Genotypic and phenotypic profiles of virulence factors and antimicrobial resistance of *Proteus mirabilis* isolated from chicken carcasses: potential zoonotic risk

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Abstract

Proteus mirabilis is an opportunistic pathogen often associated with a variety of human infections acquired both in the community and in hospitals. In this context, the present work aimed to evaluate the genotypic and phenotypic characteristics of the virulence factors and antimicrobial resistance determinants of 32 *P. mirabilis* strains isolated from chicken carcasses in a poultry slaughterhouse in the north of the state of Paraná, Brazil, in order to assess a potential zoonotic risk. The isolates presented a variety of virulence genes that contribute to the development of infection in humans. The *mrpA*, *pmfA*, *atfA* (fimbriae), *ireA* (siderophores receptor), *zapA*, *ptA* (Proteases), and *hpmA* (hemolysin) genes were found in 32 (100%) isolates and *ucaA* (fimbriae) in 16 (50%). All isolates showed aggregative adherence in HEp-2 cells and formed biofilms. Of all strains, 27 (84.38%) showed cytotoxic effects in Vero cells. Antimicrobial susceptibility was tested using 20 antimicrobials, in which 25 (78.13%) strains were considered multidrug-resistant. The presence of *bla*_{ESBL} and *bla*_{ampC} genes conferring resistance to β -lactams and *qnr* to quinolones were also detected in the isolates after presumption in the phenotypic test, in which 7 (21.88%) isolates contained the CTX-M-2 group, 11 (34.38%) contained CIT group and 19 (59.38%) contained *qnrD*. Therefore, chicken carcasses contaminated with *P. mirabilis* may pose a health risk to the consumer, as these isolates have a variety of virulence and antimicrobial resistance characteristics that can be found in *P. mirabilis* strains isolated from human infections.

Keywords Foodborne · Poultry · Public health · Multidrug-resistant · ESBL · AmpC

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Introduction

The Brazilian poultry industry has been outstanding in both the national and international markets, with Brazil being considered the second largest producer and leader in the world export of chicken meat. The southern region of Brazil is responsible for the majority of the production of broilers, with Paraná state being the largest national producer and accounting for more than 35% of the exports of all chicken meat produced in the country [1].

In view of the commercialization and relevance of the product throughout the world, it is essential to ensure the microbiological quality of poultry meat. In fact, all processing steps during and after the chicken slaughter can contaminate the meat with bacteria present in the avian microbiota and in

the slaughterhouse, especially in equipment that helps in the handling of carcasses, cuts, and by-products [2].

The fact that *Proteus* spp. is isolated from chicken feces along with other enteric bacteria, such as *Escherichia coli*, indicates its function as a component of the normal intestinal microbiota [3]. This facilitates the transfer of these bacteria to the slaughter line and cross-contamination, especially in the carcass evisceration process [4].

Some of these bacterial contaminants may remain after carcass processing and survive storage. It is therefore essential for the consumer to be aware that chicken meat can be a source of transmission of microorganisms. Therefore, in order to avoid possible cross-contamination, it is essential for taking care of the disinfection of the handling tools after the preparation of the meat [4].

The isolation of *P. mirabilis* from food products is documented in some studies, especially in those of animal origin, such as chicken meat, and some of these isolates are considered multidrug-resistant (MDR) [5, 6]. Bacterial resistance is a global public health threat and the excessive use of antimicrobials in poultry accelerates this phenomenon [7]. The problem is predicted to increase considerably in the coming years due to the intensification of the production of foods from animal origin and the non-rational use of antimicrobials [8].

P. mirabilis is a Gram-negative bacterium widely found in the environment and isolated from the intestinal tract of humans and other animals [3]. Although it is considered a commensal, the reports of occasional cases of infection and the description of nosocomial outbreaks point to its opportunistic pathogenic potential. Urinary tract infection (UTI) stands out as the most prevalent *P. mirabilis* infection, which has the capacity to cause other diseases in humans [9].

Several virulence factors may be associated with the pathogenicity of *P. mirabilis* in humans, such as fimbriae mannose-resistant *Proteus*-like (MR/P), *Proteus mirabilis* fimbriae (PMF), and uroepithelial cell adhesin (UCA), which allow bacterial-cell adhesion to host cells [9], PtA and ZapA proteases that degrade structural proteins and the immune system, respectively [10, 11], hemolysins HpmA and HlyA, considered toxins for host cells and involved in colonization of the urinary tract [12, 13], siderophores receptor IreA, considered of great importance in the acquisition of Fe⁺³ of the host [14]. In addition, adhesion capacity, formation of biofilms, and cytotoxicity are also important in the infectious process [9, 15].

Additionally, *P. mirabilis* has been associated with foodborne disease, despite their primary pathogenic role has not been confirmed [16–18], and may be a public health threat to society due to its strong association with a variety of human infectious diseases [19]. Therefore, more research is indicated to discuss and elucidate the zoonotic potential of *P. mirabilis*.

The present study aimed to characterize *P. mirabilis* strains isolated from chicken carcasses in the slaughter line, as well as

the genotypic and phenotypic characteristics of virulence factors and antimicrobial resistance. To our knowledge, this is the first study from Brazil that explores the general virulence and antimicrobial resistance characteristics of *P. mirabilis* strains isolated from carcasses of broiler chickens, in order to explore their zoonotic potential.

Materials and methods

Food samples

A total of 42 chicken carcasses were evaluated through sterile swabs scrubbed on the surface of the thighs and abdomen of non-eviscerated chickens in two time periods separated by a 15-day interval. All carcasses were randomly selected in the slaughter line during a sanitary inspection of a poultry slaughterhouse in the north of Paraná, Brazil. The isolates were collected using sterile Swabs and the collected material was transported in Cary-Blair medium (Difco™, USA).

