Korean Journal of Microbiology (2022) Vol. 58, No. 3, pp. 150-156
DOI https://doi.org/10.7845/kjm.2022.2040

pISSN 0440-2413
eISSN 2383-9902

Prevalence of multidrug-resistant (MDR) *Escherichia coli* in untreated effluents from a wastewater treatment plant (WWTP) in Dhaka, Bangladesh[§]

Md Naeem Hossain[1]*, Asim kumar Roy[1], Humayra Habib[1], Zenat Zebin Hossain[1,2], Humaira Akhter[1], and Anowara Begum[1]

[1]*Department of Microbiology, University of Dhaka, Dhaka 1000, Bangladesh*
[2]*Department of Public Health, School of Pharmacy and Public Health, Independent University, Dhaka 1229, Bangladesh*

(Received June 28, 2022; Revised August 27, 2022; Accepted August 27, 2022)

The widespread use of antibiotics in human and animal husbandry results in the potential release of antibiotics into surrounding environments and leads to the emergence of antibiotic-resistant bacteria and antibiotic resistance genes. With this concern, we aimed to study the molecular mechanisms associated with multidrug resistance (MDR) and biofilm formation capacity in *Escherichia coli* isolates from Dhaka City's wastewater treatment plant. A total of 37 antimicrobial-resistant *E. coli* isolates were analyzed for the presence of genes associated with antibiotic resistance and biofilm formation using several phenotypic and polymerase chain reaction (PCR) assays and curing plasmids through the SDS method. Among the 37 isolates, most prevalent gene detected was *qnr*S (32.43%), followed by *bla*TEM (29.73%), *bla*CTX-M-15 (24.32%), *dfr*A17 (21.62%), *sul*2 (8.1%), *qnr*B (5.41%), *sul*1 (5.41%), and *dfr*A1 (2.70%). Twelve *E. coli* isolates (32.43%) possessed only class 1 integrons. A comparison of results prior to and after plasmid curing revealed most *E. coli* had plasmid-mediated antibiotic resistance. Among the 37 isolates, nine strains showed weak biofilm production and one showed strong biofilm production. All of the isolates were curli producers; *fim*H and *csg*A genes were present in 72.9% and 64.9% of the isolates, respectively. The dynamic antibiotic resistance diversity revealed in this study may pose concern regarding the potential development of drug-resistant bacteria and their dissemination into the surrounding environment via WWTPs.

Keywords: antibiotic-resistant bacteria (ARBs), antibiotic resistance genes (ARGs), extended spectrum β-lactamase (ESBL)

***For correspondence.** E-mail: nhossain995@gmail.com; Tel.: +880-1937565285
[§]Supplemental material for this article may be found at http://www.kjom.org/main.html.

Wastewater treatment plants (WWTPs) are an ideal place to monitor and investigate the development of antibiotic resistance and its dissemination into the environment and thus the biotic ecosystem (Hendriksen *et al.*, 2019). Wastewater treatment plants are also recognized as "hotspots" for spreading antibiotic resistance traits among bacteria through horizontal gene transfer and selective pressure by the residual antibiotics (Kim *et al.*, 2018).

Antibiotic-resistant bacteria are never completely eliminated from sewage via treatment processes at the WWTPs. Thus, it will be released into both the aquatic and terrestrial surrounding environments. In the end, through the food chain, ARBs will enter the human system (Kumar and Pal, 2018). Of greater concern is that the emergence of antibiotic resistance genes (ARGs) and ARBs gradually reduces the therapeutic potential of antibiotics, since a large quantity of antibiotics are released into the environment (Fair and Tor, 2014).

As a Southeast Asian developing country, Bangladesh poses a regional and global threat with a high prevalence of ARBs and ARGs (Ahmed *et al.*, 2019). Several studies have shown that indiscriminate and irrational use of antibiotics in health care, farming and agriculture leads to therapeutic failure in this country (Chowdhury *et al.*, 2021; Hosain *et al.*, 2021). As a consequence, multidrug-resistant (MDR) pathogenic bacteria will continue to emerge in the future, posing a potential risk for combating infectious diseases in developing countries like Bangladesh (Rabbani *et al.*, 2017). Unfortunately, no national antimicrobial resistance (AMR) data are currently available to support this finding, according to the World Health Organization

(WHO, 2014). Currently, treatment failure due to drug-resistant infections results in 0.7 million deaths per year, worldwide, and it is anticipated that almost 10 million drug-resistance related deaths will occur globally per year in 2050 (Dadgostar, 2019).

