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RESEARCH

Clonal relationship, virulence genes, and antimicrobial resistance of *Morganella morganii* isolated from community-acquired infections and hospitalized patients: a neglected opportunistic pathogen

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Abstract

Morganella morganii is a bacterium belonging to the normal intestinal microbiota and the environment; however, in immunocompromised individuals, this bacterium can become an opportunistic pathogen, causing a series of diseases, both in hospitals and in the community, being urinary tract infections more prevalent. Therefore, the objective of this study was to evaluate the prevalence, virulence profile, and resistance to antimicrobials and the clonal relationship of isolates of urinary tract infections (UTI) caused by *M. morganii*, both in the hospital environment and in the community of the municipality of Londrina-PR, in southern Brazil, in order to better understand the mechanisms for the establishment of the disease caused by this bacterium. Our study showed that *M. morganii* presents a variety of virulence factors in the studied isolates. Hospital strains showed a higher prevalence for the virulence genes *zapA*, *iutA*, and *fimH*, while community strains showed a higher prevalence for the *ireA* and *iutA* genes. Hospital isolates showed greater resistance compared to community isolates, as well as a higher prevalence of multidrug-resistant (MDR) and extended-spectrum beta lactamase (ESBL)-producing isolates. Several *M. morganii* isolates from both sources showed high genetic similarity. The most prevalent plasmid incompatibility groups detected were FIB and I1, regardless of the isolation source. Thus, *M. morganii* isolates can accumulate virulence factors and antimicrobial resistance, making them a neglected opportunistic pathogen.

Keywords *Morganella morganii* · Opportunistic pathogen · UTI · Epidemiology · Antimicrobial resistance

Introduction

Morganella morganii, previously named *Proteus morganii* (Chen et al. 2012a, 2012b), is a Gram-negative bacterium belonging to the Morganellaceae family (Adeolu et al. 2016), found in the human intestine and in the environment (Li et al. 2018). Belonging to the tribe Proteeae, together with bacteria of the genus *Proteus* and *Providencia*, it can be classified as an opportunistic bacterium that causes nosocomial infections (Chen et al. 2012a, 2012b), mainly involving cases of urinary infections and surgical wounds (Livani and Kabir 2019), sepsis, and other extraintestinal infections (Patil et al. 2012). It has two subspecies, *Morganella morganii morganii* and *Morganella morganii sibonii* (Falagas et al. 2006).

The first report of this species causing infections in humans was in 1906 (Morgan 1906). Later, in 1939, this pathogen was reported to cause urinary tract infection (UTI)

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(Liu et al. 2016), this type of infection being the most prevalent when it comes to *M. morganii* (Leylabadlo et al. 2016), often associated with long-term urinary catheter usage (Stickler 2008, 2014; Minnullina et al. 2019). Despite not being a prevalent pathogen, this bacterium was reclassified as a rare opportunistic pathogen, due to the increase in the number of cases of infection caused by *M. morganii*, its ability to accumulate several virulence and resistance factors (Bandy 2020), and its capacity to adapt to different environments (Ghosh and LaPara 2007).

Several virulence-related genes are described in sequences of different strains of *M. morganii*, deposited as sequenced genomes, in the NCBI database (Chen et al. 2012a, 2012b; Liu et al. 2016; Minnullina et al. 2019). These available described sequences reveal genes associated with fimbriae, adhesins, proteases, lipopolysaccharide (LPS), hemolysins, urease, and siderophores (Liu et al. 2016). The presence of these genes may be associated with the ability of biofilm formation, cytotoxicity, and adhesion and may contribute to the infectious process caused by this bacterium.

Although infections by *M. morganii* are not as prevalent, this bacterium exhibits a broad intrinsic resistance (CLSI 2021). The emergence of antimicrobial resistance represents a major public health problem, as well as a significant obstacle to the treatment of pathologies associated with microorganisms (Andersson et al. 2020). Several mechanisms can result in the emergence of antimicrobial resistance, whether intrinsic, acquired (through plasmid transfer), or even adaptive (Wilson et al. 2020).

In fact, *M. morganii* should be considered a clinically significant pathogen, with a broad infectious and potentially emerging spectrum, due to its resistance and virulence mechanisms, which make treatment and the immune system response more complex and difficult (Bandy 2020).

Materials and methods

Bacterial isolation

A total of 138 *M. morganii* isolates were studied. These microorganisms were isolated from urine of patients treated at the University Hospital of Londrina and from patients in the community (Centrolab Laboratory) in Londrina, PR, Brazil. Of the 138 isolates, 68 isolates belonged to hospitalized patients and 70 from the community, over a period of 5 years (2016 to 2020).

The antibiogram and identification of bacterial isolates were performed using the Vitek® 2 COMPACT system (BioMérieux, MarcyL'Etoile, France). The samples were tested for the following antimicrobials: ertapenem, imipenem, meropenem, amikacin, gentamicin, sulfamethoxazole plus trimethoprim, ciprofloxacin, norfloxacin, nalidixic acid, aztreonam, cefepime, ceftriaxone, ceftazidime, and piperacillin plus tazobactam. Only samples with $\geq 10^5$ colony forming units (CFU) were selected for the study. This research was approved by the Research Ethics Committee (CEP-UEL), opinion 1.590.120.

