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ARTICLE

Prevalence, Antimicrobial Resistance, and Distribution of blaCTX-M and blaTEM Genes Among ESBL-Producing Morganella morganii Isolated from Clinical Samples in Bangladesh

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ABSTRACT

Background: Morganella morganii, once regarded as a benign intestinal commensal, now poses a serious threat due to its intrinsic AmpC β-lactamase production and increasing multidrug resistance. **Objective:** This study aimed to assess the efficacy of antibiotic combination therapies against MDR Morganella morganii isolates, specifically evaluating ceftazidime plus amikacin and amikacin plus imipenem regimens to improve treatment outcomes. **Methods:** A cross-sectional observational and experimental study was conducted from November 2022 to June 2023 at Dhaka Medical College Hospital, Bangladesh. Seven clinical Morganella morganii strains were isolated from urine, wound, blood, and stool samples. Identification was performed using culture, biochemical tests, and PCR. Antimicrobial susceptibility was determined by disc diffusion and MIC via agar dilution following CLSI (2022) guidelines using standard protocols. **Results:** Seven isolates of Morganella morganii were recovered, representing an overall prevalence of 1.72%. Urine samples yielded 42.85% of isolates, while wound samples contributed 28.57%. Males accounted for 71.42% of cases, predominantly in the 26–35-year age group. ESBL production was observed in 28.57% of isolates, with PCR confirming bla_CTX-M and bla_TEM genes in 50% each among ESBL-positive strains. Carbapenemase production was detected in 50% and 25% of imipenem-resistant isolates by CD assay and DDS test, respectively. Overall, 57.14% were MDR and 14.28% XDR. Detailed calculations indicate significant associations between risk factors and resistance patterns with high significance. **Conclusion:** This study underscores the urgent need for early detection and targeted combination therapy to combat MDR and XDR Morganella morganii, with ceftazidime-amikacin and amikacin-imipenem regimens showing promising clinical potential.

Keywords: Prevalence, Antimicrobial Resistance, blaCTX-M and blaTEM Genes, Morganella morganii

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INTRODUCTION

Morganella morganii is a gram-negative bacillus belonging to the Enterobacteriaceae family, and it is a common inhabitant of the environment and intestinal tracts of humans, mammals, and reptiles [1]. Morganella morganii is recognized as an unusual opportunistic pathogen that is isolated specifically in urinary tract or wound infections. However, Morganella morganii has recently been regarded as an increasingly important pathogenic bacterium due to

its virulence and increased drug resistance, which causes a variety of clinical infections, such as urinary tract infections, bacteremia and sepsis, and results in a high mortality rate in some infections [2]. Biologically, Morganella morganii is a motile, non-lactose fermenting bacterium, which shares with the Proteus members the capacity for urease production and the presence of phenylalanine deaminase3. Morganella morganii is an unusual opportunistic pathogen that is clinically and often isolated as a cause of nosocomial infection in adults, specifically in urinary tract or wound infections.

The urinary tract is the major portal for Morganella morganii entry, followed by the hepatobiliary tract, skin and soft tissue, and blood [3]. A variety of virulence factors including fimbrial adhesins, lipopolysaccharides (LPS), IgA protease, hemolysins, ureases, insecticidal and apoptotic toxins, iron acquisition system, type-III secretion system, and two-component systems allows Morganella morganii to cause various invasive infections [4]. Morganella morganii has chromosomally encoded blaAmpC, which confers resistance to cephalosporins and penicillins [5]. As a result of its ability to cause invasive disease, the presence of blaAmpC and virulence factors, and its propensity to acquire resistance determinants, Morganella morganii has been labeled an emerging "superbug" [6]. Enterobacterales resistant to third-generation cephalosporins due to the production of AmpC β-lactamase encoded by resident chromosomal genes (e.g., Enterobacter spp., Serratia marcescens, Citrobacter freundii, Providencia spp., Morganella morganii – ESCPM group) are frequent agents of bloodstream infection. In the ESCPM group, AmpC β-lactamase can be expressed at high levels by mutation. Overexpression confers resistance to broad-spectrum cephalosporins including cefotaxime, ceftazidime, and ceftriaxone [7]. AmpC expression among these organisms is inducible in response to β-lactam exposure. On removal of β-lactam exposure, AmpC production generally decreases; however, if mutations have occurred in certain regulatory genes (e.g., ampD, ampR), selection of mutants with stable AmpC de-repression can occur [8].