Multidrug-resistant *Escherichia coli* has been categorized as a priority pathogen by WHO due to emerging AMR (Vaiyapuri *et al.*, 2021). In addition, *E. coli* is the biggest culprit in enteric infections, with a total of 50,800 children dying from diarrhea in Bangladesh annually (Talukdar *et al.*, 2013).

The data on the prevalence of MDR *E. coli* and their biofilm-forming abilities in urban WWTP effluents, especially in Dhaka, Bangladesh, is scanty. Therefore, this study is an effort to illustrate the background of the AMR patterns and biofilm formation capacity of *E. coli* recovered from WWTP effluents in Dhaka, Bangladesh. *Escherichia coli*, as fecal indicator bacteria, were screened for antibiotic resistance and the plausible public health concern linked to the transfer of ARGs including integrons and plasmids, since WWTPs are known to be an early warning system.

In this study, we characterize *E. coli* according to their antibiotic resistance profile, plasmid profile, and biofilm formation capacity using various phenotypic methods and gene detection via PCR techniques.

Materials and Methods

Sample collection

Every month from June 2017 to May 2018 (except January and April 2018), 10 samplings of two-liter representative wastewater grab samples were retrieved from Dhaka City's WWTP (Pagla WWTP) (N23°41.004"/ E90°27.080" with 7 m elevation). The wastewater samples were collected from the main sewage flow after the first filtering step before the WWTP inlets, without effluent processing. After collection, each sample was transferred to the lab within 1 h, stored at 4°C and processed the sample within the day (Hossain *et al.*, 2022).

Isolation and identification of *E. coli* strains: each sample was studied to detect thermotolerant *E. coli* by streaking 10 µl of wastewater onto m-TEC (Oxoid) agar plates and incubating them overnight at 45°C. The presumptive colonies were selected for further identification using colony morphology and characteristics. A total of 37 presumptive *E. coli* isolates were identified on the basis of species-specific *uid*A genes (Supplementary data Table S1).

Antimicrobial susceptibility pattern for different antibiotic groups: thirty-seven selected *E. coli* isolates were tested for antimicrobial susceptibility by disk diffusion using the standardized Kirby Bauer method (Bauer *et al.*, 1966) and commercially available disks (Oxoid). The isolates were tested against the following 15 antimicrobials which covered eight different classes (penicillin [β-lactam], cephalosporin [extended spectrum β-lactam], quinolone, tetracyclines, folate pathway inhibitor, aminoglycoside, chloramphenicol, macrolides): ampicillin (AMP/10 µg), amoxicillin (AML/10 µg), nalidixic acid (NA/30 µg), ciprofloxacin (CIP/5 µg), tetracycline (TE/30 µg), trimethoprim/sulfamethoxazole (SXT/25 µg), gentamicin (CN/l0 µg), kanamycin (K/30 µg), streptomycin (S/10 µg), chloramphenicol (C/30 µg), erythromycin (E/15 µg), ceftriaxone (CRO/30 µg), cefixime (CFM/5 µg), cefotaxime (CTX/30 µg), and cefuroxime (CXM/30 µg).

Extended spectrum β-lactamase production detection method: double disk synergy tests (DDSTs) were performed using ceftazidime (CAZ/30 µg), cefotaxime (CTX/30 µg), ceftriaxone (CRO/30 µg) and amoxicillin/clavulanic acid (AUG/30 µg) disks to detect the ability of 37 *E. coli* isolates to produce ESBL (Jarlier *et al.*, 1988).

Detection of antibiotic resistance gene(s) by the molecular method: genomic DNA was extracted from 37 isolates using the boiled template method (De Medici *et al.*, 2003). These isolates were characterized through the PCR method using a thermal cycler (MJ Research PTC-200). Primer names and sequences, resistance genes, primer position, annealing temperature, amplicon sizes, and references are listed in Supplementary data Table S1.

Plasmid profiling

Isolation of plasmid DNA and plasmid curing: for plasmid extraction, the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific Inc.) was used for the 37 *E. coli* isolates. Following extraction, these plasmid harboring isolates were subjected to curing by using 10% SDS (sodium dodecyl sulphate) adopted the method developed by Zaman *et al.* (2010).