Phenotypic screening of ESBL by combined disc

To identify possible ESBL-producing isolates, we performed the combined disk assay. For this, in addition to the inoculum carried out in saline solution, respecting the 0.5 McFarland scale, disks of ceftazidime and cefotaxime associated or not with clavulanic acid were used on a Mueller Hinton agar plate. Halos were measured after 24 h. The discs that obtained halos with a size greater than or equal to 5 mm between the disc without and with association with clavulanic acid were considered ESBL (CLSI 2021).

In silico determination of virulence genes and design of oligonucleotide primers

Fourteen complete and detailed sequenced genomes available on the NCBI website were analyzed (NZ_CP027177.1, NZ_CP063843.1, NZ_CP023505.1, NZ_CP048806.1, NZ_CP026046.1, NZ_CP048275.1, NZ_CP014026.2, NZ_LS483498.1, NZ_CP033056.1. NZ_CP025933.1, NC_020418.1, NZ_CP034944.1, NZ_CP064833.1, NZ_CP032295.1). We selected genes that were predominant among the strains and of great significance for bacterial virulence. The genetic sequences of the virulence genes were obtained through analysis combined with the Virulence Factor of Pathogenic Bacteria (VFDB) webserver using the VFanalyzer tool, which is available at http://www.mgc.ac.cn/cgi-bin/VFs/v5/main.cgi?func=VFanalyzer.

After the combined analyses, we performed the construction of the oligonucleotides initiators of the virulence genes (Table 1) selected according to their function and homology. The construction of the primer oligonucleotides was performed using the "PrimerQuest Tool" offered by Integrated DNA Technologies® (IDT), available at (https://www.idtdna.com/Primerquest/Home/Index).

Research of virulence genes

Nine genes associated with virulence of *M. morganii* obtained from the analysis of genomes and the VFDB webserver, *ireA* and *iutA* (siderophores), *zapA* (protease), *hlyA* and *shA* (hemolysins), *mrp*A and *fim*H (fimbriae), and *invA* and *tibA* (adhesins/invasins), were investigated in our isolates. The DNA used was extracted by the boiling method, followed by cooling. The PCR reaction was performed on the GeneAmp PCR System 9700 thermocycler (Applied Biosystems™) with a final volume of 25 µL. The reaction

Table 1 Oligonucleotides used to detect virulence genes of *Morganella morganii*

Genes		Oligonucleotide sequence	Amplicon size	Temp (°C)	Reference
Adhesins/invasins	***invA***	TCGCGGTACTGCTGATTATG (F)	736 bp	57	This study
		CTGACCCGGGTGACAATAAA (R)			
	tibA	CATCACCAACAAAGCGGATAAA (F)	583 bp	55	This study
		GGTGACATAGTGCTGACCATAA (R)			
Fimbriae	***mrpA***	AACGTGAACCCAGTTCGATAG (F)	354 bp	55	This study
		TGCAACCGCCAGAAGTAAA (R)			
	fimH	GCCGCTGTAACTGATGGTATAA (F)	448 bp	55	This study
		CAGCTTTACCTCCGCCAATAA (R)			
Hemolysin	***hlyA***	CTGTCAAAGGTGTCAGGGATAA (F)	273 bp	57	This study
		CCAGTTCACGGGTAACAGTATAA (R)			
	shlA	ACGGGAGACTTTGCTGTAATC (F)	205 bp	55	This study
		CACTCAGGTGCAGGGTAATC (R)			
Protease	***zapA***	CTCAGTGATTCGGGTTTGGT (F)	270 bp	55	This study
		CGTTCCATGCGCGTTAATTG (R)			
Siderophores	***iutA***	GACGAAACCCAGACCTTCTATC (F)	232 bp	55	This study
		CGTTGCTTTCCTTCGCTTTC (R)			
	ireA	CTGAATCGGTGAAGGTGTAGTT (F)	254 bp	55	This study
		CGTTACCGAAGAACTGGGTATT (R)			

F forward, *R* reverse, *bp* bases pairs, *Temp* annealing temperature (in °C)

components included 12.5 µL of the mix (containing 2mM $MgCl_2$ — Invitrogen™, 2.5 µL of 10X-Invitrogen™ buffer, 0.2 mM dNTPs (10mM) — Invitrogen™), 1 µL (20 pMol) of forward and reverse primers (IDT™), 0.2 µL (1U) of Taq DNA polymerase (Invitrogen™), 2.0µL of bacterial lysate, and 8.3µL of ultrapure water. The products of this experiment were evaluated after the electrophoresis using a 1.5% agarose gel stained with SYBR® SAFE (Invitrogen™) and observed by means of an ultraviolet light transilluminator.

Research of resistance genes

In order to carry out research on antibiotic resistance genes, the presence of the following genes was verified through the PCR reaction: CTX-M-1, 2, 8, 9, and 25 (Woodford et al. 2006); TEM and SHV (Arlet 1991); *aac'(6)-lb-cr* (Chen et al. 2012a, 2012b); *sul*1, *sul*2 (Li et al. 2007); *qnr*A, *qnr*B, *qnr*S (Cattoir et al. 2007); *qnr*D (Wang et al. 2009), and *fos*A3 (Sato et al. 2013). For this reaction, we used the following: 12.5 µL of the mix (containing 2mM $MgCl_2$ — Invitrogen®, 2.5 µL of 10X-Invitrogen® buffer, 0.2 mM of dNTPs (10mM) — Invitrogen™), 1 µL (20 pMol) of forward and reverse primers (IDT™), 0.2 µL (1U) of Taq DNA polymerase (Invitrogen™), 2.0 µL of bacterial lysate, and 8.3 µL of ultrapure water. The products of this experiment were evaluated after the electrophoresis run, in a 1.5% agarose gel, stained with SYBR® SAFE (Invitrogen™), and observed via an ultraviolet light transilluminator.