Antimicrobial resistance in Morganella morganii has been mainly acquired via plasmids and class 1 integrons. Enzymes conferring resistance to beta-lactams and carbapenems, such as TEM, CTX-M, KPC, OXA, VIM, and NDM, have been detected in clinical isolates of Morganella morganii, representing a challenge to therapeutic success [9]. The wide spectrum of β-lactamases represented by TEM-1 and SHV-1 gave rise to the name "extended spectrum" β-lactamases (ESBL), which later involved CTX-M and OXA-type enzymes. These enzymes are capable of hydrolyzing and inactivating a wide variety of therapeutic β-lactam antimicrobials [10]. MDR and extensively drug-resistant clinical strains of Morganella morganii are frequently reported around the globe [11]. The detectable presence of extensively resistant Morganella morganii strains exclusively on the artificial substrate may depict the potential role of plastic in amplifying the pathogenicity of bacteria. Plastics provide a hydrophobic surface for the attachment of microorganisms, promoting colonization and biofilm formation. Under this condition, commonly found on medical implant surfaces, bacteria form a complex and multi-layered three-dimensional architecture, which offers protection from a wide range of environmental challenges and favors the exchange of drug-resistance genes [12].

Morganella morganii has developed a remarkable ability to adhere to different surfaces, form biofilms, and acquire highly efficient MDR. In the clinical sector, the biofilm-forming potential of such pathogenic bacteria is a significant problem, which is well documented and refers to hospital-acquired infections transmitted by catheters [13]. Like Proteus mirabilis, Morganella morganii is a urease-positive organism. However, it produces a urease enzyme that is distinct from that of Proteus mirabilis, and the urine pH change mediated by Morganella morganii urease activity rarely results in the development of struvite crystals or catheter blockage. Morganella morganii is more commonly isolated from unobstructed catheters than blocked catheters in patients catheterized long-term [13]. Urease production serves as a fitness factor that facilitates bacterial growth and biofilm formation during urinary tract infections, which may explain why Morganella morganii mainly causes urinary tract infections. Morganella morganii utilizes D-serine degradation metabolic pathways to improve its fitness during polymicrobial catheter-associated urinary tract infections (CAUTIs), emphasizing its dominant role in CAUTI in the future [14]. The increasing problem of multidrug-resistant (MDR) bacteria in recent years has created the need to judge and reconsider new antibiotic options for the treatment of these infections [15].

However, monotherapy may lead to the development of resistance and often treatment failure. Combination antimicrobial therapies can be a good alternative. Selecting the appropriate combination therapy is a challenging task in the clinical setting. In this situation, in vitro antimicrobial synergy tests can shed valuable light on effective combinations with acceptable/minimal side effects in the management of these difficult-to-treat infections [16]. Animal models are considered the most important in vivo models in terms of basic pharmacokinetic parameters like drug efficiency, safety, and toxicological studies, as these pre-clinical data are required before translating into humans. In most instances, both in vitro and in vivo models are corroborated before proceeding to medical trials. In vivo models are mostly conducted in mice, rats, and rabbits [17]. Until better antibiotics are being developed, novel antibiotic combinations that yield some in vitro and in vivo synergistic activity are perhaps the best options we have to manage this grave condition. No study on Morganella morganii has yet been carried out at the microbiology department of DMCH. Therefore, this study has been designed to evaluate the efficacy of antibiotic combinations against multidrug-resistant Morganella morganii both in vitro and in vivo.