Antimicrobial susceptibility of post-plasmid curing isolates:

the Kirby Bauer disk diffusion method was used for antibiotic susceptibility of post-plasmid curated isolates. The pre- and post-plasmid curing antimicrobial susceptibility pattern was tested against 15 antibiotics from eight different groups, as listed above. The resulting zones of inhibition were compared to the initial zones of inhibition for the plasmid-harboring *E. coli*.

Statistical analysis

For statistical analysis, IBM SPSS Statistics version 22 software was used, with prime data divided into two categories: pre- and post-plasmid curing. Then, essential recoding was done accordingly. The Chi-square test was used to determine the statistical significance of plasmids in antibiotic resistance.

Determination of biofilm formation ability

Detection of cellulose and curli fimbriae production: cellulose and curli fimbriae production was detected in 37 *E. coli* isolates cultured on Luria-Bertani (LB) agar plates supplemented with 40 μg/ml Congo red and 20 μg/ml Brilliant Blue R250 without salt, and incubated for 24 h at 37°C and 48 h at 28°C. After incubation, the morphotypes were determined and classified. Three independent tests of each isolate for cellulose and curli production were performed (Römling *et al.*, 2000).

Quantification of biofilm by crystal violet staining assay: the method developed by Christensen *et al*. (1985) was used for staining. The extent of biofilm formation was interpreted based on the method established by Stepanović *et al*. (2000), and was classified into three categories: 1) non-biofilm producer: OD ≤ ODc, 2) weak biofilm producer: ODc < OD ≤ 2x ODc, and 3) strong biofilm producer: 4x ODc < OD, based on the mean value of the measured OD595nm.

Biofilm formation in the liquid-air interface: biofilm formation in liquid (i.e., pellicle formation at the liquid-air interface) was observed by using 4.5 ml of LB inoculated with 0.5 ml of freshly grown culture and incubated at 200 rpm and 37°C for seven days.

Detection of biofilm-associated genes: biofilm-associated genes including *csg*A, *pap*C, and *fim*H were detected by PCR techniques, as described above.

Results

Multidrug resistance profile of *E. coli*

All 37 strains of *E. coli* were tested against 15 antibiotic agents from 8 different antibiotic classes. Results showed that the highest resistance was to erythromycin, at 99% (n = 36), followed by 91.9% (n = 34) to cefotaxime and amoxicillin, 89.2% (n = 33) to streptomycin, 75.7% (n = 28) to ampicillin, 67.6% (n = 25) to cefuroxime, 64.9% (n = 24) to ceftriaxone, 62.2% (n = 23) to cefixime, 59.5% (n = 22) to kanamycin, 54.1% (n = 20) to ciprofloxacin, 51.4% (n = 19) to nalidixic acid and tetracycline, 40.5% (n = 15) to trimethoprim/sulfamethoxazole, 29.7% (n = 11) to gentamicin, and 21.6% (n = 8) to chloramphenicol (Fig. 1).

Detection of antibiotic resistance markers (ARMs) and ESBL production

Thirty-six isolates of MDR *E. coli* were found (only one isolate resistant to a single antibiotic was found), among which ESBL producers were identified by phenotypic methods. Therefore, certain ARG markers were detected to correlate the phenotypic data with the genotypic inheritance of ARMs (Supplementary data Table S2).

Through the phenotypic DDST method, 13 of the 37 isolates were found to be ESBL-positive. The presence of ESBL genes was detected using the gene-specific PCR method in those positive isolates. The PCR assay revealed that all but one of the ESBL-positive isolates contained one or more ESBL producing genes (*bla*TEM and *bla*CTX-M-15); however, the *bla*SHV gene was not found. The results showed that 46% (n = 6) of the ESBL-positive isolates contained the *bla*TEM gene, whereas

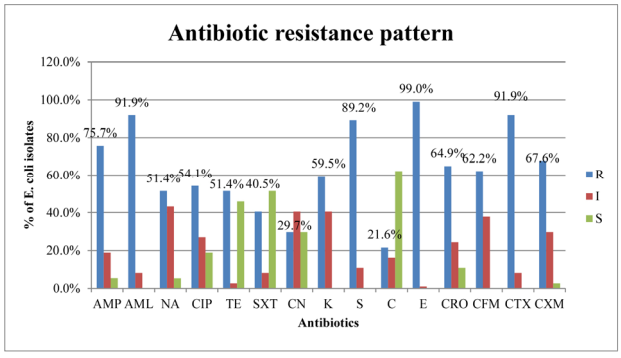

Fig. 1. Antibiotic resistance profile of *E. coli*.