ERIC-PCR

Bacterial isolates were tested according to their genetic similarity using the ERIC-PCR technique, following the methodology described by Versalovic et al. (1991). The oligonucleotide primers used were ERIC-1 (5′-ATGTAAGCT CCTGGGGATTCAC-3′) and ERIC-2 (5′-AAGTAAGTG ACTGGGGTGAGCG-3′). For this reaction, the following components were used: 12.5 µL of the mix (containing 2 mM $MgCl_2$ — Invitrogen, 2.5 µL of 10X-Invitrogen® buffer, 0.2 mM of dNTPs (10 mM) — Invitrogen™), 1 µL (20pMol) of primers forward and reverse (IDT™), 0.2 µL (1U) of Taq DNA polymerase (Invitrogen™), 2.0 µL of bacterial lysate, and 8.3 µL of ultrapure water. The products of this experiment were evaluated after the electrophoresis run, in agarose gel with a concentration of 2%, followed by ethidium bromide staining. The genetic similarity dendrogram was constructed using the software Gel J 2.0 (HERAS et al. 2015), using the method of unweighted pairs group with arithmetic mean (UPGMA) and the coefficient of similarity of data for the analysis of the clusters (Jaccard), with index tolerance of 1.5. To determine the clusters, we used 85% similarity as a cut-off point.

Biofilm formation

Based on the protocol established by Kwiecinska-Piróg et al. (2014), with modifications, the biofilm formation assay was performed. To evaluate the biofilm formation capacity of

the study bacteria, we used 96-well polystyrene plates. The bacteria were previously cultured in trypticasein soy broth (TSB; Difco™). Subsequently, the samples were centrifuged at 4000 rpm for 15 min, their supernatant was discarded and the sedimented material washed in phosphate-buffered saline (PBS). The bacterial suspension was centrifuged at 4000 rpm for 10 min, and the sedimented material was used to prepare a 0.5 McFarland suspension in TSB broth (Difco™). In each well of the plate, we added 20 µL of the bacterial suspension and 180 µL of sterile TSB, in quadruplicate, for each isolate. For the negative controls, we used 200 µL of pure sterile TSB, and for the positive control, we used the *E. coli* strain (EAEC 042) (Nataro et al. 1995). The plate was incubated at 37 °C for 24 g. After this period, the wells were washed with distilled water and fixed with 200 µL of methanol in each well of the plate. At the end of this process, the methanol was removed, and the plate was dried at 37 °C for 20 min. After this procedure, we added 200 µL of 0.01% crystal violet to each well for 20 min. Subsequently, the plate was washed with distilled water until the wells became colorless. The plates were again taken to drying. For fixation, 200 µL of methanol were added to each well. After the entire process, the plate was taken to the spectrophotometer to read the absorbance at 570 nm. Biofilm was classified as absent, weak, moderate, strong, and very strong based on the value obtained for the arithmetic mean of the negative control and a triple value of its standard deviation ($T = Xnc + 3\delta$).

HeLa cell adhesion protocol

The technique was performed according to the protocol developed by Cravioto et al. (1979). To perform the test, we used HeLa cells in a confluent layer on a round glass coverslip, in 24-well polystyrene plates. Three washes were performed by a PBS solution. Subsequently, 1 mL of Dulbecco's modified Eagle's medium (DMEM; Difco™ made with and without 2% D-mannose) was added to each well, along with a 40-µL aliquot of the bacterial culture, into each of the wells of the plate. The plate was incubated for 3 h at 37 °C. After this period, the wells were washed with 1 mL of PBS to remove non-adherent bacteria, and then 1 mL of DMEM (Difco™) was added to the wells. The plate was incubated for an additional 3 h at 37 °C. At the end of these processes, the plate wells were washed again with another 1 mL of PBS. The coverslips were fixed with the aid of 100% methanol and later stained with May-Grünwald. The coverslips were deposited on glass slides, which were observed and photographed with the aid of a photomicroscope. For the negative control, only HeLa cells were used for the test, while for positive controls, we used *E. coli* strain E2348/69 (Levine et al. 1978) for localized adhesion, *E. coli* C1845 for diffuse adhesion (Bilge et al. 1989), and *E. coli* 042 (Nataro et al. 1995) for aggregative-type adhesion.

Cytotoxicity Protocol

The assay was performed based on the protocol of Konowalchuk et al. (1977), with some modifications. For this experiment, we used a confluent layer of Vero cells in a 96-well polystyrene plate. *M. morganii* isolates were incubated in TSB broth (Difco™) under agitation. After the incubation period, the isolates were submitted to centrifugation at 13,000 g for 10 min. The supernatant obtained was filtered through a syringe filter with a membrane (Durapore™) with pores of 0.22 µm and 47 mm in diameter. The filtered content was transferred to a polystyrene plate at a 1:10 dilution. The plate was incubated for 72 h at 37 °C and 5% CO_2. The cytotoxicity of the analyzed samples was quantified through absorbance, from the measurement of the metabolic rate of Vero cells with the MTT test (3-(4,5dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide), a compound that evaluates the cells viable from MTT conversion, as suggested by Murakami et al. 2000. Wells containing Vero cells with no bacterial supernatant were used as a negative control, and EDL 933 strain (Yu and Kaper 1992) was used as a positive control for the test. Cell death of 50% or more, compared to the negative control, was considered highly toxic.