METHODOLOGY

The study was carried out at the Department of Microbiology, Dhaka Medical College, from November 2022 to June 2023, to inquire the prevalence, antimicrobial resistance, and distribution of blaCTX-M and blaTEM genes among ESBL-producing Morganella morganii isolates. The sample size was 550, determined based on the estimated infection prevalence. Blood, urine, stool, wound swabs, and pus samples were collected from patients with clinically suspected infections admitted to Dhaka Medical College Hospital. Participants were included regardless of sex or prior antibiotic intake, following informed written consent. Patients unwilling to provide consent were excluded. Data on patient demographics, duration of hospital stay, and laboratory findings were documented on a structured data collectionsheet. Sample collection followed strict aseptic protocols. Wound and pus samples were collected with sterile swabs, venous blood was drawn with proper skin disinfection, and midstream clean-catch urine was obtained. Stool samples were collected during acute diarrheal episodes. All samples were promptly sent to the laboratory for examination. In the laboratory, the samples were cultured on blood agar, MacConkey agar, Mueller-Hinton agar, SS agar, and chromogenic agar as required. Biochemical identification was performed by triple sugar iron (TSI) agar, Simmons' citrate agar, and motility-indole-urease (MIU) agar. Automated identification was performed with the VITEK® 2 COMPACT system. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method, interpreted according to CLSI guidelines (2022). ESBL-producing organisms were identified by double-disc synergy tests, and MDR and XDR strains were categorized by standard definitions. Checkerboard assays were used for the detection of synergistic interactions between antibiotics and MBL was detected with disc combination assays in addition to EDTA. The method enabled the detection of Morganella morganii, confirmed its resistance pattern, and tested potential antibiotic combinations and provided insightful information on antimicrobial stewardship and infection control.

RESULT

The present study included 550 samples. 205 were urine samples, 148 were wound swabs/pus samples, 80 were blood samples, and 117 were stool samples. Of these, 407 (74%) yielded culture-positive results, as shown in Table 1 Culture yielded growth of 152 (74.14%) in urine samples, followed by 115 (77.71%) in wound swab/pus samples. Table 2 demonstrates the distribution of different species of bacteria isolated from different samples. Among the isolated bacteria, 118 (28.99%) were Esch. coli, 7 (1.72%) were Morganella morganii. Table 3 shows the distribution of Morganella morganii isolated from culture-positive samples. Among the 152 culture-positive urine samples, 3 (1.97%) isolates were M. morganii; among 115 culture-positive wound swab/pus samples, 2 (1.74%) isolates were M. morganii; among 54 culture-positive blood samples, one (1.85 %) isolated was M. morganii and among 86 culture positive stool samples, one (1.16%) isolated was M. morganii.

Table 1: Culture-positive results from various clinical samples (N = 550)

Samples	Number of samples	Culture positive n (%)
Urine	205	152 (74.14)
Wound swab and pus	148	115 (77.71)
Blood	80	54 (67.50)
Stool	117	86 (73.50)
Total	550	407 (74.00)

N = Total number of samples.
n = number of culture-positive samples.

Table 2: Distribution of organisms isolated from different samples by biochemical tests (N =407).

Organisms	n (%)
Escherichia coli	118 (28.99)
Pseudomonas spp.	73 (17.94)
Klebsiella spp.	56 (13.76)
Acinetobacter spp.	19 (4.67)
Enterobacter spp.	41 (10.07)
Citrobacter spp.	28 (6.88)
Proteus spp.	21 (5.16)
Salmonella spp.	6 (1.47)
Staphylococcus aureus	26 (6.39)
Morganella morganii	7 (1.72)
Providencia spp.	6 (1.47)
Enterococcus spp.	6 (1.47)
Total	407 (100.00)

N= Total number of bacteria.
n = number of bacterial species.