Bacterial isolation and identification

The collected material was transferred to Brain Heart Infusion broth (Difco™, USA) and cultured at 37 °C for 24 h. After bacterial growth, the samples were plated on MacConkey Agar plates (Difco™, USA), and incubated at 37 °C for 24 h. Suspected colonies were confirmed to be *P. mirabilis* with the biochemical tests EPM, MILi (PROBAC™, BR), and Simmons citrate (Difco™, USA).

Detection of virulence genes

We investigated nine genes associated with the virulence of *P. mirabilis* in humans. The selected genes included *ireA* (siderophore receptor), *mrpA*, *ucaA*, *pmfA*, *atfA* (fimbriae), *ptA* and *zapA* (proteases), and *hmpA* and *hlyA* (hemolysins), as shown in Table 1. The bacterial DNA was obtained by the boiling extraction method. The polymerase chain reaction (PCR) was performed on a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems™) containing the final volume of 25 µL, composed of 2 mM MgCl₂ (Invitrogen™), 10× buffer (Invitrogen™), 0.2 mM dNTPs (Invitrogen™), 20 pmol Primer Forward, 20 pmol Primer Reverse (Invitrogen™), 1.25 U Taq DNA polymerase (Invitrogen®), bacterial DNA, and Milli-Q ultrapure water (Millipore™). PCR products were size separated by gel electrophoresis on a 2% agarose gel stained with SYBR SAFE (Invitrogen™) and emerged in TBE buffer (89 mM Tris base, 89 mM Boric Acid, 2 mM EDTA, pH 8.3). The molecular marker used was a 1 Kb Ladder (Invitrogen™). The amplicons were observed in a transilluminator with ultraviolet light (Vilbert Loumart™). The primers designed in this study were based on the complete

Table 1 Genes associated with virulence of *P. mirabilis* screened by PCR

Genes	Primers sequences (5'→3')	(bp)	Hybridization temperatures	References
<i>ireA</i>	(F) ACTACGATAACGAGCGCCAG (R) GCCCTAACTGGGGGAATACG	681	60 °C	This study
<i>mrpA</i>	(F) GAGCCATTCAATTAGGAATAATCCA (R) AGCTCTGTACTTCCTTGACAGA	648	58 °C	[20]
<i>ucaA</i>	(F) GCTTTTACATCCCCAGCGGT (R) GCTGCATTTGCTGGCTCATC	476	60 °C	This study
<i>pmfA</i>	(F) CAAATTAATCTAGAACCCTC (R) ATTATAGAGGATCCCTTGAAGGTA	617	54 °C	This study
<i>atfA</i>	(F) CATAATTCTAGACCTGCCCTAGCA (R) CTGCTTGATCCGTAATTTTAAACG	382	50 °C	[21]
<i>ptA</i>	(F) CCACTGCGATTATCCGCTCT (R) ATCGGCAGAAAGTGACAAGCA	686	60 °C	This study
<i>zapA</i>	(F) TATCGTCTCCTTCGCCTCCA (R) TGGCGCAAATACGACTACCA	332	59 °C	This study
<i>hpmA</i>	(F) GTTGAGGGGCGTTATCAAGAGTC (R) GATACTGTTTTGCCCTTTTGTGC	709	55 °C	[12]
<i>hlyA</i>	(F) AACAAAGGATAAGCACTGTTCTGGCT (R) ACCATATAAGCGGTCATTCCCGTCA	1177	63 °C	[12]

bp base pairs

genome sequence of the *P. mirabilis* strain HI4320 [22] and were used in the PCR following the conditions: 95 °C/5 min, 30 cycles of 95 °C/1 min, hybridization temperature/1 min, 72 °C/1 min, and final extension at 72 °C/7 min. The strain *P. mirabilis* HI4320 was used as positive control [22] and the reaction without DNA as a negative control.

Adherence in HEp-2 cells

The adherence capacity of the isolates of *P. mirabilis* to human larynx carcinoma (HEp-2) cells was analyzed as previously described [23]. Bacterial-cell interactions were assayed for 6 h. HEp-2 cells were grown in 24-well plates with 13 mm coverslips containing 1 mL DMEM (Dulbecco's Modified Eagle Medium). After the formation of a HEp-2 cell monolayer, the medium was discarded and the plates were washed three times with phosphate-buffered saline (PBS), containing 9.7 mM Na₂HPO₄, 1.25 mM KH₂PO₄, 137.93 mM NaCl, and 2.68 mM KCl. Next, 1 mL DMEM without antibiotics supplemented with 2% fetal bovine serum (FBS) was added to each well. Subsequently, a 40 µL aliquot of the bacterial culture overnight in tryptic soy broth (TSB) was added to each well and the plates were incubated for 3 h at 37 °C. After this step, the wells were washed five times with 1 mL sterile PBS to remove non-adhering bacteria. Once more, 1 mL of DMEM with 2% SFB was added to the wells and incubated for an additional 3 h at 37 °C. At the end of the 6 h period, the wells were washed five times with PBS, then the coverslips were fixed with 100% methanol and stained with May-Grünwald for 5 min and Giemsa for 20 min. The coverslips were observed under a light microscope at 1000x magnification. Cells

incubated without bacteria were used as negative control and *E. coli* E2348/69 [24], 042 [25] and C1845 [26] as positive controls for the patterns of localized, aggregative and diffuse adhesion, respectively.

Cytotoxicity in Vero cell cultures

All 32 bacterial isolates were tested in Vero cells (African green monkey kidney) to evaluate cytotoxic capacity as demonstrated [27], with modifications. The isolates were cultured in 3 mL TSB broth at 37 °C for 18 h under shaking at 180 rpm. After bacterial growth, the samples were centrifuged at 13000 g for 10 min. The supernatants were collected and filtered through a syringe filter with a PVDF membrane (Durapore™) with 0.22-µm pore size and 47-mm diameter. Filtered supernatants were added in four repetitions each to a 96-well polystyrene plate at a dilution of 1:10 and subsequently incubated for 72 h at 37 °C and 5% CO₂.