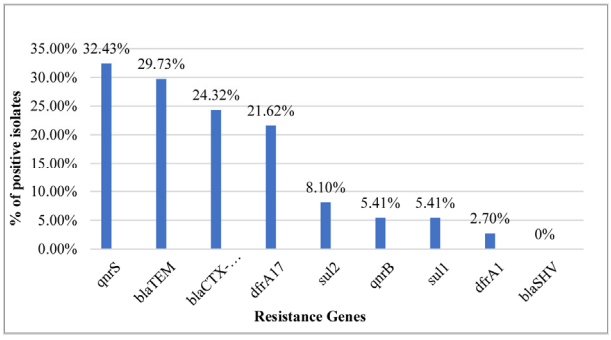

Fig. 2. Resistance gene(s) detection in *E. coli*.

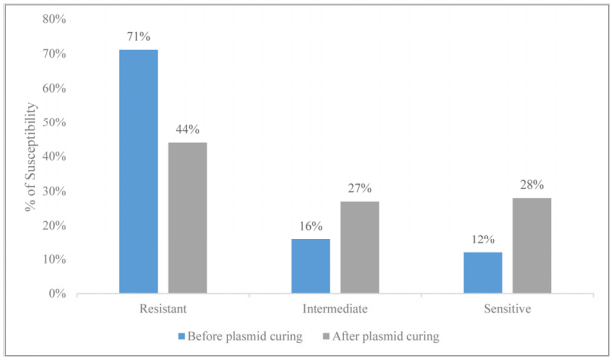

Fig. 3. Antibiotic susceptibility of *E. coli* before and after plasmid curing.

the *bla*CTX-M-15 gene was present in 70% (n = 9) of the isolates. Of 37 isolates analyzed, 29% (n = 11) were positive for *bla*TEM and 24% (n = 9) were positive for *bla*CTX-M-15 genes.

Of the *E. coli* strains which showed resistance to quinolone antibiotics, two were positive for the *qnr*B gene and 12 were positive for the *qnr*S gene. Results for trimethoprim/sulfamethoxazole (SXT)-specific resistance genes showed that one and eight isolates were positive for the *dfr*A1 and *dfr*A17 genes, respectively, and two and three isolates were positive for the *sul*1and *sul*2 genes, respectively.

Among the 37 isolates, the most prevalent gene detected was *qnr*S (32.43%), followed by *bla*TEM (29.73%), *bla*CTX-M-15 (24.32%), *dfr*A17 (21.62%), *sul*2 (8.10%), *qnr*B (5.41%), *sul*1 (5.41%), *dfr*A1 (2.70%) and *bla*SHV (0.00%) (Fig. 2). Among 37 isolates, 13 (35.1%) were ESBL positive by DDST.

Detection of integron classes

The PCR results showed that, out of 37 isolates, 12 harbored Class 1 integron resistance genes but none of them carried Class 2 and 3 integron resistance genes. Among the twelve class 1 integron positive *E. coli*, seven were ESBL-positive isolates.

Plasmid profile analysis

This analysis revealed that 13 of the isolates (35%) carried plasmids with less than 10 kb in size. Plasmid profile analysis showed that *E. coli* isolates harbored different size plasmids ranging from > 1 kb to 8 kb. Of these, two isolates (N6EC1 and N7EC2) showed double bands and the rest showed a single band.

Multidrug resistance profile of the plasmid–containing *E. coli* isolates

Thirteen plasmid-carrying *E. coli* isolates were found to be resistant to most of the antibiotics tested. All 13 isolates were completely resistant to AMP, AML, E, and third generation cephalosporins: CTX.

Antibiotic susceptibility of the cured *E. coli* isolates

A significant reduction in resistance, from 71.2% to 44.1%, was also found (from 139 resistant isolates before curing to 86 after curing). For AMP, AML, and E, all isolates remained resistant after plasmid curing. Furthermore, the isolates which were initially sensitive remained sensitive (Fig. 3).

