Research Article

Antibiotic Resistance and Phenotypic and Genotypic Detection of Colistin Resistance among *Enterobacter Species* Isolated from Patients of a Tertiary Care Hospital, Bangladesh

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Abstract

The prevalence of antimicrobial resistance among *Enterobacter* species is alarmingly high. Colistin as a last resort antibiotic reintroduced in the treatment pipeline to combat the drug resistant *Enterobacter* infection has led to emergence of colistin resistance. The study was designed to determine the prevalence of antibiotic resistance among isolated *Enterobacter* and to assess the occurrence of colistin resistance both phenotypically and genotypically in a tertiary care hospital, Bangladesh.

This cross-sectional study was conducted during the period July2018 to June 2019. A total 350 different samples were processed. *Enterobacter* were identified phenotypically and were processed for antimicrobial susceptibility test (AST) by using modified disk diffusion method. Colistin resistance was determined by minimum inhibitory concentration (MIC) using agar dilution method. Colistin resistant strains were further processed for colistin resistance genes using polymerase chain rection (PCR).

Among the total samples processed, 65.14% (228/350) yielded culture positive growth of which 12.28% (28/228) were *Enterobacter* species. Among isolated *Enterobacter* 22 (9.64%) were *E. cloacae* and 6 (2.63%) were *E. aerogenes*. In AST, 89.29%, 57.14% and 42.86% were resistant to ciprofloxacin, amikacin and imipenem respectively. Tigecycline and colistin were most effective antibiotics. Among colistin resistant *Enterobacter* isolates, 85.71%, 71.42%, 42.85% and 57.14% were positive for *PmrC, PmrA, PhoP and PmrB* respectively detected by PCR.

Our study reports identification and distribution of drug resistant *Enterobacter* strains in a major tertiary care hospital. High prevalence of drug resistance and emergence of colistin resistance is indicative of deteriorating situation. Therefore, further extensive surveillance is mandatory.

Keywords: *Enterobacter*; Antimicrobial susceptibility; Colistin resistance; Polymerase chain reaction; Agar dilution method; *E.cloacae, E.aerogenes*

1. Introductıon

Enterobacter spp., including *Enterobacter cloacae* and *E. aerogenes*, are opportunistic pathogens that cause infections in the blood, respiratory, urinary, and gastrointestinal tracts [1] and are a member of the ESKAPE group of significant bacterial pathogens in humans [2]. *Enterobacter* is associated with hospital acquired infection that has been ranked as the third most frequent isolate following *Escherichia coli* and *Klebsiella* species [3].

These organisms seem to have innate resistance to older anti-microbial agents and have the propensity to rapidly develop resistance to newer anti-microbial agents [4]. Treatment of infections with *Enterobacter spp.* is difficult and broad resistance to third generation cephalosporins, penicillin and quinolones is an increasing problem [5]. Gram negative bacteria, mainly Enterobacterales, Acinetobacter baumannii and Pseudomonas aeruginosa are able to produce enzymes such as extended-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases, carbapenemase and metallo-beta-lactamases (MBL), which enable the host bacteria to develop resistance to most classes of antibiotics in use [6]. *Enterobacter spp*. is increasingly associated with multidrug resistance, including the resistance to the last-resort carbapenems [7]. *Enterobacter spp*. is second most common carbepenem resistant *Enterobacteriaceae* (CRE) in the United States [8]. The rapid increase of serious infections by resistant Enterobacter necessitates the availability of an alternative antimicrobial agent for treatment [9].

As multidrug resistance and carbapenem resistance increase in Gram-negative pathogens including Enterobacter spp., colistin, a polycationic antimicrobial peptide, is often recommended as a last-line therapeutic option [10]. Although colistin resistance rate is relatively low worldwide, resistance has begun to emerge [11]. In addition to the steady emergence of colistin resistance due to chromosomal mutations, plasmid-borne colistin resistance has recently been reported, which is a great threat to public health [12]. Implementation of effective surveillance programs and infection controls are considered as the two pillars to check the growth and spread of AMR [13]. Accurate and timely information on local and national patterns of organisms causing infection and their drug-

resistance profile is therefore paramount for good clinical management and benchmarking [14].

However, few studies have investigated on the colistin resistance of *Enterobacter spp*. in comparison with studies of other Gram-negative pathogens. Therefore, our study was designed to determine the prevalence of drug resistant strains of *Enterobacter* species from clinical isolates to recognize the possible therapeutic options for combating these ICU bugs. At the same time, we also report colistin resistant *Enterobacter cloacae* and *E. aerogenes* and presence of colistin resistant gene among them in Bangladesh.