Research of plasmid incompatibility groups (INCs)

To carry out the plasmid incompatibility group gene research assay, the genes described by Carattoli et al. (2005) were searched. For this reaction, we used 12.5 µL of the mix (containing 2 mM $MgCl_2$ — Invitrogen™, 2.5 µL of 10X-Invitrogen™ buffer, 0.2 mM of dNTPs (10 mM) — Invitrogen™), 1 µL (20pMol) of forward and reverse primers (IDT™), 0.2 µL (1 U) of Taq DNA polymerase (Invitrogen™), 2.0 µL of bacterial lysate, and 8.3 µL of ultrapure water. The products of this experiment were evaluated after the electrophoresis run, in a 1.5% agarose gel stained with SYBR® SAFE (Invitrogen™) and observed via an ultraviolet light transilluminator.

Statistics

The evaluation of the relationship between the different factors researched was performed through multivariate logistic regression analysis and odds ratio calculation from the prediction model (Menard 2002). The significance level considered was $\alpha = 5\%$. Statistical analysis was performed using the R statistical software (version 4.1.1).

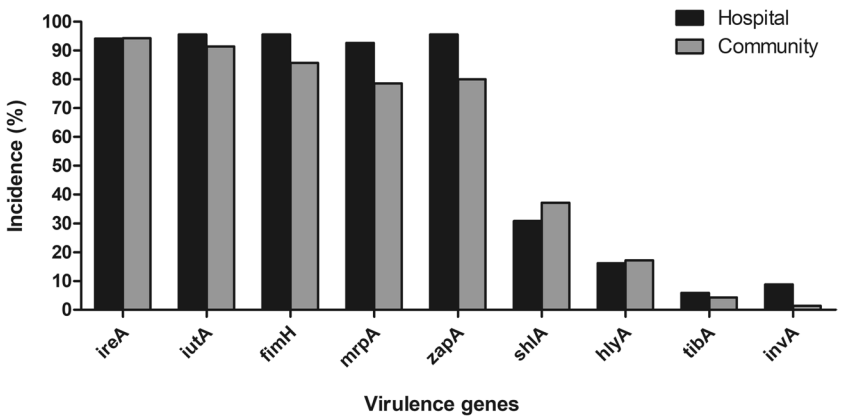

Fig. 1 Incidence of virulence genes in *M. morganii* isolates from hospital and community settings

Results

Virulence

All isolates were tested for the detection of virulence genes, which contribute to pathogenicity in infections caused by *M. morganii* (Supplementary Figure 1 and Fig. 1). Of the 70 isolates from the community, 66 (94.28%) were positive for the *ireA* gene, 64 (91.42%) for the *iutA* gene, 60 (85.71%) for the *fimH* gene, 56 (80%) for the *zapA* gene, 55 (78.57%) for the *mrpA* gene, and 25 (37.14%) for the *shlA* gene; 12 (17.14%) were positive for the *hlyA* gene, 3 (4.28%) for *tibA*, and 1 (1.42%) for *invA*.

Of the 68 hospital isolates, 65 (95.58%) were positive for the *zapA*, *iutA*, and *fimH* genes; 64 (94.11%) for the *ireA* gene; 63 (92.64%) for the *mrpA* gene; 21 (30.88%) for the *shlA* gene; 11 (16.17%) for the *hlyA* gene; 6 (8.82%) for the *invA* gene; and 4 (5.88%) for the *tibA* gene.

Phenotypic characteristics of virulence

Based on the analyses, we can conclude that *M. morganii* isolates have the ability to phenotypically express aggregative adhesion (Fig. 2) in HeLa cells. Isolates from the hospital environment are more likely to exhibit adherence compared to community isolates (OR: 2.35; CI: 1.26,4.39), with an adherence rate of 63 (92.64%) isolates, while community isolates expressed adherence in 55 (78.57%) isolates.

Regarding cytotoxicity, 24 (35.29%) of hospital isolates and 24 (34.28%) of community isolates showed cytotoxic effects on Vero cells.

Regarding biofilm formation, the isolates were classified according to the protocol of Kwiecinska-Piróg et al. (2014), in biofilm intensities: absent, weak, moderate, strong, and very strong, according to the result of their absorbance (Fig. 3). The results of biofilm intensity found in hospital isolates were as follows: 20 (29.41%) classified as weak, 32 (47.05%) moderate, 13 (19.12%) strong, and 3 (4.42%) very strong. While in the isolates of community origin,

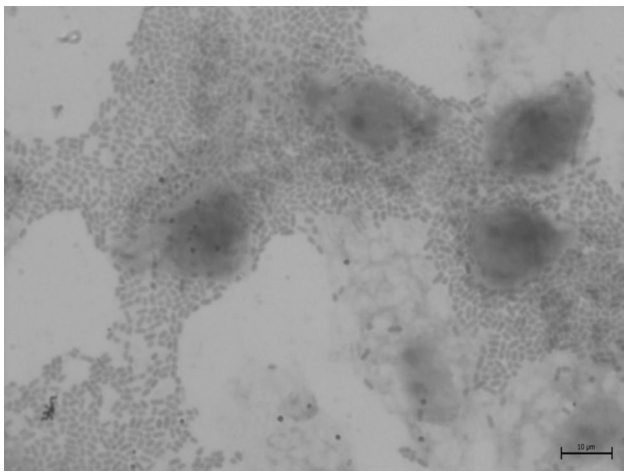

Fig. 2 Aggregative adhesion pattern expressed by UTI isolate caused by *M. morganii* in HeLa cells