The cytotoxicity of the isolates was quantified measuring the metabolic activity of Vero cells with the MTT (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay [28]. Cells without bacterial supernatant were used as a negative control and *E. coli* O157:H7 (EDL933) was used as a positive control for cytotoxicity in Vero cells [29]. The isolate was considered highly cytotoxic when 50% or more of cell death were measured in comparison with the negative control.

Biofilm formation

The biofilm assay was performed on 96-well polystyrene plates as previously described [30] using crystal violet.

E. coli 042 (O44:H18) [25] was used as positive control for biofilm formation and TSB Broth (Difco™) only was used as negative control. The absorbance readings (A) were performed in a spectrophotometer at a wavelength of 570 nm. The threshold value of the absorbance (T) confirmed the biofilm formation and was defined as the sum of the arithmetic mean of the negative control (nc) and three times the standard deviation (δ), according to the formula ($T = xnc + 3\delta$).

Susceptibility to antimicrobials

The resistance of all 32 isolates was evaluated with the disc diffusion method in Mueller-Hinton agar, using the antimicrobials as recommended by the Clinical and Laboratory Standards Institute [31]. The antimicrobials (Oxoid™) used were Ampicillin (AMP) 10 µg, amoxicillin + clavulanate (AMC) 20/10 µg, cephalotin (CFL) 30 µg, cefoxitin (CFO) 30 µg, ceftazidime (CAZ) 30 µg, Ceftriaxone (CRO) 30 µg, cefotaxime (CTX) 30 µg, cefepime (CPM) 30 µg, nalidixic Acid (NAL) 30 µg, norfloxacin (NOR) 10 µg, ciprofloxacin (CIP) 5 µg, trimethoprim-sulfamethoxazole (SUT) 1.25/23.75 µg, aztreonam (ATM) 30 µg, chloramphenicol (CLO) 30 µg, gentamicin (GEN) 10 µg, amikacin (AMI) 30 µg, ertapenem (ETP) 10 µg, and imipenem (IPM) 10 µg. Additionally, ceftiofur (CTF) 30 µg, enrofloxacin (ENO) 5 µg were used [32]. The isolate was considered multidrug-resistant when it exhibited resistance to three or more classes of antimicrobials [33]. *E. coli* ATCC® 25922™ was used for quality control purposes.

Detection of resistance genes

The detection of resistance genes was performed by PCR assay. All isolates that exhibited resistance to cephalosporins in the phenotypic assay were screened for the presence of ESBL-encoding groups of the type CTX-M 1, 2, 8, 9, and 25 [34], TEM and SHV [35], as well as the six plasmid-mediated AmpC-specific families, MOX, FOX, EBC, ACC, DHA, and CIT [36]. The quinolone resistance genes *qnrA*, *qnrB*, *qnrS* [37], and *qnrD* [38] were also investigated in isolates that exhibited resistance to quinolones in the phenotypic assay.

Results

Genotypic and phenotypic profile of virulence

From a total of 42 chicken carcasses evaluated, 32 were positive for the presence of *P. mirabilis*. Only one strain was isolated from each positive carcass, totalizing 32 strains. The bacterial isolates studied presented a variety of virulence genes that enable and contribute to the development of

infection in humans. The *mrpA*, *pmfA*, *atfA* (fimbriae), *zapA*, *ptA* (proteases), *hpmA* (hemolysin), and *ireA* (siderophore receptor) genes were detected in all isolates, evidencing a high prevalence of these genes in *P. mirabilis* isolated from chicken carcasses. The gene *ucaA* (fimbriae) was the least prevalent among fimbrial genes, being found in 16 (50%) isolates. The *hlyA* (hemolysin) gene was not detected in any isolate.

The phenotypic characterization of the virulence factors investigated in our study showed that all 32 (100%) isolates expressed a pattern of aggregative adhesion to HEp-2 cells cultures (Fig. 1) and showed a capacity for biofilm formation in polystyrene plates, in which 17 isolates (53.12%) formed a strong biofilm and 15 (46.88%) a very strong biofilm (Fig. 2). In addition to these phenotypic characteristics of virulence, a highly cytotoxic effect on Vero cell culture was measured for a total number of 27 (84.38%) isolates. The genotypic and phenotypic profile of the virulence factors of these strains is presented in Table 2.

Genotypic and phenotypic profile of resistance to antimicrobials

Twenty antimicrobials belonging to several classes were tested. Resistance profiles of isolates ranged of strains susceptible to all antimicrobials tested to strains resistant to 15 different antimicrobials. Of all the antimicrobials tested, the isolates presented a higher frequency of resistance (> 50%) to ampicillin, amoxicillin-clavulanic acid, cefotaxime, nalidixic acid, enrofloxacin, trimethoprim-sulfamethoxazole, and gentamicin. The isolates showed greater susceptibility to the antimicrobials aztreonam, chloramphenicol, amikacin, ertapenem, and imipenem, highlighting the last two in which none of the isolates presented resistance. The frequency of antimicrobial resistance of the isolates is shown in Fig. 3. Of all the

Fig. 1 Aggregative adhesion pattern exhibited by *P. mirabilis* strain isolated from chicken carcass (magnification $\times 1000$)

Fig. 2 Intensity of *P. mirabilis* biofilm isolated from chicken carcasses

strains, 25 (78.13%) were MDR, being resistant to three or more classes of antimicrobials.

In relation to the *bla*_{ESBL} and AmpC genes responsible for conferring resistance to β -lactam antimicrobials, eight (25%) isolated had the CTX-M-2 group, while 11 (34.38%) had only the CIT group. The plasmidial gene *qnrD* that confers resistance to quinolones was found in 23 (71.88%) isolates, demonstrating the high prevalence of this gene in *P. mirabilis* isolated from chicken carcasses. The CTX-M-2 and CIT groups were found together in two isolates, CIT and *qnrD* in nine, *qnrD* and CTX-M-2 in four isolates. The CTX-M-2, CIT, and *qnrD* groups were found together in two strains. (Table 2).