2. Methods

2.1 Study design

This cross- sectional study was conducted from July 2018 to June 2019 in the department of Microbiology, Dhaka Medical College Hospital Bangladesh. Samples was collected from adult patients having clinically suspected infections admitting in Dhaka Medical College Hospital or were received in the Microbiology department for culture and sensitivity after taking informed written consent irrespective of sex and antibiotic intake. Patients who did not give consent were excluded from this study.

2.2 Isolation and Identification of *Enterobacter* Species

All the samples were aseptically collected following the standard microbiological procedure. A total 350 clinical sample consisting of urine (n=124), wound swab (n=113), endotracheal aspirates (n=58), and blood (n=53) was collected. Clinical samples were cultured on blood agar and MacConkey agar media. Isolates were identified phenotypically by standard microbiological techniques (Colonial morphology, Gram stain, and biochemical test. Genus *Enterobacter* was identified by characteristics colonies (Lactose-fermenting, sometimes mucoid colonies), Gram staining pattern as Gram negative bacilli, motility as motile, and standard biochemical reactions (catalase, oxidase, indole production, citrate utilization, urease activity, reaction in triple sugar iron medium and lysine decarboxylase test). Common *Enterobacter spp* (*E. cloacae* and *E. aerogenes*) were isolated [15].

2.3 Antimicrobial Susceptibility Test

Susceptibility to antimicrobial agents of all isolated organisms were determined by Kirby-Baurer modified disc diffusion technique using Mueller-Hinton plates and zones of inhibition were interpreted according to CLSI guidelines [16]. The criteria for the United States Food and Drug Administration was used for the interpretation of zone of inhibition of tigecycline. Antibiotic discs were obtained from commercial sources (Oxoid Ltd, UK). Following antimicrobial discs were used: amikacin (30µg), piperacillin-tazobactam (100/10µg), imipenem (10µg), tigecycline (15µg), ciprofloxacin (30µg), cefepime (30µg), ceftazidime (30µg), ceftriaxone (30µg), cefoxitin (30µg), cefuroxime sodium (30µg), amoxiclav (amoxicillin 20µg & clavulanic acid 10µg) and aztreonam (10µg). In this method, broth culture of test bacteria (comparable to McFarland tube no.0.5; inoculums density 1.5× 108 bacteria/ml) was uniformly carpeted on the surface of Mueller Hinton agar (MHA). Twelve, antibiotics discs were placed onto the lawn culture of the test bacteria by sterile forceps. The inoculated and seeded MHA plates were

incubated at 37 ℃ for 24 h. After incubation, zone of inhibition was measured and results were interpreted as sensitive, intermediate and resistant [17]. Colistin susceptibility was determined by agar dilution method.

2.3.1 Colistin Susceptibility Test

Agar dilution method was used to determine the susceptibility of colistin. As 0.5 McFarland turbidity standards contain 1×10^8 cfu/ml [16] 10 times dilution of test inoculums was done to achieve 1×10^7 cfu/ml. Two concentrations of colistin 2 µg/ml and 4µg/ml were prepared in the agar medium. Bacterial inoculums was applied readily onto the agar surface and the plates were incubated at 37 °C up to 24 h. Isolates having a MIC of≤2 µg/mL is considered colistin susceptible while MIC of>2 µg/mL is considered colistin resistant [18].

2.4 Phenotypic Detection of ESBLs Producers

Using sterile cotton swab, test inoculums (compared with McFarland standard) were inoculated in Mueller-Hinton agar plate. Third generation caphalosporins (ceftriaxone, ceftazidime and cefotaxime) were placed 20 mm apart from center of the amoxiclav disc. The inoculated plate was incubated at 37^0C for 24 hours. A clear extension of the edge of the inhibition zone of cephalosporin discs towards amoxiclav disc was interpreted at ESBLs production [19].

2.5 Phenotypic Detection of MBL Producers

Confrmation of MBL production was made by inhibition method in which Ethylene Diamine Tetra Acetic Acid (EDTA) was used as an inhibitor. Two imipenem discs were placed on the inoculated Mueller-Hinton agar plate. One imipenem disc was supplemented with 5 µl of 0.5 M EDTA solution (containing approximately 750 µg EDTA) and incubated overnight at 37^0C. An increased zone of diameter of ≥ 6 mm around the disc containing imipenem supplemented with EDTA compared to the disc containing imipenem only was interpreted as MBL production [20].