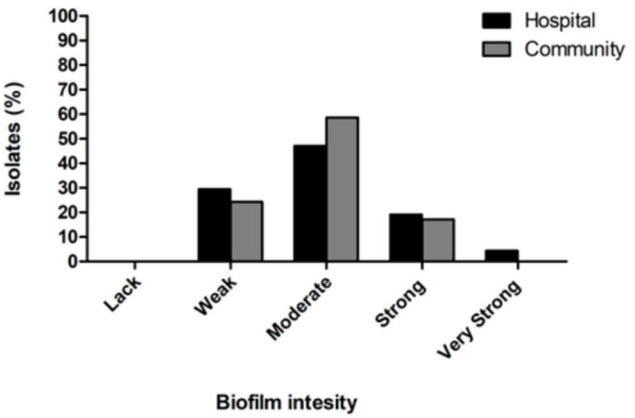

Fig. 3 Biofilm intensities expressed by *M. morganii* isolates from hospital and community UTIs

the results obtained were as follows: 17 (24.29%) of low intensity, 41 (58.57%) of moderate intensity, 12 (17.14%) of strong intensity, not obtaining absorbances compatible with

the formation of a very strong biofilm for any community isolate. Community isolates are more likely to be producers of moderate intensity biofilm when compared to hospital isolates (OR: 1.79; CI: 0.91; 3.53). Any isolate did not produce biofilm, regardless of its place of origin.

Antimicrobial resistance

Based on the analysis obtained using the Vitek 2 system, the isolates were classified according to their susceptibility and resistance to the tested antimicrobials. The isolates were tested for antimicrobials belonging to different classes (Fig. 4).

Hospital isolates proved to be more resistant to the antibiotics tested in relation to the community, showing 14.70% resistance to amikacin, 61.00% gentamicin resistance, 79.41% to aztreonam, 69.11% ceftazidime, 64.70% ceftriaxone, 45.58% to cefepime, 5.88% piperacillin associated with tazobactam, 57.35% to ciprofloxacin ($p < 0.05$); 38.23% to norfloxacin, 35.29% to nalidixic acid, and 63.23% to sulfazotrim.

Community isolates indicate 100% susceptibility to amikacin. They showed 28.75% resistance to gentamicin, 12.5% to aztreonam and ceftazidime, 28.75% ceftriaxone, 15% cefepime, 10% piperacillin associated with tazobactam, 47.14% to ciprofloxacin, 45.71% to norfloxacin, 75.71% nalidixic acid, and 44.28% to sulfazotrim. Despite community isolates being more susceptible to antimicrobials, some antibiotics showed greater resistance when compared to hospital isolates, as in the case of piperacillin associated with tazobactam (OR: 3.57 CI: 1, 12, 67), ciprofloxacin and nalidixic acid (OR: 9, 31 CI: 3.72, 23.31). Regardless of the site of infection, the isolates did not show resistance to any carbapenem tested.

Based on combined disk ESBL phenotypic screening, the isolates were genotypically tested for CTX-M-1, 2, 8, 9, and 25 groups using the PCR technique. Of the 68 hospital isolates, 32 (47.05%) were positive for ESBL, of which 9 were positive for the M-1 group, 22 for the M-2 group, and 2 for the M-9 group. Regarding the isolates of community origin, of the 70 isolates, 9 (12.85%) were positive for ESBL, being these, 7 for the M-1 group, 1 isolated for M-2, and 2 for M-9.

When comparing the antimicrobial resistance of ESBL isolates, we showed that these isolates are predominantly more resistant in relation to non-ESBL (Table 2). In hospital isolates, we showed that resistance to antimicrobials gentamicin (OR: 59.93 CI: 0, > 100), ceftriaxone (OR: 341 CI: 33.66, > 100), cefepime (OR: 400 CI: 0, > 100), acid nalidixic (OR: 7.31 IC: 2.37, 22.51), and sulfazotrim (OR: 30 IC: 6.12, > 100) are remarkably superior in ESBL isolates. In the community, ESBL-producing isolates are > 400 times more likely to be resistant to aztreonam than non-ESBL isolates (OR: 400 IC: 0, > 100).

Regarding the search for resistance genes not related to ESBL, in the isolates from the hospital environment, we evidenced the presence of 21 (30.88%) isolates resistant to *qnrD*; 10 (24.39%) isolates showed resistance to the *sul*1 and 30 (73.17%) to the *sul*2. In the isolates of community origin, we detected the presence of *qnrD* genes in 23 (32.85%) isolates, *sul1* in 11 (32.35%) isolates, and *sul2* gene in 19 (55.88%) isolates. No presence of SHV, TEM, fosA3 genes, other variants of the *qnr* gene, or aac′(6)-lb-cr was found in the studied isolates, regardless of the site of isolation.