A contrasting scenario was encountered for isolate N4EC4, which was initially moderately resistant and sensitive to NA and C, respectively, but became resistant after plasmid curing. Plasmid bands were found in isolates N6EC10 and N9EC1 after curing; however, each of them lost resistance to four different antibiotics (Supplementary data Table S3).

Statistical analysis

Data analysis showed 71.4% (139 isolates) resistant (R) and 28.7% (56 isolates) intermediate/sensitive (I/S) isolates prior to plasmid curing. However, after curing, the scenario was changed to 44.1% (86 isolates) R and 55.9% (109 isolates) I/S. Again, among the Rs (139 isolates) in the previous phase, we found that 60.4% (84 isolates) remained Rs and 38.1% (53 isolates) changed to I/Ss in the post phase. But among I/Ss (56 isolates) in the previous phase, 3.5% (2 isolates) shifted to Rs and 96.5% (54 isolates) remained as I/Ss. This figure seems to

suggest a positive change with the plasmid curing treatment. The Chi-square test result was statistically significant, as the *p*-value found (0.000) was less than 0.05 (5% level of significance).

Determination of biofilm formation capability

The brown, rough and dry characteristic morphotype of curli production was found in all 37 isolates. Biofilm was visualized in two isolates following incubation at 37°C for five consecutive days, as a ring of cells adhered to the glass wall at the air-liquid interface. The mean OD595 nm value was found to be 0.00 for the sterile LB medium (negative control) and the cut-off value (ODc) was found to be 0.113. Twenty-seven *E. coli* isolates were found to be non-biofilm forming (OD595 ranged from 0.046 to 0. 113). Nine (24.32%) were found to be weak biofilm producers ($0.113 < OD595\ nm \leq 0.226$) and one was a strong biofilm producer ($OD\ 595 > 0.452$). The results showed that the *pap*C gene was absent in all the isolates, *fim*H was present in 72.9% (27 out of 37), and *csg*A was present in 64.9% (24 out of 37) (Supplementary data Table S4).

Discussion

The purpose of this study is to identify multidrug resistant *E. coli*, which WHO has listed as a priority pathogen (Vaiyapuri *et al.*, 2021), and characterize its antimicrobial resistance gene(s) as well as their determinants and biofilm formation, from samples obtained at the WWTP in Dhaka, Bangladesh.

The unrestrained use of antibiotics in health care sectors and animal husbandry creates high selective pressure in favor of developing antibiotic resistance in countries like Bangladesh (Faiz and Basher, 2011). This study provides evidence to support this view, since more than 97.3% (n = 36) of the *E. coli* isolates are MDR. A comparable finding reports the presence of high levels of MDR *E. coli* in hospital and domestic WWTPs in South India (Praveenkumarreddy *et al.*, 2020).

Clinically significant Class A β-lactamase producing *bla*CTX-M-15 positive *E. coli* are often found in environmental sources (Peirano and Pitout, 2019). Similarly, the presence of the *bla*TEM gene (30%) and *bla*CTX-M-15 gene (24%) is identified in those isolates. Qi *et al*. (2010) found CTX-M-15 genes (64%) in their isolates, consistent with our study showing more CTX-M-15 genes (70%) in ESBL-positive isolates than TEM and SHV genes. Several studies have observed the co-resistance of ESBL-positive *E. coli* to various antibiotics including non-β-lactam antibiotics (Balkhed *et al.*, 2013; Tacão *et al.*, 2014). This is relevant to our study, as 12 *qnr*S positive isolates are found, among which seven harbor the ESBL gene.

In this study, almost half of the isolates are resistant to first- and second-generation quinolones, similar to findings from sewage samples reported in Spain (Colomer-Lluch *et al.*, 2013). Our prevalence of *qnr*B and *qnr*S genes, indicating quinolone resistance, are similar to the findings from hospital wastewater in Tehran, Iran (Ranjbar and Farahani, 2017). However, half of the *qnr*S positive isolates has possessed plasmids.

In our study, a higher prevalence of *dfr*A17 gene is found in SXT resistance. However, some isolates have showed resistance against SXT without having the *sul* and *dfr* genes. The paradox might be explained by the fact that more than 30 different SXT resistance genes already exist, and may have conferred resistance in those isolates (Šeputienė *et al.*, 2010).