2.6 Determination of MIC of Colistin

Minimum inhibitory concentration of colistin was determined by agar dilution method. Different concentrations of colistin ranging from 2µg/ml to 256µg/ml were prepared in the agar medium. For each plate 50ml Mueller-Hinton media was prepared.50 ml of sterile Mueller-Hinton agar was impregnated with 12.5 µl, 25 µl, 50 µl, 100 µl, 200 µl, 400 µl, 800 µl, 1600 µl of colistin to achieve the concentration 2µg/ml, 4µg/ml, 8µg/ml, 16 µg/ml, 32µg/ml, 64µg/ml, 128 µg/ml and 256 µg/ml per plate respectively. Bacterial inoculum was applied readily onto the agar surface and the plates were incubated at 37 ℃ overnight. The MIC end point was determined as the lowest concentration of antibiotics that completely inhibits the visible growth [21]. *Escherichia coli* ATCC 25922 was used as control strain [16]. Commercially available colistin injection vial (Forest pharma Limited) was used

2.7 Molecular Method [22]

Polymerase chain reaction (PCR) was done for the detection of colistin resistance genes in colistin resistant *Enterobacter species*.

2.7.1 Procedure of Bacterial Pellet Formation and DNA Extraction

A loop full of bacterial colonies from MHA media was inoculated into a micro centrifuge tube having sterile TSB and incubated overnight at 37ºC. Incubated tube was centrifuged at 4000g for 10 minutes. Supernatant was discarded and tubes containing bacterial pellets were kept at -20ºC for DNA extraction. Three hundred microlitre of sterile distilled water was added to micro centrifuge tubes having pellets and vortexed until mixed well. Then the mixture was heated at 100° C for 10 minutes in a heat block. After heating, tubes were immediately placed on ice for 5 minutes and centrifuged at 14000 g for 6 minutes at 4ºC. Finally, the supernatant was taken into another micro centrifuge tube. This extracted DNA was preserved at 4ºC for 7-10 days and -20ºC for a long time.

2.7.2 Mixing of Master Mix with Primer and DNA Template

PCR was performed in a final reaction volume 25 µl in a PCR tube, containing 12.5 µl of master mix (mixture of dNTP, taq polymerase, $MgCl_2$ and PCR buffer), 2 µl forward primer, 2 µl reverse primer (Promega Corporation, USA, 2 µl of extracted DNA and 6.5 µl of nuclease free water. After a brief vortex, the tubes were centrifuged. The pair of primers were used to yield PCR products depicted in (Table 1).

Table I: Primers used in this study [23]

Genes	Sequence (5'-3')		Size (bp)
1.*PmrA*	F	CGC AGG ATA ATC TGT TCT CCA	808
	R	GGT CCA GGT TTC AGT TGC AA	
2.*PmrB*	F	GCG AAA AGA TTG GCA AAT CG	659
	R	GGA AAT GCT GGT GGT CAT CTG A	
3.*PmrC*	F	CTC TCG CCT CGT TCT GAA	140
	R	CGG AGT GGT GTC GAG GAT A	
4.*mgrB*	F	ACC ACC TCA AAG AGA AGG CGT T	347
	R	GGC GTG ATT TTG ACA CGA ACA C	
5.*PhoP*	F	GAG CGT CAG ACT ACT ATC GA	942
	R	GTT TTC CCA TCT CGC CAG CA	
6.*PhoQ*	F	CCA CAG GAC GTC ATC ACC A	1594
	R	GCA GGT GTC TGA CAG GGA TT	
7.*mcr1*	F	CGG TCA GTC CGT TTG TTC	309
	R	CTT GGT CGG TCT GTA GGG	
8.*mcr2*	F	TGTTGCTTGTGCCGATTGGA	567
	R	AGATGGTATTGTTGGTTGCTG	

2.7.3 Amplification in Thermal Cycler (Gene Atlas, Master cycler gradient, Japan, Model482)

PCR assays were performed in a DNA thermal cycler. After amplification products were processed for gel documentation or kept at -20ºC till tested.

2.7.4 Agarose gel Electrophoresis and Visualization

PCR products were detected by electrophoresis on 1.5% agarose gel. Gel was prepared with 1 X TBE buffer (Tris EDTA). For 1.5% agarose gel preparation, 0.18 gram agarose powder (LE, analytic grade, Promega, Madison, USA) was mixed with a 1.25 ml TBE buffer. A comb was placed in a gel tray, the gel was poured. After solidification, 1 µl of loading dye and 5 µl of amplicon was mixed on parafilm and was loaded in agarose well. Similarly, 2 µl of 100bp DNA ladder was mixed with 1µl loading dye and was loaded. Gel electrophoresis was done in 230 voltages for 30 minutes. After electrophoresis, the gel was stained with ethidium bromide (20µl ethidium bromide in 200 ml distilled water). The gel was observed under UV transilluminator (Gel Doc, Major Science, Taiwan) for DNA bands. The DNA bands were identified according to their molecular size by comparing with the molecular weight marker (100bp DNA ladder) loaded in a separated lane.