Discussion

The bacterial isolates studied presented a variety of virulence genes that enable and contribute to the development of infection. Our results evidenced a variety of genes responsible for encoding fimbriae (Table 2) that contribute to the UTI in humans, such as fimbriae MR/P, PMF, and UCA [39, 40]. A study conducted by Barbour et al. [41] detected the *mrpA* gene in all strains of *P. mirabilis* isolated from chicken and human and identified a high nucleotide similarity between them, suggesting a possible zoonotic potential of these strains. The ambient temperature fimbriae (ATF) do not contribute to UTI, but its expression may allow the permanence of *P. mirabilis* in the environment [21].

Besides fimbrial genes, a high prevalence of genes *zapA*, *ptA* (proteases), *hpmA* (hemolysin), and *ireA* (siderophores receptor) was also found in our isolates (Table 2), evidencing that chicken carcasses may contain *P. mirabilis* with a diversity of virulence genes. In relation to the genes coding hemolysins, all isolates had *hpmA*, whereas *hlyA* was not detected in any of these. These findings are in agreement with other studies, which report a higher prevalence of *hpmA* than *hlyA* in *P. mirabilis* [12, 42]. Few studies involving research on virulence factors in *P. mirabilis* strains of food and infections are reported. This is the first study that shows evidence for the presence of the virulence genes *pmfA*, *ucaA*, *atfA*, *hpmA*,

zapA, *ptA*, and *ireA* in *P. mirabilis* isolated from chicken carcasses.

We have observed that most of the virulence factors investigated are related to an extraintestinal pathogenic profile, such as the extraintestinal pathogenic *Escherichia coli* (ExPEC). This clue may be supported by the fact that our isolates possessed a diversity of virulence factors that are commonly found in ExPEC, such as fimbriae, hemolysins, proteases, and the iron uptake system [43–45]. Other studies also report a high prevalence of virulence factors in *E. coli* isolated from chicken carcasses and highlight the pathogenic potential of these strains in developing extraintestinal infections in humans [46, 47]. This hypothesis is strongly supported by other works [48–50], as well as a study conducted by Vincent et al. [51], which reports an intimate similarity between strains of *E. coli* isolated from chicken meat and UTIs.

We believe that chicken meat can be contaminated with *P. mirabilis* in the slaughter line during the carcass evisceration process or by cross contamination. It is also believed that these strains can be transmitted to consumers, colonize the human intestinal tract, and possibly develop extraintestinal infections, such as UTIs, similarly to what it is believed to occur with *E. coli* from food sources [48–51].

The colonization of the epithelium is important for *P. mirabilis* uropathogenicity. This ability can be demonstrated by experiments using different cell lines [52]. The aggregative adherence pattern has been previously reported in uropathogenic *P. mirabilis* [20]. It is known that the expression of the MR/P fimbriae contributes to this adhesion pattern [20]. This evidence supports our results since all the isolates had the *mrpA* gene and expressed the aggregative adherence pattern. This is the first report of *P. mirabilis* isolated from chicken carcasses expressing the aggregative adherence pattern in HEp-2 cells. This pattern of adhesion has also been demonstrated in *E. coli* isolated from extraintestinal infections [43, 45].

The cytotoxic effect is also considered important in the infectious process, especially in uropathogenicity, and was exhibited by most of our isolates (84.38%). There is evidence that the hemolysin HpmA is responsible for most of the

Table 2 Genotypic and phenotypic characteristics of virulence and antimicrobial resistance of *P. mirabilis* isolated from chicken carcasses

