enterocolitica strains biotype 1A (n = 2), 2 (n = 12), and 4 (n = 20) were studied. These strains were selected from the collection of the "Brazilian Reference Center on Yersinia spp. other than Y. pestis" isolated from 1979 to 2012, based on the resistance profiles found for some other strains of the biotypes mentioned above in previous studies of our group.5–7 The antimicrobial susceptibility profile was determined using the disk diffusion method and interpreted according to the breakpoints for Enterobacteriaceae.2,3,9 Moreover, double-disk synergy test (DDST) was performed to detect extended-spectrum beta-lactamase (ESBL) production,10 enzymes able to hydrolyze third- and fourth-generation cephalosporins.

Plasmid acquired genes coding for resistance to extended-spectrum cephalosporins (blaCTX-M, blaTEM and blasYNJ), tetracyclines (tet), aminoglycosydes (aac(6’)-Ib), and fluoroquinolones (qnr, aac(6’)-Ib-cr, qepA and qoxA) were searched.11–14 Moreover, plasmids were searched following the PCR-based replicon typing (PBRT) scheme targeting replicons of the major incompatibility groups (Inc) harboring/disseminating antibiotic resistance genes in Enterobacteriaceae.15

Y. enterocolitica samples showed recognized intrinsic resistance to cefazolin (CFZ) (34/34), cephalotin (CF) (34/34), ampicillin (AMP) and ticarcillin (TIC) (32/34), and amoxicillin-clavulanic acid (AMC) (19/34). This inherent non-susceptibility pattern has been displayed by all or almost all strains from different biotypes and isolation sources. However, cefoxitin (FOX) intrinsic resistance, additionally also recognized by EUCAST,16 was not detected (Table 1).

Most Y. enterocolitica isolates harbored chromosomal genes blaA and blaB encoding for two beta-lactamases, respectively,

### Table 1 – General data of the 34 Yersinia enterocolitica strains studied.

| Strains | Biotype | Source            | Year | Resistance profile |
|---------|---------|-------------------|------|--------------------|
| IP 7382 | 4       | Animal feces      | 1979 | AMP, TIC, CFZ, CF, NIT |
| IP 7884 | 4       | Animal feces      | 1979 | AMP, TIC, CFZ, CF |
| FCF 57  | 1A      | Milk              | 1980 | AMP, TIC, AMC, CFZ, CF, NIT |
| FCF 86  | 2       | Fresh water       | 1982 | AMP, TIC, AMC, CFZ, CF |
| FCF 88  | 2       | Fresh water       | 1983 | AMP, TIC, AMC, CFZ, CF |
| FCF 268 | 1A      | Chicken meat      | 1984 | AMP, TIC, AMC, CFZ, CF |
| FCF 90  | 2       | Fresh water       | 1984 | AMP, TIC, AMC, CFZ, CF |
| FCF 376 | 4       | Human feces       | 1985 | AMP, TIC, AMC, CFZ, CF |
| FCF 93  | 2       | Fresh water       | 1986 | AMP, TIC, CFZ, CF |
| FCF 94  | 2       | Fresh water       | 1987 | AMP, TIC, CFZ, CF |
| FCF 100 | 2       | Fresh water       | 1988 | AMP, TIC, AMC, CFZ, CF, PTZ |
| FCF 418 | 4       | Human diarrheic feces | 1988 | AMP, TIC, CFZ, CF |
| FCF 103 | 2       | Fresh water       | 1989 | AMP, TIC, CFZ, CF |
| FCF 105 | 2       | Fresh water       | 1990 | AMP, TIC, CFZ, CF |
| FCF 110 | 2       | Fresh water       | 1991 | AMP, TIC, AMC, CFZ, CF |
| FCF 113 | 2       | Fresh water       | 1992 | AMP, TIC, CFZ, CF |
| FCF 115 | 2       | Fresh water       | 1993 | AMC, CFZ, CF |
| FCF 600a| 4       | Human diarrheic feces | 1998 | AMP, TIC, CFZ, CF, CPM |
| FCF 601 | 4       | Animal feces      | 1999 | AMP, TIC, CFZ, CF, NIT |
| FCF 605a| 4       | Human diarrheic feces | 1999 | AMP, TIC, AMC, CFZ, CF, NAL, NIT |
| FCF 606a| 4       | Human diarrheic feces | 1999 | AMP, TIC, AMC, CFZ, CF, CPM, NIT |
| FCF 607a| 4       | Human diarrheic feces | 1999 | AMP, TIC, AMC, CFZ, CF, CPM, NIT |
| FCF 609a| 4       | Human diarrheic feces | 2000 | AMP, TIC, CFZ, CF, NAL |
| FCF 612a| 4       | Human diarrheic feces | 2000 | AMP, TIC, AMC, CFZ, CF |
| FCF 613a| 4       | Human diarrheic feces | 2000 | AMP, TIC, AMC, CFZ, CF |
| FCF 614a| 4       | Human diarrheic feces | 2003 | AMP, TIC, CFZ, CF, NAL |
| FCF 615a| 4       | Human blood       | 2004 | AMP, TIC, AMC, CFZ, CF |
| FCF 618a| 4       | Human diarrheic feces | 2008 | AMP, TIC, CFZ, CF, NAL, KAN |
| FCF 619a| 4       | Human diarrheic feces | 2008 | AMP, TIC, AMC, CFZ, CF, SXT |
| FCF 620a| 4       | Human blood       | 2008 | AMC, CFZ, CF |
| FCF 624a| 4       | Lymph node swab   | 2010 | AMP, TIC, AMC, CFZ, CF, CFX, NAL, NOR, CIP, LEV, TET, DOX, SXT, NIT, FOS |
| FCF 625a| 4       | Lymph node swab   | 2010 | AMP, TIC, CFZ, CF |
| FCF 626a| 4       | Human blood       | 2012 | AMP, TIC, AMC, CFZ, CF, NAL |

Bold and underline type means acquired resistance other than plasmid acquired resistance investigated here.