ERIC-PCR

When analyzing the genetic similarity between hospital and community isolates, we observed the presence of 18 clusters, where 14 of them presented isolates from both sources (C3, C4, C5, C6, C8, C9, C10, C11, C12, C13, C14, C16, C17, and C18), three of them exclusive to hospitals (C1, C2 and C15), and one exclusive of community isolates (C7)

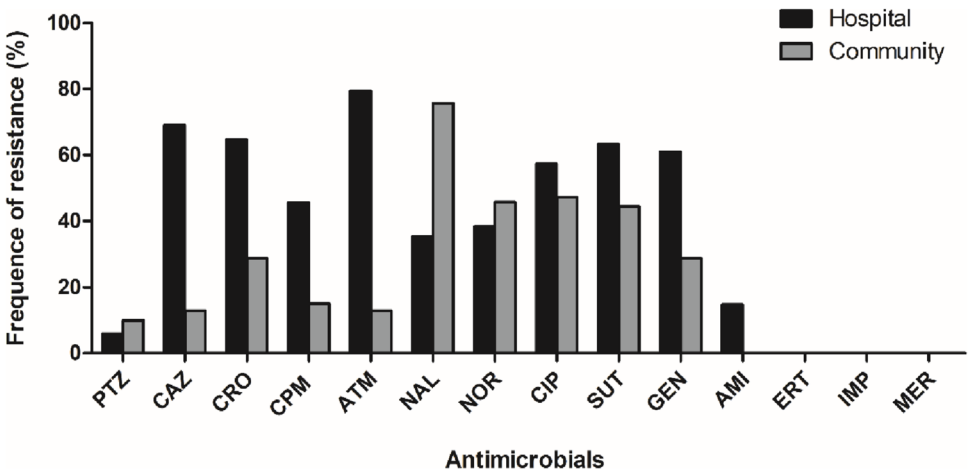

Fig. 4 Frequency of antimicrobial resistance of hospital and community isolates of UTI caused by *M. morganii*. PTZ piperacillin associated with tazobactam; CAZ ceftazidime; CRO ceftriaxone; CPM cefepime; ATM aztreonam; NAL nalidixic acid; NOR norfloxacin; CIP ciprofloxacin; SUT sulfamethoxazole associated with trimethoprim; GEN gentamicin; AMI amikacin; ERT ertapenem, IMP imipenem, MER meropenem

Table 2 Comparative table of antimicrobial resistance comparing ESBL and non-ESBL isolates of *M. morganii* in the hospital and in the community from Londrina, PR, Brazil

Antimicrobials	Hospital		Community	
	ESBL = 32	NON ESBL = 36	ESBL = 9	NON ESBL = 61
AMI	25%	5.56%	11.11%	0%
GEN	90.63%	16.67%	44.44%	26.23%
ATM	46.88%	5.56%	100%	0%
CAZ	43.75%	8.33%	100%	0%
CRO	100%	13.89%	100%	21%
COM	87.50%	8.88%	88.88%	8.20%
ERT	0%	0%	0%	0%
IPM	0%	0%	0%	0%
MPM	0%	0%	0%	0%
PTZ	12.50%	0%	44.44%	4.92%
CIP	96.88%	27.78%	55.55%	47.54%
NOR	59.38%	19.44%	55.55%	45.90%
NAL	53.14%	19.44%	77.77%	63.93%
SUT	93.75%	33.33%	44.44%	49.18%

AMI amikacin, *GEN* gentamicin, *ATM* aztreonam, *CAZ* ceftazidime, *CRO* ceftriaxone, *CPM* cefepime, *ERT* ertapenem, *IMP* imipenem, *MER* meropenem, *PTZ* piperacillin associated with tazobactan, *CIP* ciprofloxacin, *NOR* norfloxacin, *NAL* nalidixic acid, *SUT* sulfamethoxazole associated with trimethoprim

(Supplementary Figure 2). The cut-off point for cluster formation was set at 85% similarity.

Plasmid incompatibility groups (INCs)

Based on the results obtained through the PCR technique, we evidenced the presence of incompatibility groups in the studied isolates. The hospital isolates presented the FIB, I1, and A/C groups in 19 (27.94%), 14 (20.58%), and 2 (2.94%), respectively. While in the community, we revealed the presence of incompatibility groups FIB in 19 (27.14%), I1 in 12 (17.14%), and FIA in 3 (4.28%).

Discussion

This study found the presence of several virulence and resistance factors present in isolates of hospital and community origin, which corroborate the pathogenicity and efficacy of the infection. Despite being a poorly isolated microorganism, the presence of *M. morganii* in both nosocomial and community-acquired infections is emerging and requires attention. In our study, we evaluated the presence and incidence of genes (*mrpA*, *fimH*, *invA*, *tibA*, *zapA*, *ireA*, *iutA*, *hlyA*, *shlA*) related to virulence of *M. morganii* in hospital and community isolates from the city of Londrina, PR, Brazil.

The *zapA* gene encodes a metalloprotease commonly present in *P. mirabilis*, capable of degrading IgA, an important defense component present in mammalian mucosa, by hydrolyzing it (Tolulope et al. 2021; Walker et al. 1999). Studies evaluating the presence of the *zapA* gene detected the high prevalence of this gene in *P. mirabilis* (Pathirana et al. 2018). Our study showed a greater presence of this gene in hospital isolates (OR:2.65 CI:1.27, 5.52), which can be justified by the fact that in the hospital environment, this metalloprotease, especially in immunocompromised individuals, favors the infectious process.