3. Result

Among the total samples processed, 65.14% (228/350) had bacterial growth. Out of 228 culture positive samples, 28 (12.28%) were *Enterobacter species* of which 22(9.64%) were E. cloacae and 6 (2.63) were E. aerogenes. Among 61 urine samples 11(18.03) were Enterobacter species, among 93 wound swab and pus 9(9.68%) were *Enterobacter species* (Table II).

Table II: Distribution of isolated *Enterobacter cloacae* and *Enterobacter aerogenes* from different culture positive samples (N=228)

Samples	***E.cloacae***	***E.aerogenes***
Urine (N=61)	8 (13.11)	3 (4.91)
Wound swab and pus (N=93)	7 (7.53)	2 (2.15)
Endotracheal aspirates (N=53)	6 (11.32)	1 (1.07)
Blood (N=21)	1 (4.16)	0 (0.00)
Total (N=228)	22 (9.64)	6 (2.63)

Among the 28 isolated *E. cloacae* and *E. aerogenes*, all were resistant to amoxiclav and cefoxitin, 89.29% were resistant to ciprofloxacin and cefuroxime, 57.14% were resistant to amikacin, 50% were resistant to piperacillin-tazobactam, 42.86% were resistant to imipenem, 25% were resistant colistin and 3.57% were resistant tigecycline (Table III).

Table III: Antibiotic resistance pattern of isolated *E. cloacae* and *E. aerogenes* (N=28)

Antimicrobial drugs	***E. cloacae*** **N=22** **n(%)**	***E. aerogenes*** **N=6** **n(%)**	**Total** **N=28** **n(%)**
Amoxiclav	22 (100.00)	6 (100.00)	28 (100.00)
Cefoxitin	22 (100.00)	6 (100.00)	28 (100.00)

Ciprofloxacin	21 (95.45)	4 (66.67)	25 (89.29)
Cefuroxime	22 (100.00)	3 (50.00)	25 (89.29)
Ceftriaxone	21 (95.45)	3 (50.00)	24 (85.71)
Ceftazidime	19 (86.36)	3 (50.00)	22 (78.57)
Aztreonam	15 (53.57)	2 (33.33)	17 (60.71)
Amikacin	13 (59.09)	3 (50.00)	16 (57.14)
Imipenem	11 (50.00)	1 (16.67)	12 (42.86)
Piperacilin-tazobactam	11 (50.00)	3(50.00)	14 (50.00)
Cefepime	7 (31.82)	2 (33.33)	9 (32.14)
*****Colistin	6 (27.27)	1 (16.67)	7 (25.00)
Aztreonam	15 (53.57)	2 (33.33)	17 (60.71)
Tigecycline	1 (4.54)	0 (0.00)	1 (3.57)

N= Total number of isolated bacteria

n =Total number of resistant bacteria

*****Colistin resistance was determined by MIC by agar dilution method.

Out of 28 isolated *E. cloacae* and *E. aerogenes*, 15 multidrug resistant strains were detected. Of which, 5 (45.45%) were detected from urine, 5 (55.55%) from wound swab, 4 (57.14%) from endotracheal aspirates, one (100%) from blood sample (Table IV).

Table IV: Distribution of multidrug resistant (MDR) *E. cloacae* and *E.aerogenes* isolated from different samples

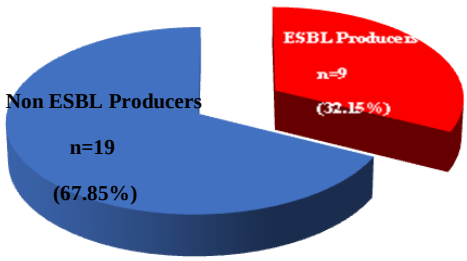
Sample	Total isolates N=28	MDR isolates n =15 (%)
Urine	11	5 (45.55)
Wound swab and pus	9	5 (55.55)
ETA	7	4 (57.14)
Blood	1	1 (100.00)
Total	28	15 (53.57)

ETA= Endotracheal aspirate

N= Total number of isolated *E. cloacae* and *E. aerogenes*

n = Total number of MDR isolates.

Among isolated Enterobacter cloacae and E. aerogenes 32.15% were ESBLs producer detected by double disc synergy test (Figure 1).



N= Total number of *E.cloacae* and *E.aerogenes.*

n= Number of ESBL producers & non ESBL producers.