Table 3: Distribution of Morganella morganii isolated from different culture-positive samples (N = 407)

Source of the samples	M. morganii n (%)
Urine (N=152)	3 (1.97)
Wound swab and pus (N=115)	2 (1.74)
Blood (N=54)	1 (1.85)
Stool (N=86)	1 (1.16)
Total	7 (1.72)

N = Total number of culture-positive samples.
n = number of isolated M. morganii from different culture-positive samples.

Table 4 demonstrates the distribution of isolated Morganella morganii among in-patient and out-patient departments. Among the 7 M. morganii isolates, 6 (85.71%) were from the in-patient department (from urine, wound swab/pus, blood samples) and one (14.29%) was from the out-patient department (from stool samples). Table 5 shows the distribution of isolated Morganella morganii according to gender among patients of different age groups. Five were isolated from male and 2 were from female patients. Four (57.14%) were from the age group 61-80 years and 3 (42.86%) were from age group 41-60 years. Table 6 shows the antimicrobial

resistance pattern of the isolated Morganella morganii. Among the 7 isolated M. morganii, four (57.14%) each was resistant to amikacin and imipenem and 5 (71.42 %) each were resistant to ceftriaxone, ceftazidime, cefepime, ciprofloxacin, amoxiclav, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, aztreonam, and cefoxitin.

Table 4: Distribution of isolated Morganella morganii among in-patient and out-patient departments (N=7)

Isolated M. morganii	n (%)
In-patient department	6 (85.71)
Out-patient department	1 (14.29)

Table 5: Distribution of isolated Morganella morganii according to gender among patients of different age groups

Age group (In year)	Male (N=5) n (%)	Female (N=2) n (%)	Total (N=7) n (%)
41-60	2 (40.00)	1 (50.00)	3 (42.86)
61-80	3 (60.00)	1 (50.00)	4 (57.14)

Table 6: Antibiotic resistance pattern of isolated Morganella morganii by disc diffusion method (N=7)

Antimicrobial drugs	Resistance, n (%)
Amoxiclav	5 (71.42)
Ceftriaxone	5 (71.42)
Aztreonam	5 (71.42)
Cefepime	5 (71.42)
Ceftazidime	5 (71.42)
Cefoxitin	5 (71.42)
Piperacillin-tazobactam	5 (71.42)
Ciprofloxacin	5 (71.42)
Amikacin	4 (57.14)
Imipenem	4 (57.14)
Trimethoprim-Sulfamethoxazole	5 (71.42)

N = Total number of isolated M. morganii.
n = Number of resistant bacteria.

Figure 1 demonstrates ESBL producers among the isolated Morganella morganii. Among 7 isolated M. morganii, 2 (28.57%) were ESBL producers and 5 (71.43%) were non-ESBL producers. Table 7 summarizes the distribution of ESBL-producing Morganella morganii in different samples identified by the DDS test. Among 2 ESBL-producing M. morganii, one (50%) was detected from urine samples, and one (50%) was detected from pus/wound swab samples. Table 8 summarizes the distribution of blaCTX-M and blaTEM, genes among phenotypically confirmed ESBL-producing Morganella morganii in different samples by PCR. Among the 2 ESBL producers, the blaCTX-M gene was present in one (50%) isolated wound swab/pus sample and the blaTEM gene was present in one (50%) isolated urine sample.

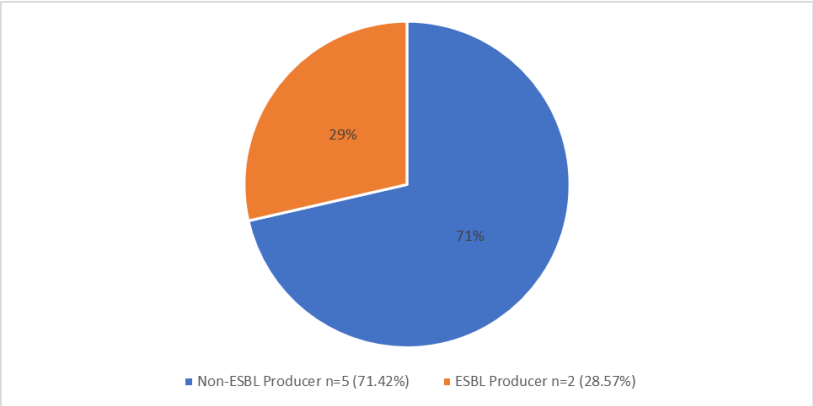

Figure 1: ESBL producers among the isolated Morganella morganii (N=7)