In mammals, extracellular iron is associated with the protein transferrin, which delivers iron throughout the body. This association reduces serum iron levels, which is an essential component for bacteria, especially pathogenic ones (Saha et al. 2016). Siderophores are necessary for this acquisition to occur, favoring bacterial survival (Skaar 2010). The *iut*A gene encodes an aerobactin ferric receptor protein, aiding in iron acquisition (Robinson et al. 2018). In a recent study carried out by Ikeda et al. (2021) with *E. coli*, the values found for the *iutA* gene are similar to our study. Like *iutA*, the *ireA* gene is also related to iron acquisition (Russo et al. 2001). In a study carried out with *P. mirabilis* from community-acquired UTIs, all isolates (100%) presented the *ireA* gene, which is consistent with the high prevalence shown in our study (de Oliveira et al. 2021).

The presence of fimbriae genes such as *mrpA* is essential for the establishment of the initial phase of infection, favoring adhesion, which is an important factor during the installation of the microorganism and its maintenance in the host (Rocha et al. 2007). In a recent study of *P. mirabilis* isolates from UTIs, the presence of this gene was detected in all the isolates examined (Sanches et al. 2021), which is consistent with the high prevalence of this gene in our study. Type 1 fimbriae, such as *fimH*, also support the bacterial adhesion

process, establishing an important role in infectious processes, especially in UTI. In a study with uropathogenic *E. coli* (UPEC), the *fim*H gene was detected in more than 90% of the strains studied (Haghighatpanah and Mojtahedi 2019), which aligns with the findings of our study.

All *M. morganii* isolates that showed the ability to adhere to HeLa cells showed the presence of the *mrpA* gene, regardless of the use or not of D-mannose. However, our results show that the presence of only type 1 fimbriae in the bacterial adhesion test, using D-mannose, inhibited the cell adhesion process, supporting the relationship of type 3 fimbriae with bacterial adhesion capacity, with the aggregative feature. Hospital isolates showed greater adherence capacity when compared to community isolates (OR: 2.35; CI: 1.26, 4.39), which may justify the ability to stay in the hospital environment.

Hemolysins are considered virulence factors that have cytotoxic activity, with the ability to form pores on the cell surface, as is the case with hemolysin *HlyA*. Previous studies have reported that about 50% of *M. morganii* isolates have cytotoxic activity (Koronakis et al. 1987). When compared with *E. coli* hemolysin, it showed similar hemolytic and functional activity (Eberspacher et al. 1990). The *shlA* gene is the most common virulence factor of *Serratia marcescens* (González et al. 2020), which encodes a pore-forming toxin, with cytotoxic effects on epithelial cells and fibroblasts (Di Venanzio et al. 2014). Interestingly, our study demonstrates the presence of *hlyA* or *shlA* genes in all isolates with cytotoxic activities ($p < 0.001$).

Biofilms are considered virulence mechanisms of great importance for bacterial pathogenesis (Gellatly and Hancock 2013; Verderosa et al. 2019). It is estimated that about 80% of human bacterial infections result from the formation of biofilms (Davies 2003), especially when associated with the use of long-term catheters (Stickler 2008, 2014). A study with *P. mirabilis* from community-acquired UTI reports that, like our study, all the isolates studied demonstrated the capacity for biofilm formation (de Oliveira et al. 2021). However, our study showed that the biofilms formed have moderate intensity as the most prevalent, regardless of the isolation site, unlike the results found by de Oliveira et al. (2021), in his study also carried out in the city of Londrina, where 73.2% of the isolates showed very strong intensity biofilm. The capacity for bacterial biofilm formation deserves attention, as these structures can eventually obstruct catheters, aggravating and making treatment difficult.

Normally, hospital-sourced isolates show greater resistance to antimicrobials when compared to community-acquired isolates (Caneiras et al. 2019). This can be explained by the fact that hospitalized patients are frequently exposed to the use of antimicrobials, prolonged hospitalization, and previous infections (Kader and Kumar 2005; Ferjani et al. 2015; Manyahi et al. 2017). In a study with *E. coli*, they showed that the presence of ESBL isolates is predominant in a hospital environment, and its spread to the community isolates is low (Wollheim et al. 2011), corroborating the results found in our study ($p < 0.001$).

Previous studies have reported that the most predominant group of ESBL-type enzymes in our country are those of the CTX-M group, with those of groups M-1, 2, 8, 9 being the most detected (Cyoia et al. 2019), in agreement with the findings of our study, where the most prevalent groups detected were M-1 and M-2. Hospital isolates are about 8 times more likely to have the CTX-M-2 gene compared to community *M. morganii* isolates ($p < 0.001$). Studies carried out in Japan reported the prevalence of CTX-M-2 and 9 groups in hospital Enterobacteriaceae (Yamanaka et al. 2020), while in Europe, the most predominant CTX-M group was M-1 (Reuland et al. 2016). The identification of antimicrobial resistance, particularly in specific geographic areas, is crucial to ensure effective and appropriate treatment (Ghavidel et al. 2020).

The diversity of resistance evidenced in our study reports significant data when we consider the great intrinsic resistance of *M. morganii* (CLSI 2021), which, when accompanied by acquired resistance, reduces the availability of treatment, making it difficult for the patient to recover. Our study also highlights the presence of multidrug-resistant strains (MDR) in the hospital and in the community. Hospital isolates are more likely to be MDR (OR: 1.74 CI: 1.14, 2.66) compared to community ones, showing that the hospital environment is a factor that contributes to the selection of multidrug-resistant strains. A study carried out in the same period and in the same region as ours shows that community strains of *P. mirabilis* that cause UTI (de Oliveira et al. 2021) show low resistance to the tested antimicrobials, unlike our community isolates that showed a greater potential of *M. morganii* to resistance.