Strains	Virulence genes	A.A	C.V.C	Biofilm	Phenotypic profile of resistance	Resistance genes
LB UEL – A01	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, CTF, AMC, CFL, CFO, CAZ, CTX, NAL, ENO, SUT	CIT, <i>qnrD</i>
LB UEL – A02	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	–	+	AMP, CTF, AMC, CFL, CFO, CAZ, CRO, CTX, CPM, NAL, ENO, SUT	CIT, <i>qnrD</i>
LB UEL – A03	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	–	+	AMP, AMC, CFL, CFO, CAZ, CTX, NAL, SUT, GEN	CIT
LB UEL – A04	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, CTF, AMC, CFL, CFO, CAZ, CRO, CTX, CPM, NAL, NOR, ENO, CIP, SUT, GEN	CTX-M-2 group, <i>qnrD</i>
LB UEL – A05	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, CTF, AMC, CFL, CAZ, CRO, CTX, NAL, ENO, CIP, SUT, ATM, GEN	<i>qnrD</i>
LB UEL – A06	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, NAL, NOR, ENO, CIP, SUT, GEN	<i>qnrD</i>
LB UEL – A07	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, AMC, CFL, NAL, NOR, ENO, CIP, SUT, ATM, GEN	<i>qnrD</i>
LB UEL – A08	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, CTF, AMC, CFL, CFO, CAZ, CRO, CTX	CIT
LB UEL – A09	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, CTF, AMC, CFL, CFO, CRO, CTX, CPM, NAL, ENO, SUT, ATM, CLO	CTX-M-2 group, CIT, <i>qnrD</i>
LB UEL – A10	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	–	+	AMP, CTF, AMC, CFL, CFO, CRO, CTX, CPM, NAL, NOR, ENO, CIP, SUT	CTX-M-2 group, <i>qnrD</i>
LB UEL – A11	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, CTF, CFL, CFO, CRO, CTX, CPM, NAL, NOR, ENO, CIP, SUT	CTX-M-2 group, <i>qnrD</i>
LB UEL – A12	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	CFL, NAL, SUT	Not found
LB UEL – A13	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	–	+	AMP, AMC, CTF, CFL, CFO, CAZ, CRO, CTX, NAL, ENO, SUT, GEN	CIT, <i>qnrD</i>
LB UEL – A14	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	CFL	Not found
LB UEL – A15	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, AMC, CTF, CFL, CFO, CAZ, CRO, CTX, NAL, ENO, SUT, CLO, GEN	CIT, <i>qnrD</i>
LB UEL – A16	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, AMC, CFL, CFO, CAZ, CRO, CTX, NAL, NOR, ENO, CIP, SUT, GEN, AMI	CIT, <i>qnrD</i>
LB UEL – A17	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMC, CFL, CFO, CAZ, CTX, CPM, NAL, ATM	CTX-M-2 group
LB UEL – A18	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, CTF, AMC, CFL, CRO, CTX, CPM, NAL, ENO, CIP, SUT, GEN	CTX-M-2 group, <i>qnrD</i>
LB UEL – A19	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	Not found	Not found
LB UEL – A20	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, NAL, NOR, ENO, CIP, SUT, GEN	<i>qnrD</i>
LB UEL – A21	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, CFL, NAL, NOR, ENO, CIP, SUT, CLO, GEN	<i>qnrD</i>
LB UEL – A22	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, CTF, AMC, CFL, CFO, CAZ, CRO, CTX, CPM, NAL, NOR, ENO, CIP, SUT, ATM	CTX-M-2 group, CIT, <i>qnrD</i>
LB UEL – A23	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	–	+	NAL, ENO	<i>qnrD</i>
LB UEL – A24	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, CFL, NAL, NOR, ENO, CIP, SUT, GEN	<i>qnrD</i>
LB UEL – A25	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, CTF, AMC, CFL, CFO, CAZ, CRO, CTX, NAL, ENO, SUT, CLO, GEN	CIT, <i>qnrD</i>
LB UEL – A26	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, CFL, CFO, NAL, NOR, ENO, CIP, SUT, GEN	<i>qnrD</i>
LB UEL – A27	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, AMC, CFL, CFO, CAZ, CRO, CTX, NAL, ENO, SUT, GEN	CIT, <i>qnrD</i>
LB UEL – A28	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	CTF, AMC, CFL, CFO, NAL, SUT, GEN, AMI	Not found
LB UEL – A29	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	NAL, ENO, CIP	<i>qnrD</i>
LB UEL – A30	<i>mrpA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, CTF, AMC, CFL, CFO, CRO, CTX, CPM, NAL, GEN	CTX-M-2 group
LB UEL – A31	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	CFL, CFO, NAL, ENO	<i>qnrD</i>
LB UEL – A32	<i>mrpA, ucaA, pmfA, atfA, ireA, ptA, zapA, hpmA</i>	+	+	+	AMP, AMC, CFL, CFO, NAL, SUT, GEN	Not found

A.A, aggregative adhesion; C.V.C, cytotoxicity in vero cells; AMP, ampicillin; CTF, ceftiofur; AMC, amoxicillin-clavulanic acid; CFL, cephalotin; CFO, cefoxitin; CAZ, ceftazidime; CRO, ceftriaxone; CTX, cefotaxime; CPM, cefepime; NAL, nalidixic acid; NOR, norfloxacin; ENO, enrofloxacin; CIP, ciprofloxacin; SUT, trimethoprim-sulfamethoxazole; ATM, aztreonam; CLO, chloramphenicol; GEN, gentamicin; AMI, amikacin. + positive; – negative

Fig. 3 Frequency of antimicrobial resistance of *P. mirabilis* isolated from chicken carcasses

cytotoxicity in renal cell lines since the isogenic mutant strains for this virulence factor were significantly less cytotoxic than the wild type strains [53]. Despite all of our isolates have possessed the *hpmA* gene, some of them have not expressed cytotoxic effect in Vero cells which may be due to the non-expression of this gene in vitro. However, their expression may occur in vivo and contribute to the infectious process.

Foodborne biofilm-forming bacteria pose serious risks to human health and have become increasingly frequent in the food industry, opportunizing the dissemination of biofilm-forming strains, mainly through cross-contamination [54, 55]. Our results are alarming, considering that all the strains isolated from chicken carcasses in a slaughterhouse in the north of Paraná formed a strong or very strong biofilm. In the host, biofilm formation protects bacteria from the immune system and antimicrobials, as well as contribute to the persistence of infection [56]. Biofilms in *P. mirabilis* are well studied, they are mainly associated with urinary catheters, which can block urinary flow and cause ascending infection, pyelonephritis, and possible sepsis [57]. It is important to note that some of our isolates, such as the strain LB UEL - A07, expressed a biofilm at the intensity similar to the positive control EAEC 042, a diarrheagenic *E. coli* very well known to form a very strong biofilm [25] (Fig. 2).

The isolation of bacteria that pose a threat to the health of the consumer becomes more aggravating when these, besides having factors of virulence, also present resistance to the antimicrobials, mainly when these are used for the treatment of infections in humans [58]. Our results in Fig. 3 show, that even though many antimicrobials are prohibited on poultry farms, a high frequency of *P. mirabilis* MDR was found, in which 25 (78.13%) isolates were resistant to three or more classes of antimicrobials [33].

The presence of MDR *P. mirabilis* and producers of ESBLs isolated from chicken meat has been reported in a few studies [6, 59]. The high prevalence of CTX-M-2 and CIT groups has been reported in *E. coli* isolated from chicken carcasses in Paraná, Brazil [46]. In addition, a study in the UK reported the detection of the CTX-M-2 group in *E. coli* isolated from chicken meat imported from Brazil [60]. Our study evidenced a high prevalence of the *qnrD* gene in *P. mirabilis* isolated from chicken carcasses, which has been commonly found in genera of the *Proteeae* tribe, such as *Proteus*, *Providencia*, and *Morganella* [61].