Figure 1: ESBL producers among the isolated *E.cloacae* & *E.aerogenes* (N=28)

Among 12 imipenem resistant *Enterobacter spp*. 9 (75) carbapenemase producers were detected by CD assay (Table V)

Table V: Phenotypic detection of carbapenemase producers by combined disc method among imipenem resistant *E. cloacae* and *E. aerogenes* (N=12)

Organisms	**Carbapenemase producers** CD assay n (%)
E. cloacae	8 (66.67)
E. aerogenes	1 (8.33)
Total	9 (75.00)

N= Total number of imipenem resistant bacteria.

n = Number of carbapenemase producers by phenotypic test

Among the colistin resistant *Enterobacter spp.*, 6 (85.71%) were *E.cloacae* and 1 (14.28%) were *E.aerogenes* isolated from different clinical samples (Figure 2)

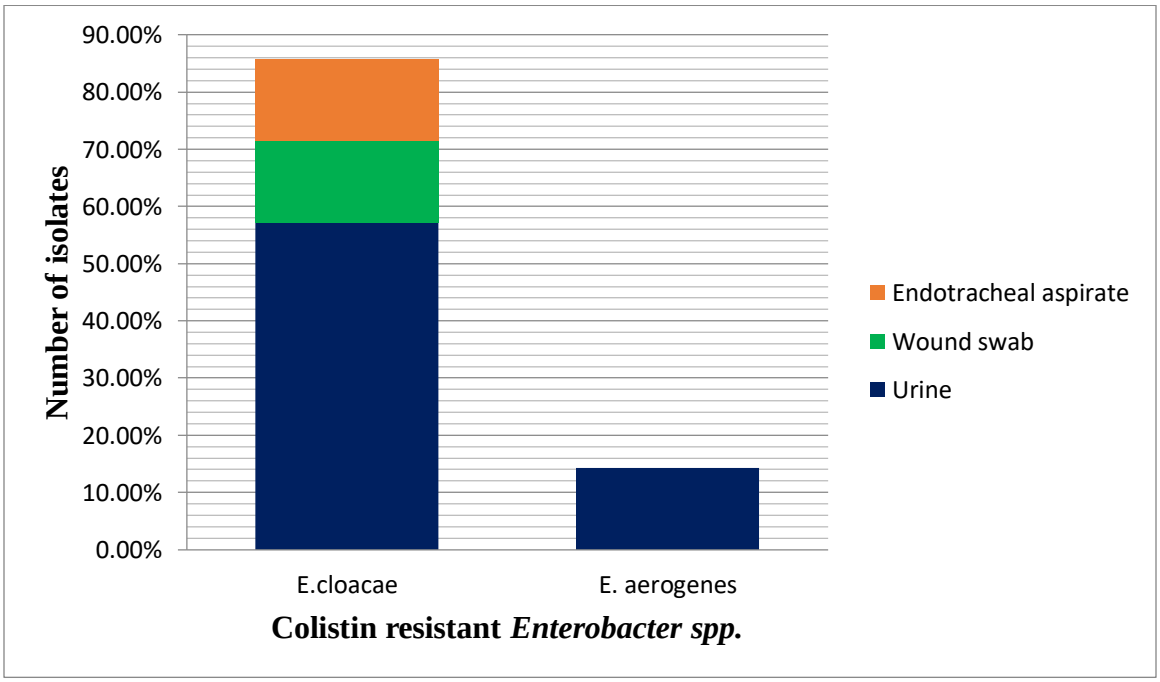


Figure 2: Colistin resistant *Enterobacter spp.* Isolated from different samples. (N=7)

Among 7 colistin resistant *Enterobacter Species* (6, *E. cloacae* and one, *E. aerogenes*), 2 (28.57) had MIC 256 µg/ml, 3 (14.29) had MIC 128 µg/ml (Figure 3).