In our study, Class I integrons is detected in 32.43% of isolates, proportionate to the percentage (31.5%) of an earlier study conducted by Wang *et al*. (2014) in China. Several previous studies have validated that resistance to a greater number of antibiotics is correlated with the presence of integrons (Grape *et al.*, 2005). All integron 1-positive isolates are MDR *E. coli*; however, the majority of MDR isolates (25 of 37) are integron-negative. These findings might indicate that integrons represent only one of several factors influencing the generation of an MDR strain.

The ARGs are frequently located on plasmids, conferring resistance to a variety of different antimicrobials (Yang *et al.*, 2020). In our study, plasmid curing therefore resulted in sensitivity to several antibiotics to which there was initial resistance. However, the N4EC4 isolate's puzzling loss of sensitivity to NA and C after curing can be ascribed to a number of plausible factors. For one thing, the curing methods (Liu *et al.*, 2012) or curing agents (Letchumanan *et al.*, 2015) being used may cause mutations in host chromosomes or increased expression of efflux mechanism might reduce susceptibility to several antimicrobials (Buckner *et al.*, 2018). However, a

significant correlation between plasmid curing and antibiotic susceptibility is asserted through statistical analysis.

However, several biofilm-associated genes is found among the isolates. Altogether, 72.90% of the isolates possessed the *fim*H gene. The major subunit of curli fimbriae encoded by *csg*A (Uhlich *et al.*, 2006), is present in 64.90% of the isolates. Thus, this study has demonstrated a high prevalence of biofilm-related genes in MDR *E. coli* isolates, posing a potential threat in the environment.

As best we know, this is the first report on the MDR *E. coli* isolates recovered from WWTP untreated effluents in Dhaka, Bangladesh. However, there are some limitations in our study. First, there was no exploration for ARB and ARGs in WWTP effluents following the treatment process. However, various studies have reported several beta-lactamase genes in hospital and city wastewater effluents following treatment, raising the potential for ARGs to be disseminated in aquatic environments (Adegoke *et al.*, 2020), since genes may persist for a long time, even after the bacteria are dead (Fouz *et al.*, 2020). Second, our study was limited to Bangladesh, and the study findings might not be applicable to global settings.

Conclusion

This study has revealed a high prevalence of MDR *E. coli* and high ARG diversity in WWTP untreated effluents in Dhaka, Bangladesh. These biological contaminants may play a pivotal role in an increased frequency of resistance transmission among both pathogenic and non-pathogenic bacteria. Therefore, if proper treatment is not implemented and monitored regularly, resistance might pose a public health threat in the treatment of infections, especially with the development of both regional and global pan-drug resistance.

Acknowledgments

This study was funded by the Government of the People's Republic of Bangladesh, Ministry of Education, Bangladesh Bureau of Educational Information & Statistics (BANBEIS) Project ID: LS2018783.

Conflict of Interest

The authors have no relevant financial or non-financial interests to disclose.

References

Adegoke AA, Madu CE, Aiyegoro OA, Stenström TA, and Okoh AI. 2020. Antibiogram and beta-lactamase genes among cefotaxime resistant *E. coli* from wastewater treatment plant. *Antimicrob. Resist. Infect. Control.* **9**, 46.

Ahmed I, Rabbi MB, and Sultana S. 2019. Antibiotic resistance in Bangladesh: a systematic review. *Int. J. Infect. Dis.* **80**, 54–61.

Balkhed ÅÖ, Tärnberg M, Monstein HJ, Hällgren A, Hanberger H, and Nilsson LE. 2013. High frequency of co-resistance in CTX-M-producing *Escherichia coli* to non-beta-lactam antibiotics, with the exceptions of amikacin, nitrofurantoin, colistin, tigecycline, and fosfomycin, in a county of Sweden. *Scand. J. Infect. Dis.* **45**, 271–278.

Bauer A, Kirby WM, Sherris JC, and Turck M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* **45**, 149–158.

Buckner MMC, Ciusa ML, and Piddock LJV. 2018. Strategies to combat antimicrobial resistance: anti-plasmid and plasmid curing. *FEMS Microbiol. Rev.* **42**, 781–804.