It is important to highlight the considerable rate of resistance to quinolones and cephalosporins of the third and fourth generation found in this study (Fig. 3), since these antimicrobials are frequently used in the treatment of human infections [62, 63]. Similar results were also found in a study in China [6]. Currently, a variety of human infectious diseases are currently treated with quinolones, including abdominal infections, chronic bronchitis, community-acquired pneumonia, nosocomial infections, sexually transmitted infections, skin infections, and urinary tract infections [62]. This is the first report of groups CTX-M-2, CIT, and *qnrD* in *P. mirabilis* isolated from chicken carcasses in Brazil.

Therefore, chicken meat can be a source of transmission and dissemination of *P. mirabilis* strains resistant to antimicrobials and carriers plasmid-mediated resistance genes to the community. Thus, it is essential that chicken producers are cautious about the prophylactic use of antimicrobial agents in poultry production, mainly as a food additive [8]. The study of resistant bacteria and plasmid-mediated resistance is important mainly because infections caused by resistant strains are more related to high morbidity and mortality rates than susceptible strains [64].

In the present study, it was demonstrated that chicken carcasses of a slaughterhouse in the north of Paraná are a potential risk for consumer health. As shown in Table 2, they carried *P. mirabilis* strains with a great diversity of virulence factors and antimicrobial resistance determinants, both phenotypically and genotypically. These findings become more aggravating when we consider the possibility of these strains colonizing the intestinal tract of the consumers and horizontally transfer the plasmid genes associated with antimicrobial resistance to the commensal strains. Our results are alarming, since the state of Paraná is responsible for most of the chicken meat exports from Brazil [1].

Although few reports, *P. mirabilis* has been associated with an outbreak of gastroenteritis and vomiting in a study that found the identical *P. mirabilis* strain in a diarrheic stool sample and vomit of a patient affected with food poisoning [17]. These associations were strongly sustained during an outbreak caused by the consumption of pork cooked in China, in which an identical strain was isolated from the hospitalized individuals, kitchen handler, waiter, and contaminated food waste, proving the clonal relation of the strains by Pulsed-field gel electrophoresis (PFGE) [16]. However, more studies need to be done to elucidate whether *P. mirabilis* can be considered the primary pathogen of food poisoning and which virulence factors contribute to it.

In addition, *P. mirabilis* has also been associated with pneumonia [65] and sepsis and infection of the central nervous system [66]. Therefore, the high frequency of *P. mirabilis* recovered from chicken carcasses in our study is alarming, considering that chicken meat may be responsible for disseminating *P. mirabilis* with virulence potential and MDR via the food chain.

Conclusions

In conclusion, *P. mirabilis* isolated from chicken carcasses have a variety of virulence factors and are resistant to multiple antimicrobials used in the treatment of human infections. Therefore, it is important that the consumer is aware of the risk when handling and preparing chicken meat, as well as performing a correct disinfection of the kitchen tools after the preparation of this meat, in order to avoid contact, cross-contamination, and possible food poisoning with this pathogen. In addition, we believe that the major risk is the colonization of the intestinal tract by these strains and further the development of extraintestinal diseases, such as UTIs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. ABPA (2017) Brazilian association of animal protein. Annual Report. http://abpa-br.com.br/storage/files/final_abpa_relatorio_anual_2017_ingles_web.pdf. Accessed 10 March 2019
2. Rouger A, Tresse O, Zagorec M (2017) Bacterial contaminants of poultry meat: sources, species, and dynamics. *Microorg* 5(3):1–16
3. Drzewiecka D (2016) Significance and roles of *Proteus* spp. Bacteria in natural environments. *Microb Ecol* 72(4):741–758
4. Firildak G, Asan A, Goren E (2015) Chicken carcasses bacterial concentration at poultry slaughtering facilities. *Asian J Biol Sci* 8(1):16–29
5. Kim SH, Wei CI, An H (2005) Molecular characterization of multidrug-resistant *Proteus mirabilis* isolates from retail meat products. *J Food Prot* 68(7):1408–1413
6. Wong MH, Wan HY, Chen S (2013) Characterization of multidrug-resistant *Proteus mirabilis* isolated from chicken carcasses. *Foodborne Pathog Dis* 10(2):177–181
7. Boeckel TPV, Brower C, Gilbert M et al (2015) Global trends in antimicrobial use in food animals. *Proc Natl Acad Sci* 112(18):5649–5654
8. Nhung NT, Chansiripornchai N, Carrique-mas JJ (2017) Antimicrobial resistance in bacterial poultry pathogens: a review. *Front Vet Sci* 4:1–17
9. Armbruster CE, Mobley HLT, Pearson MM (2018) Pathogenesis of *Proteus mirabilis* infection. *EcoSal Plus* 8(1):1–123
10. Alamuri P, Eaton KA, Himpel SD, Smith SN, Mobley HLT (2009) Vaccination with *proteus* toxic agglutinin, a hemolysin-independent cytotoxin in vivo, protects against *Proteus mirabilis* urinary tract infection. *Infect Immun* 77(2):632–641
11. Belas R, Manos J, Suvanasuthi R (2004) *Proteus mirabilis* ZapA metalloprotease degrades a broad spectrum of substrates, including antimicrobial peptides. *Infect Immun* 72(9):5159–5167
12. Cestari SE, Ludovico MS, Martins FH, da Rocha SPD, Elias WP, Pelayo JS (2013) Molecular detection of HpmA and HlyA hemolysin of uropathogenic *Proteus mirabilis*. *Curr Microbiol* 67(6):703–707
13. Mobley HLT, Chippendale GR (1990) Hemagglutinin, urease, and hemolysin production by *Proteus mirabilis* from clinical sources. *J Infect Dis* 161(3):525–530
14. Himpel SD, Pearson MM, Arewang CJ, Nusca TD, Sherman DH, Mobley HL (2010) Proteobactin and a yersiniabactin-related siderophore mediate iron acquisition in *Proteus mirabilis*. *Mol Microbiol* 78(1):138–157
15. Schaffer JN, Pearson MM (2015) *Proteus mirabilis* and urinary tract infections. *Microbiol Spectr* 3(5):1–39
16. Wang Y, Zhang S, Yu J, Zhang H, Yuan Z, Sun Y, Zhang L, Zhu Y, Song H (2010) An outbreak of *Proteus mirabilis* food poisoning associated with eating stewed pork balls in brown sauce, Beijing. *Food Control* 21(3):302–305
17. Shi X, Lin Y, Qiu Y et al (2016) Comparative screening of digestion tract toxic genes in *Proteus mirabilis*. *PLoS One* 11(3):1–12