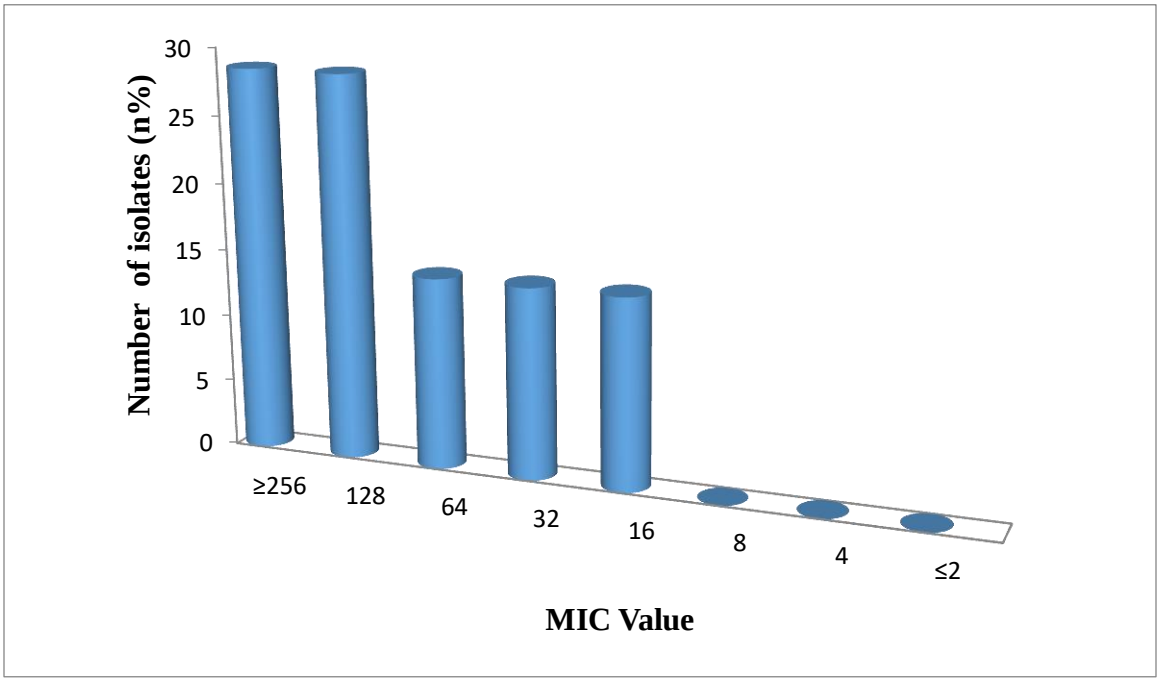


Figure 3: MIC of colistin among colistin resistant *Enterobacter* species (N=7)

Among 7 colistin resistant isolates, 3 (42.85) were positive for *phoP*, 5 (71.42) were positive for *pmrA*, 4 (57.14) were positive for *pmrB*, 6 (85.71) were positive for *pmrC* genes detected by PCR. No *mgrB*, *mcr 1* and *mcr 2* genes were detected. Multiple gene were detected from urine sample. None of these gene were detected from blood sample (Table VI).

Table VI: Detection of *phoP, phoQ, mgrB, pmrA, pmrB, pmrC, mcr-1* and *mcr-2* genes from different isolates among colistin resistant *E. cloacae* and *E. aerogenes* by PCR (N=7)

Genes	**Urine n(%)**	**Wound swab n(%)**	**ETA n(%)**	**Total n(%)**
phoP	3 (42.85)	0 (0.00)	0 (0.00)	3 (42.85)
phoQ	1 (14.29)	1 (14.29)	0 (0.00)	2 (28.57)
pmrA	4 (57.14)	1 (14.29)	0 (0.00)	5 (71.42)
pmrB	2 (28.57)	1 (14.29)	1 (14.29)	4 (57.14)
pmrC	4 (57.14)	1 (14.29)	1 (14.29)	6 (85.71)
mgrB	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
mcr 1	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
mcr 2	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

N= Total number of colistin resistant *E. cloacae* and *E. aerogenes*

n = Number of colistin resistant gene in different samples.

ETA= Endotracheal aspirates.