Table 7: Distribution of ESBL-producing Morganella morganii identified by DDS test (N=2)

Morganella morganii in different samples (N=2)

Samples	n (%)
Urine	1 (50.00)
Pus & wound swab	1 (50.00)

N=Total number of β-lactamase genes.
n=number of different β-lactamase genes.

Table 8: Distribution of blaCTX-M and blaTEM among phenotypically confirmed ESBL-producing

Gene	Pus and wound swab n (%)	Urine n (%)	Total n (%)
blaCTX-M	1 (50.00)	0 (00.00)	1 (50.00)
blaTEM	0	1 (50.00)	1 (50.00)

N=Total number of β-lactamase genes.
n=number of different β-lactamase genes.

DISCUSSION

Morganella morganii, a non-negligible opportunistic pathogen from the Enterobacteriaceae family, has recently been listed by the World Health Organization (WHO) as a global priority pathogen due to its alarming capacity to acquire drug-resistance genes, which leads to increased mortality rates [18]. Antibiotic resistance has emerged as a pressing global health issue over the past two decades, with resistance now affecting a substantial portion of bacteria responsible for hospital-acquired infections. As many as 70% of hospital-acquired infections are caused by bacteria resistant to at least one commonly used antibiotic, with many strains now exhibiting multidrug resistance (MDR) [19]. In Bangladesh, data on M. morganii is scarce, with only one study conducted on cattle in Dhaka [20]. The present study aims to highlight the significance of M. morganii as an emerging nosocomial pathogen, focusing on its MDR profile and evaluating potential antibiotic combinations as alternative treatments for this challenging pathogen. In the current study, 74% of the 550 samples yielded culture-positive results. Among these, Escherichia coli was the most commonly isolated organism (28.99%), followed by Pseudomonas spp. (17.94%), a result consistent with previous findings in Dhaka Medical College Hospital (DMCH) [21]. M. morganii showed an overall prevalence of 1.72% among the culture-positive samples, which aligns closely with findings from Taiwan (1.47%) and India (0.89%) [22, 23]. The relatively high prevalence in this study might be attributed to the frequent use of broad-spectrum cephalosporins in hospitalized patients at DMCH, to which M. morganii is often resistant due to the presence of chromosomal AmpC β-lactamase. This makes M. morganii more prone to cause nosocomial infections. Other studies reporting lower prevalence may stem from difficulties in identifying M. morganii in

routine laboratories due to its similarity to other species, such as Providencia spp. and Proteus spp., leading to a higher chance of misidentification. A study in Iraq reported a prevalence rate of 4%, which may be explained by their focus on high-risk patients, such as those with diabetes, trauma, catheterization, or elderly individuals [24]. The present study also found that 85.72% of M. morganii isolates came from the in-patient department, underscoring the hospital-acquired nature of most infections, a finding echoed by a study in China [25].