AMP, ampicillin; TIC, ticarcillin; AMC, amoxicillin-clavulanic acid; PTZ, piperacillin-tazobactam; CF, cephalotin; CFZ, cefazolin; CFX, cefuroxime; FOX, cefoxitin; CTX, cefotaxime; CZA, cefazidime; CPM, cefepime; ATM, aztreonam; ERT, etampenem; GEN, gentamicin; AMI, amikacin; KAN, kanamycin; SXT, trimethoprim-sulfamethoxazole; NAL, nalidixic acid; NOR, norfloxacin; CIP, ciprofloxacin; LEV, levofloxacin; C, chloramphenicol; NIT, nitrofurantoin; TET, tetracycline; DOX, doxycyclin; TGC, tigecycline; PB, polymyxin B; FOS, fosfomycin. All manufactured by Oxoid (Basingstoke, Hampshire, UK).

* Presence of IncFII plasmid replicon.
BlaA (a non-inducible broad-spectrum carbenicillinase) and BlaB (an AmpC-type inducible cephalosporinase). The differential expression and activities of these two enzymes determine the differential beta-lactam intrinsic resistance among biotypes of Y. enterocolitica. Moreover, a small percentage of strains may appear susceptible due to laboratory method variation, mutation or resistance expression. Therefore, in vitro susceptible results should be viewed with caution because in vivo non-susceptibility could lead to therapeutic failure. Approximately half of the strains (14/34) showed also acquired resistance (Table 1). Beta-lactam resistance to piperacillin-tazobactam (PTZ) and cefuroxime (CFX) could have been due to BlaB (AmpC) overproduction. However, BlaB overproduction does not explain cefepime (CPM) resistance, once AmpC beta-lactamases are not able to hydrolyze fourth-generation cephalosporins (like CPM). Thereby, once ESBL production was not detected and the recognized enzymatic intrinsic resistance is not able to confer this phenotype, other mechanism of resistance like porin loss and/or over expression of efflux system could have been responsible to CPM as well as PTZ and CFX resistance in the isolates studied here. Nitrofurantoin (NIT) resistance was detected in 17.6% (6/34) of strains; other studies reported 36% resistant as well as 100% susceptible strains. Trimethoprim-sulfamethoxazole (SXT) resistance was founded in 8.8% (3/34) of strains, and 4% to 10% of resistance were seen in other studies. Nalidixic acid (NAL) resistance was observed in 20.5% (7/34) of strains and other studies have described an increasing number of strains showing NAL resistance over recent years reaching 23%. In addition, fluoroquinolones like norfloxacin (NOR), ciprofloxacin (CIP), and levofloxacin (LEV) resistance was found in just one strain named FCF624, corroborating the findings of other studies where rates of fluoroquinolone resistance were significantly lower than those observed for NAL alone. Tetracycline (TET), doxycyclin (DOX), kanamycin (KAN), and fosfomycin (FOS) resistance were also detected in the FCF624 strain (Table 1), showing multiple resistance phenotypes, rarely reported in Y. enterocolitica.

No plasmid acquired genes coding for resistance to extended-spectrum cephalosporins, tetracyclines, aminoglycosides, and fluoroquinolones were found. Thereby, resistance to these antimicrobial classes could have chromosomal origin.

In this study, just IncFIIV plasmid replicon were detected mostly in strains of the pathogenic biotype 4 isolated from human diarrheic feces; however, it did not relate to any acquired resistance genes (Table 1). IncFII-like plasmids are mostly considered as virulence plasmids, such as PYY harboring type III secretion system (ops), that can be present alone or co-resident and compatible with other FII-positive resistance plasmids (typable by the FII, FIA, FIB, FIC loci) within the same bacterial cell (http://pubmlst.org/plasmid/). Plasmids carrying transferable antibiotic resistance genes have been detected from a variety of Enterobacteriaceae; however reports in Y. enterocolitica are rare, such as the conjugative plasmid (30–40 kb) transferring chloramphenicol, streptomycin, and sulfonamide resistance phenotypes from a sporadic Y. enterocolitica 4/O:3 strain.

Y. enterocolitica is a non-hospital pathogen and the absence of acquired resistance genes could be explained by little interaction with hospital pathogens and consequently a reduced possibility to genetic exchanges, such as horizontal gene transfer mediated by plasmids. Nevertheless, resistance genes and resistant bacteria have been alarmingly and increasingly found in food and food-producing animals, such as mcr-1 gene in ESBL-producing Escherichia coli strains isolated from pig. Y. enterocolitica is a zoonotic pathogen that causes gastrointestinal disease and porcine animal seems to be the main carrier of this bacterial species. Likewise, the food chain could boost Y. enterocolitica antimicrobial resistance.

In summary, our study showed that some Brazilian Y. enterocolitica strains of different biotypes remain susceptible to drugs used for treating gastroenteritis, as well as extra-intestinal and hospital infections. In addition, variation in the in vitro intrinsic resistant pattern was detected, except non-resistance to FOX in all strains. Moreover, neither acquired resistance genes nor diversity of plasmids replicons searched were found. Notwithstanding, due to epidemiological link between Y. enterocolitica and pork production chain, monitoring plasmid acquired resistance in Y. enterocolitica could also be considered for antimicrobial resistance control purposes and food safety measures.

Conflicts of interest

The authors declare no conflicts of interest.

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