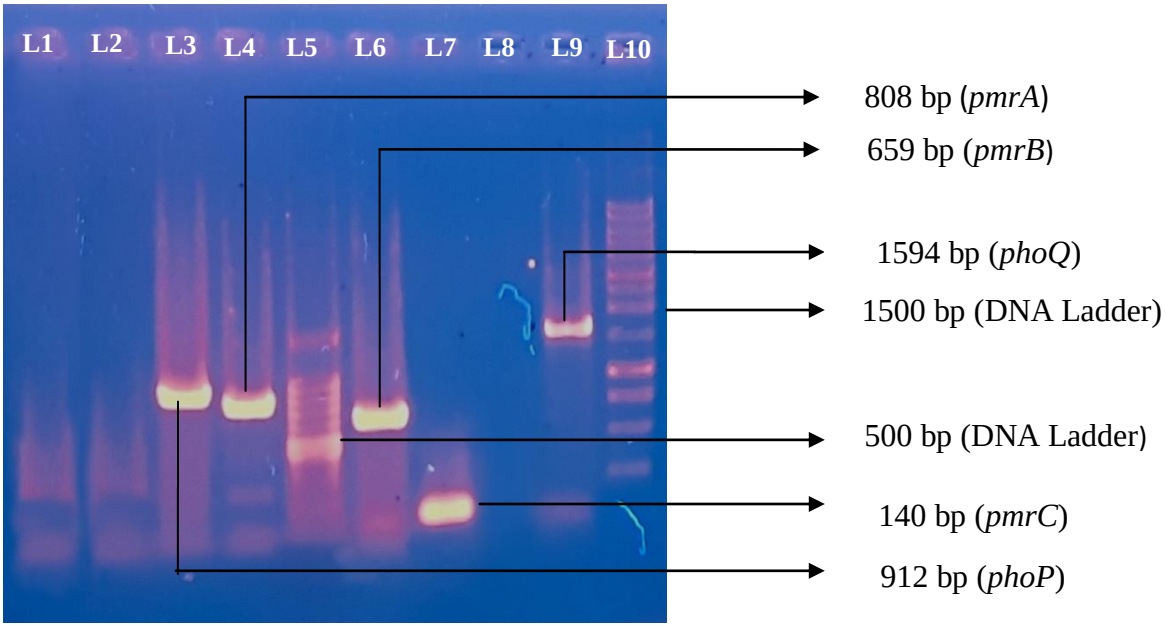


Figure 4: Photograph of gel electrophoresis of amplified DNA of 912bp for *phoP*, Gene (lane 3), amplified DNA of 808bp for *pmrA* (lane 4), 100 bp DNA ladder (lane 5), amplified DNA of 659 bp *pmrB* (lane 6), amplified DNA of 140 bp for *pmrC* (lane 7), amplified DNA of 1594 bp *phoQ* (lane 9). One kbp DNA ladder (lane 9), negative control without DNA (TE buffer) (lane one), negative control *Escherichia coli* ATCC 25922 (lane 2), blank (lane 8).

4. Discussion

Enterobacter has been identified as an important pathogen causing many types of hospital-acquired infections (HAIs) [24]. The emergence of *Enterobacter* as a worrying resistant pathogen is an important health concern, especially when the scarcity of new antibiotics active against Gram-negative bacteria is considered .As surveillance of AMR and early response to the infection control are crucial steps to bypass the issues, this study aimed to determine the antimicrobial resistance pattern of *Enterobacter species* isolated from different clinical specimens and also to find out colistin resistant *Enterobacter spp*. along with resistance gene in Bangladesh.

Out of 350 samples, 228 (65.14 %) samples were culture positive of which 28 (12.28%) were *Enterobacter species*. These finding are in agreement with the recent study [25] in DMCH who reported that 63.20% of samples (urine, wound swab, ETA, blood, sputum, pus) were culture positive. A study [26] in India reported that a National Nosocomial Infection Surveillance System (NNIS) showed that *Enterobacter* accounts for 5 to 11% of all nosocomially acquired blood, wound, respiratory tract infection and urinary tract infection. These findings are nearly close to the present finding. In the present study, among 28 isolated *Enterobacter* species, 22 (78.57%) were identified as *Enterobacter cloacae* and 6 (21.43%) were identified as *Enterobacter aerogenes* by biochemical tests. These findings were similar to a study [26] in India who reported that 77.94% were *E. cloacae* and 22.05% were *E. aerogenes*. This similarity may be attributed to the fact that these two studies were conducted in same geographic area.

In the present study, thirteen commonly used antibiotics were used on isolated *Enterobacter spp*, to observe the antimicrobial resistance pattern by disc diffusion method. Among the antibiotics used, *Enterobacter spp.* was 100%

resistant to cefoxitin and amoxiclav. These finding can be correlated with a study [27] who reported that *Enterobacter* species are intrinsic resistance to aminopenicillins, amoxicillin/ clavulanic acid, cefoxitin and first generation cephalosporins because of the production of chromosomally encoded, inducible AmpC β-lactamase. In the present study, ceftriaxone and ceftazidime had resistance rate 85.71% and 78.57% a study in palestine [28] reported 68.3% resistance to ceftriaxone and 63.14% to ceftazidime which are lower than the present study. CLSI states that, *Enterobacter* may develop resistance during prolong therapy by third generation cephalosporins as a result of derepression of AmpC β-lactamase. Therefore, isolates that are initially susceptible may become resistant within 3 to 4 days after initiation of therapy [17], which might be true for higher proportion of resistance for *Enterobacter spp.* to cefuroxime in the present study. In the present study, 57.17% *Enterobacter spp*. were resistant to amikacin. In contrast to present findings a study in Iran [29] reported 48.60% *Enterobacter* resistant to amikacin in a study in Iran which is lower than the present findings. The reason behind the higher resistance rate in the present study might be due to the fact that, there was a significant effect of selection pressure of primary antibiotic in the following cases: Amikacin in *E. cloacae* and *E. coli*, piperacillin/tazobactam in *K.pneumoniae* [30]. Among the isolated *Enterobacter spp.* 32.14% were detected as ESBL producers. A study in India [31], reported the prevalence of ESBL were 33.33% which are in agreement with the present findings. The prevalence of ESBLs among the *Enterobacter spp*. in Pakistan from different hospital ranged from 14.93% -79% [32]. In case of other members of *Enterobacteriacae* ESBL production rate has decreased. The prevalence of the ESBL producers had been decreasing over the last few years. Exact reason of such reduction of ESBL is not clear but it can be explained by the fact that the use of ampicillin and cephalosporin has been decreased due to resistant strains and the use of carbapenems and polymyxin has been increased by the physicians which might have some role in decreasing ESBL production. Moreover, the prevalence of ESBL producer varies with time as well as from country to country and even hospital to hospital [33].