18. Wu Y, Liu X, Chen Q, Liu H, Dai Y, Zhou YJ, Wen J, Tang ZZ, Chen Y (2018) Surveillance for foodborne disease outbreaks in China, 2003 to 2008. *Food Control* 84:382–388
19. Hamilton AL, Kamm MA, Ng SC et al (2018) *Proteus* spp. as putative gastrointestinal pathogens. *Clin Microbiol Rev* 31(3):1–19
20. Rocha SPD, Elias WP, Cianciarullo AM et al (2007) Aggregative adherence of uropathogenic *Proteus mirabilis* to cultured epithelial cells. *FEMS Immunol Med Microbiol* 51(2):319–326
21. Zunino P, Geymonat L, Allen AG, Legnani-Fajardo C, Maskell DJ (2000) Virulence of a *Proteus mirabilis* ATF isogenic mutant is not impaired in a mouse model of ascending urinary tract infection. *FEMS Immunol Med Microbiol* 29(2):137–143
22. Pearson MM, Sebahia M, Churcher C, Quail MA, Seshasayee AS, Luscombe NM, Abdellah Z, Arrowsmith C, Atkin B, Chillingworth T, Hauser H, Jagels K, Moule S, Mungall K, Norbertczak H, Rabinowitsch E, Walker D, Whithead S, Thomson NR, Rather PN, Parkhill J, Mobley HLT (2008) Complete genome sequence of uropathogenic *Proteus mirabilis*, a master of both adherence and motility. *J Bacteriol* 190(11):4027–4037
23. Cravioto A, Gross RJ, Scotland SM, Rowe B (1979) An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. *Curr Microbiol* 3(2):95–99
24. Levine MM, Bergquist EJ, Nalin DR, Waterman DH, Hornick RB, Young CR, Sotman S (1978) *Escherichia coli* strains that cause diarrhoea but do not produce heat-labile or heat-stable enterotoxins and are non-invasive. *Lancet* 1:1119–1122
25. Nataro JP, Yikang D, Cookson S, Cravioto A, Savarino SJ, Guers LD, Levine MM, Tackett CO (1995) Heterogeneity of enteroaggregative *Escherichia coli* virulence demonstrated in Volunteers. *J Infect Dis* 171(2):465–468
26. Bilge SS, Clausen CR, Lau WE (1989) Molecular characterization of a fimbrial adhesin, F1845, mediating diffuse adherence of diarrhea-associated *Escherichia coli* to HEp-2 cells. *J Bacteriol* 171(8):4281–4289
27. Konowalchuk J, Speirs JL, Stavric S (1977) Vero response to a cytotoxin of *Escherichia coli*. *Infect Immun* 18(3):775–779
28. Murakami J, Kishi K, Hirai K, Hiramatsu K, Yamasaki T, Nasu M (2000) Macrolides and clindamycin suppress the release of Shiga-like toxins from *Escherichia coli* O157:H7 in vitro. *Int J Antimicrob Agents* 15(2):103–109
29. Yu J, Kaper JB (1992) Cloning and characterization of the *eae* gene of enterohemorrhagic *Escherichia coli* O157:H7. *Mol Microbiol* 6(3):411–417
30. Kwiecinska-Piróg J, Bogiel T, Skowron K, Wieckowska E, Gospodarek E (2014) *Proteus mirabilis* biofilm - qualitative and quantitative colorimetric methods-based evaluation. *Braz J Microbiol* 45(4):1423–1431
31. Clinical and Laboratory Standards Institute (CLSI) (2018) Performance standards for antimicrobial susceptibility testing. 28th edn. CLSI supplement M100. Wayne
32. Clinical and Laboratory Standards Institute (CLSI) (2008) Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. 3rd edn. CLSI Document M31-A3. Wayne
33. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 18(3):268–281
34. Woodford N, Fagan EJ, Ellington MJ (2006) Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β -lactamases. *J Antimicrob Chemother* 57(1):154–155
35. Arlet G, Philippon A (1991) Construction by polymerase chain reaction and use of intragenic DNA probes for three main types of transferable beta-lactamase (TEM, SHV, CARB). *FEMS Microbiol Lett* 66(1):19–25
36. Pérez-pérez FJ, Hanson ND (2002) Detection of plasmid-mediated AmpC β -lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol* 40(6):2153–2162
37. Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P (2007) Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. *J Antimicrob Chemother* 60(2):394–397
38. Cavaco LM, Hasman H, Xia S, Aarestrup FM (2009) *qnrD*, a novel gene conferring transferable quinolone resistance in *Salmonella enterica* Serovar Kentucky and Bovismorbificans strains of human origin. *Antimicrob Agents Chemother* 53(2):603–608
39. Zunino P, Sosa V, Schlapp G, Allen AG, Preston A, Maskell DJ (2007) Mannose-resistant *Proteus*-like and *P. mirabilis* fimbriae have specific and additive roles in *P. mirabilis* urinary tract infections. *FEMS Immunol Med Microbiol* 51(1):125–133
40. Pellegrino R, Scavoane P, Umpiérrez A et al (2013) *Proteus mirabilis* uroepithelial cell adhesin (UCA) fimbria plays a role in the colonization of the urinary tract. *Pathog Dis* 67(2):104–107
41. Barbour EK, Hajj ZG, Hamadeh S, Shaib HA, Farran MT, Araj G, Faroon O, Barbour KE, Jirjis F, Azhar E, Kumosani T, Harakeh S (2012) Comparison of phenotypic and virulence genes characteristics in human and chicken isolates of *Proteus mirabilis*. *Pathog Glob Health* 106(6):352–357
42. Swihart KG, Welch RA (1990) The Hpma hemolysin is more common than Hly_a among *Proteus* isolates. *Infect Immun* 58(6):1853–1860
43. Toval F, Köhler CD, Vogel U et al (2014) Characterization of *Escherichia coli* isolates from hospital inpatients or outpatients with urinary tract infection. *J Clin Microbiol* 52(2):407–418
44. Cyويا PS, Rodrigues GR, Nishio EK, Medeiros LP, Koga VL, Pereira APD, Vespero EC, Houle S, Dozois CM, Nakazato G, Kobayashi RKT (2015) Presence of virulence genes and pathogenicity islands in extraintestinal pathogenic *Escherichia coli* isolates from Brazil. *J Infect Dev Ctries* 9(10):1068–1075
45. Cergole-Novella MC, Pignatari ACC, Guth BEC (2015) Adhesion, biofilm and genotypic characteristics of antimicrobial resistant *Escherichia coli* isolates. *Braz J Microbiol* 46(1):167–171
46. Koga VL, Scandorieiro S, Vespero EC, Oba A, de Brito BG, de Brito KCT, Nakazato G, Kobayashi RKT (2015) Comparison of antibiotic resistance and virulence factors among *Escherichia coli* isolated from conventional and free-range poultry. *Biomed Res Int* 2015:1–8
47. Cyويا PS, Koga VL, Nishio EK et al (2019) Distribution of ExPEC virulence factors, *bla*_{CTX-M}, *fosA3*, and *mcr-1* in *Escherichia coli* isolated from commercialized chicken carcasses. *Front Microbiol* 9:1–9
48. Manges AR, Smith SP, Lau BJ, Nuval CJ, Eisenberg JNS, Dietrich PS, Riley LW (2007) Retail meat consumption and the acquisition of antimicrobial resistant *Escherichia coli* causing urinary tract infections: a case-control study. *Foodborne Pathog Dis* 4(4):419–431
49. Manges AR, Johnson JR (2012) Food-borne origins of *Escherichia coli* causing extraintestinal infections. *Clin Infect Dis* 55(5):712–719
50. Manges AR (2016) *Escherichia coli* and urinary tract infections: the role of poultry-meat. *Clin Microbiol Infect* 22(2):122–129
51. Vincent C, Boerlin P, Daignault D, Dozois CM, Dutil L, Galanakis C, Reid-Smith RJ, Tellier PP, Tellis PA, Ziebell K, Manges AR (2010) Food reservoir for *Escherichia coli* causing urinary tract infections. *Emerg Infect Dis* 16(1):88–95
52. Różalski A, Sidorczyk Z, Kotelko K (1997) Potential virulence factors of *Proteus* bacilli. *Microbiol Mol Biol Rev* 61(1):65–89