In our study, all antimicrobials except nalidixic acid, norfloxacin, and piperacillin-tazobactam showed higher resistance rates in hospital isolates ($p < 0.05$). We also noticed that carbapenems proved to be efficient alternatives for the treatment of infections caused by *M. morganii*, followed by amikacin and piperacillin-tazobactan, regardless of the place of origin, similar to the results found by Mostafavi et al. (2021) in a study also carried out with UTI.

Quinolone and fluoroquinolone antimicrobials are widely prescribed for the treatment of UTIs caused by enterobacteria (Stuck et al. 2012), justifying the high rate of resistance found for these antimicrobials both in hospitals and in the community. In Brazil, quinolones are widely prescribed, making the scenario even more worrisome (Wirtz et al. 2010; Vieira et al. 2020). A previous study with the Proteeae tribe reported the high prevalence of *qnrD* genes in hospital isolates in China (Chen et al. 2018). In our study,

it was possible to observe that the presence of *qnrD* may contribute to quinolone resistance and has been commonly found in *M. morganii* (Mazzariol et al. 2012; Yaiche et al. 2014; Chen et al. 2018). Another class of antimicrobial frequently associated with the treatment of UTI is sulfonamides (Arredondo-García et al. 2004; Naquin et al. 2021). Our study showed high resistance to these antimicrobials, especially in the hospital environment, where the rate was higher compared to the community (OR: 2.28 CI: 1.05, 4.96). Together with this data, we noted the high prevalence of sulfonamide resistance genes, especially the *sul2* gene, especially in hospital settings compared to the community (OR: 2.12 CI: 1.2, 3.74), suggesting that the main resistance gene for sulfonamides in our region is the *sul2* gene.

The ERIC-PCR technique is indicated for the evaluation of the genetic similarity of enterobacteria, highlighting its high discriminatory power (Versalovic et al. 1991; Michelim et al. 2008). To the best of our knowledge, this is the first study evaluating the clonal relationship of hospital and community *M. morganii* urinary isolates through the ERIC-PCR technique, also relating the phenotypic and genotypic profile of virulence and antimicrobial resistance. ERIC-PCR technique has already been used to assess the clonal relationship of other members of the Proteeae tribe (Sanches et al. 2021; de Oliveira et al. 2021). These studies reveal the presence of circulating clones with 100% identity, corroborating the results of our study, which show the circulation of possible clones of *M. morganii* within the hospital and also in the community of Londrina, PR. Interestingly, most of our clusters comprise isolates from both hospital and community sources (C3, C4, C5, C6, C8, C9, C10, C11, C12, C13, C14, C16, C17, and C18). Furthermore, we can see the presence of clone formation with 100% identity between hospital and community isolates, with the same genotypic and phenotypic virulence profile in some clusters (C3, C4, C5, C9, C11, and C12), highlighting the possibility of a circulation of clones between hospital and community of Londrina, PR, being necessary more studies to better evaluate the genetic similarity of these isolates.

Although our isolates showed plasmid incompatibility groups, regardless of the isolation site, the detected replicons were differentiated between community and hospital. Notably, the most present replicons in our study were FIB and I1, being the most prevalent, both in the hospital and in the community. In a recent study carried out in the USA with UPEC isolates from CAUTI, Tarlton et al. (2019) detected a higher prevalence of plasmid incompatibility groups FIB, FIA, and B/O in their isolates. In our study, the plasmid incompatibility group of the highest prevalence detected was also FIB; however, we did not detect B/O in our study, and FIA had a low prevalence among our community isolates. In a study carried out with *E. coli* isolates from CAUTI in Colombia, the FIB replicon was also the most prevalent detected (De La Cadena et al. 2020), similar to our study. However, further studies involving plasmid incompatibility groups in the hospital setting with UTI need to be performed.

Another important information that should be considered in our study is the presence of the plasmid incompatibility group I1 associated with the CTX-M1 group, both in the hospital (OR: 3.48 CI: 0.98, 12.39) and in the community (OR: 5.75 CI: 1.74, 19.06). These results are similar to those evidenced by Irrgang et al. (2018) and Valcek et al. (2019), suggesting that the main plasmid element carrying the CTX-M-1 group in our region is plasmid I1. In addition, we can also note the relationship of the FIB plasmid incompatibility group often associated with the *sul*2 gene, both in the hospital environment and in the community ($p < 0.05$); however, more studies relating these genes are needed.

Conclusion

Our results indicate that *M. morganii* isolates, regardless of the isolation site, have several virulence genes such as invasins, hemolysins, fimbriae, proteases, and iron acquisition systems, which favor the infectious process provided by this bacterium. The presence of resistance to several antimicrobials and the dissemination of important resistance genes in these isolates make treatment difficult, especially through the circulation of MDR isolates, both in the hospital and in the community. Another relevant factor that must be taken into account is the neglected emerging potential of *M. morganii*, because in addition to being able to cause different types of opportunistic diseases, they can also accumulate several virulence and antimicrobial resistance factors, necessitating enhanced surveillance measures in healthcare and infection control.

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Declarations

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University State of Londrina (CEP-UEL 1.590.120).

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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