In the present study, imipenem resistance in *Enterobacter spp.* was 42.86%. Imipenem resistance among the species varies widely in different parts of the world. A study [34] in India reported 53.8% imipenem resistance where as a study [28] from Palestine showed resistance rates as low as 12.2%. The frequency of imipenem resistance *Enterobacter spp.* is increasing in Bangladesh which is reflected by these studies. Risk factors for the acquisition of carbapenem resistant *Enterobacteriaceae* in a tertiary care hospital may include, prior exposure to antimicrobials, long term hospital stay especially in ICU, high dependency units, renal, hematology, oncology unit, presence of indwelling devices such as central line, urinary catheter, endotracheal tube and enteral feeding tube, co-morbid or immune compromised patients [35]. In the present study, among 12 imipenem resistant *Enterobacter spp.* 75% carbapenemase producers were detected by CD assay. In a previous study [36] in Dhaka Medical College hospital, 71.43% carbapenamase producing *Enterobacter spp.* was detected by CD assay which is close with the present findings.

Among the isolated *Enterobacter spp*. 7 (25%) colistin resistant *Enterobacter spp* were identified. A study in Korea [37] reported that 16% of *Enterobacter spp* were resistant to colistin. Another study [38] reported 13.9%- 20.1% colistin resistances in *Enterobacter spp*. However, colistin resistance is rare upon first isolation but often arises

during treatment via mutations [39]. Now a day, the use of colistin has increased due to increased prevalence of CRE which might be the reason for emergence of resistance of this reserve drug. In the present study, 15 (53.57%) multidrug resistant *Enterobacter spp.* were isolated from different samples (urine, wound swab, ETA, blood). A study [40] in Nepal reported that 52.90% *Enterobacter spp.* were MDR.

Among colistin resistant *Enterobacter species*, 6 (85.71%) *E.* cloacae and 1 (14.28%) E. *aerogenes* were isolated from different clinical samples. A study in Korea[37] reported among colistin resistant *Enterobacter* 23.9% were *E. cloacae* and 4.2% were *E.aerogenes* which is similar to our study. MIC range of colistin among colistin resistant *Enterobacter species* were 16µg/ml- ≥ 256µg/ml. This result suggests that colistin resistance in *Enterobacter* might be associated with chromosomal mutations in mgrB, phoP/phoQ, pmrA, pmrB, pmrC and crrABC which also described in *K. pneumoniae* [41]. High MIC may also be due to strong selective pressure in the isolates [42].

Among the colistin resistant *Enterobacter* species, 85.71% were positive for *pmrC*, 71.42% were positive for *pmrA*, 57.14% were positive for *pmrB*, 47.61% were positive for *phoP*. In the present study, *mgrB*, *mcr-1* and *mcr-2* genes were also searched but among colistin resistant *Enterobacter spp*. none of the isolates was positive for any of the genes. In *Enterobacter spp.* mutations of the pmrAB and phoPQ genes are mainly involved in colistin hetero resistance [43]. To best our knowledge no available data was found to compare the prevalence of colistin resistance genes among colistin resistant *Enterobacter spp*.

5. Conclusion

Our study result shows *Enterobacter species* seem to be emerged increasing resistance to multiple antibiotics may be due to the pervasive and irrational practices of antibiotic prescription and use. In our study we have also identified a high colistin resistance rate in *E. cloacae* but not in *E. aerogenes.* Therefore, prompt action is recommended for proper infection control. More emphasis should be given on routine AMR surveillance and antibiotic stewardship programs for the better management of patients.

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8. Conflict of Interest

There are no conflicts of interest in this study

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