53. Mobley HLT, Chippendale GR, Swihart KG, Welch RA (1991) Cytotoxicity of the HpmA hemolysin and urease of *Proteus mirabilis* and *Proteus vulgaris* against cultured human renal proximal tubular epithelial cells. *Infect Immun* 59(6):2036–2042
54. Zhao X, Zhao F, Wang J, Zhong N (2017) Biofilm formation and control strategies of foodborne pathogens: food safety perspectives. *RSC Adv* 7(58):36670–36683
55. Galié S, García-gutiérrez C, Miguélez EM et al (2018) Biofilms in the food industry: health aspects and control methods. *Front Microbiol* 9:1–18
56. Kostakioti M, Hadjifrangiskou M, Hultgren SJ (2013) Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harb Perspect Med* 3(4):1–23
57. Armbruster CE, Mobley HLT (2012) Merging mythology and morphology: the multifaceted lifestyle of *Proteus mirabilis*. *Nat Rev Microbiol* 10(11):743–754
58. Marshall BM, Levy SB (2011) Food animals and antimicrobials: impacts on human health. *Clin Microbiol Rev* 24(4):718–733
59. Shrestha A, Bajracharya AM, Subedi H et al (2017) Multi-drug resistance and extended spectrum beta lactamase producing gram negative bacteria from chicken meat in Bharatpur Metropolitan, Nepal. *BMC Res Notes* 10(1):1–5
60. Warren RE, Ensor VM, O'Neill P et al (2008) Imported chicken meat as a potential source of quinolone-resistant *Escherichia coli* producing extended-spectrum β -lactamases in the UK. *J Antimicrob Chemother* 61(3):504–508
61. Kraychete GB, Campana EH, Picão RC, Bonelli RR (2019) *qnrD*-harboring plasmids in *Providencia* spp. recovered from food and environmental Brazilian sources. *Sci Total Environ* 646:1290–1292
62. Aldred KJ, Kerns RJ, Osheroff N (2014) Mechanism of quinolone action and resistance. *Biochem* 53(10):1565–1574
63. Rahman SU, Ali T, Ali I et al (2018) The growing genetic and functional diversity of extended spectrum beta-lactamases. *Biomed Res Int* 2018:1–14
64. Gopinathan A, Mukhopadhyay C, Vandana KE (2017) Characterization of antimicrobial resistance mechanisms of multi-drug resistant gram negative bacterial wound infections and their clinical epidemiology from a tertiary care hospital in Karnataka, India. *Int J Res Med Sci* 5(3):824–828
65. Okimoto N, Hayashi T, Ishiga M, Nanba F, Kishimoto M, Yagi S, Kurihara T, Asaoka N, Tamada S (2010) Clinical features of *Proteus mirabilis* pneumonia. *J Infect Chemother* 16(5):364–366
66. Kassim Z, Aziz AA, Haque QM, Cheung HAS (2003) Isolation of *Proteus mirabilis* from severe neonatal sepsis and central nervous system infection with extensive pneumocephalus. *Eur J Pediatr* 162(9):644–645

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