Urinary tract infections (UTIs) (42.85%) and wound infections (28.57%) were identified as the most common infections caused by M. morganii, which aligns with findings from China [26]. A study from Serbia reported a higher isolation rate from pus samples than urine, but this difference could be due to factors such as catheterization, which facilitates bacterial colonization in the urinary bladder, or the proximity to the perianal region, where M. morganii can colonize even in healthy individuals [4]. These factors may also explain the higher isolation rates from pus collected from lesions in the lower body. However, the current study did not record data on catheterization or pus collection sites. Male patients accounted for 71.42% of the M. morganii infections in this study, a finding consistent with studies from Taiwan, which reported a significant male predominance [27, 28]. The exact cause of this male predominance remains unclear, but it could be related to gender disparities in healthcare-seeking behaviors or increased trauma in males due to outdoor activities, resulting in more wound infections and hospitalizations. The antibiotic resistance patterns in this study revealed that M. morganii isolates exhibited maximum sensitivity to amikacin (42.85%) and imipenem (42.85%), which is consistent with findings from Iraq [10]. However, the study observed high resistance rates to cephalosporins, with 71.42% of isolates resistant to ceftriaxone, ceftazidime, cefepime, and cefoxitin.

This high resistance may be due to the presence of chromosomally mediated inducible β-lactamases, such as cephalosporinase and penicillinase, which lead to treatment failures despite initial susceptibility. Imipenem resistance was found in 57.14% of M. morganii isolates, with MICs ranging from 16 to 128 μg/ml. This is consistent with a recent study from DMCH, which reported 25% imipenem resistance in Proteus mirabilis isolates with similar MIC values [29]. A study from Nepal also reported similar MIC values for imipenem resistance [30]. The rising resistance to carbapenems may be attributed to the increased use of these drugs in response to high resistance rates in broad-spectrum cephalosporins and other β-lactam antibiotics. Amikacin resistance was found in 57.14% of M. morganii isolates, with MICs ranging from 256 to 2048 μg/ml. A study at DMCH reported 68.18% resistance to amikacin in Proteus mirabilis isolates, with similar MIC values [29]. In contrast, a study in Iraq reported only 17.6% resistance to amikacin [24]. The increasing resistance in this study may be due to DMCH being a tertiary care hospital, where critically ill patients, including those in the ICU and burn units, are at higher risk of developing nosocomial infections and transmitting multidrug-resistant pathogens. Ceftazidime resistance was observed in 57.14% of M. morganii isolates, with MIC values ranging from 64 to 512 μg/ml, similar to findings from Pakistan, where 60% of M. morganii isolates were resistant to ceftazidime [31]. In this study, 28.57% of M. morganii isolates were detected as ESBL producers by the DDS test. A study at DMCH detected 22.73% ESBL-producing Proteus mirabilis, a finding comparable to this study. A study at DMCH also found 38.46% ESBL-producing Citrobacter freundii, and a study in Pakistan reported 12% ESBL production in M. morganii [32-37]. The high prevalence of ESBL-producing strains in this study could be attributed to the indiscriminate and overuse of extended-spectrum antibiotics. The present study also detected the presence of ESBL encoding genes, including blaCTX-M and blaTEM, consistent with a study at DMCH, and findings from Iraq. However, no SHV ESBL gene was detected in the present study.In conclusion, this study highlights the emerging significance of M. morganii as a nosocomial pathogen in Bangladesh, with a notable prevalence of MDR strains. The findings emphasize the need for continued surveillance and the prudent use of antibiotics in hospital settings to mitigate the spread of resistant pathogens.

CONCLUSION

Morganella morganii has emerged as a medically important pathogen because of the increasing multidrug resistance by this organism. This organism was most commonly isolated from urine and wound swab samples.

DNA sequences from PCR product was 95% identical with DNA sequences of 16S ribosomal RNA gene available at gene bank and phylogenetic tree showed Morganella morganii isolated in this study was clustered with Morganella morganii strain LMG 7874. Morganella morganii is resistant to commonly used antibiotics in the present study with increased resistance to imipenem. Resistance to imipenem is increasing due to production of carbapenemase enzyme. Among the phenotypic tests for carbapenemase detection, CD assay was found to be most sensitive and specific. Present study observed that combination therapy might serve as a good option for the treatment of MDR Morganella morganii. Ceftazidime plus amikacin and imipenem plus amikacin were the most effective combination, both in vitro and in vivo.

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