Chowdhury S, Ghosh S, Aleem MA, Parveen S, Islam MA, Rashid MM, Akhtar Z, and Chowdhury F. 2021. Antibiotic usage and resistance in food animal production: what have we learned from Bangladesh? *Antibiotics* **10**, 1032.

Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, and Beachey EH. 1985. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. *J. Clin. Microbiol.* **22**, 996–1006.

Colomer-Lluch M, Mora A, López C, Mamani R, Dahbi G, Marzoa J, Herrera A, Viso S, Blanco JE, Blanco M, *et al.* 2013. Detection of quinolone-resistant *Escherichia coli* isolates belonging to clonal groups O25B: H4-B2-ST131 and O25B: H4-D-ST69 in raw sewage and river water in Barcelona, Spain. *J. Antimicrob. Chemother.* **68**, 758–765.

Dadgostar P. 2019. Antimicrobial resistance: implications and costs. *Infect. Drug Resist.* **12**, 3903–3910.

De Medici D, Croci L, Delibato E, Di Pasquale S, Filetici E, and Toti L. 2003. Evaluation of DNA extraction methods for use in combination with SYBR green I real-time PCR to detect *Salmonella enterica* serotype enteritidis in poultry. *Appl. Environ. Microbiol.* **69**, 3456–3461.

Fair RJ and Tor Y. 2014. Antibiotics and bacterial resistance in the 21st century. *Perspect. Medicin. Chem.* **6**, 25–64.

Faiz MA and Basher A. 2011. Antimicrobial resistance: Bangladesh experience. *Reg. Health Forum* **15**, 1–8.

Fouz N, Pangesti KNA, Yasir M, Al-Malki AL, Azhar EI, Hill-Cawthorne GA, and Abd El Ghany M. 2020. The contribution of wastewater to the transmission of antimicrobial resistance in the environment: implications of mass gathering settings. *Trop. Med. Infect. Dis.* **5**, 33.

Grape M, Farra A, Kronvall G, and Sundström L. 2005. Integrons and gene cassettes in clinical isolates of co-trimoxazole-resistant Gram-negative bacteria. *Clin. Microbiol. Infect.* **11**, 185–192.

Hendriksen RS, Munk P, Njage P, van Bunnik B, McNally L, Lukjancenko O, Röder T, Nieuwenhuijse D, Pedersen SK, Kjeldgaard J, *et al.* 2019. Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. *Nat. Commun.* **10**, 1124.

Hosain MZ, Kabir SML, and Kamal MM. 2021. Antimicrobial uses for livestock production in developing countries. *Vet. World* **14**, 210–221.

Hossain MN, Hossain ZZ, Roy AK, Akhter H, and Begum A. 2022. Detection of oxytetracycline and chloramphenicol in untreated wastewater effluents by reverse phase-high performance liquid chromatography (RP-HPLC) technique. *Afr. J. Pharm. Pharmacol.* **16**, 11–18.

Jarlier V, Nicolas MH, Fournier G, and Philippon A. 1988. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Rev. Infect. Dis.* **10**, 867–878.

Kim YJ, Park JH, and Seo KH. 2018. Presence of *Stenotrophomonas maltophilia* exhibiting high genetic similarity to clinical isolates in final effluents of pig farm wastewater treatment plants. *Int. J. Hyg. Environ. Health* **221**, 300–307.

Kumar A and Pal D. 2018. Antibiotic resistance and wastewater: correlation, impact and critical human health challenges. *J. Environ. Chem. Eng.* **6**, 52–58.

Letchumanan V, Chan KG, and Lee LH. 2015. An insight of traditional plasmid curing in *Vibrio* species. *Front. Microbiol.* **6**, 735.

Liu X, Wang D, Wang H, Feng E, Zhu L, and Wang H. 2012. Curing of plasmid pXO1 from *Bacillus anthracis* using plasmid incompatibility. *PLoS ONE* **7**, e29875.

Peirano G and Pitout JDD. 2019. Extended-spectrum β-lactamase-producing enterobacteriaceae: update on molecular epidemiology and treatment options. *Drugs* **79**, 1529–1541.

Praveenkumarreddy Y, Akiba M, Guruge KS, Balakrishna K, Vandana KE, and Kumar V. 2020. Occurrence of antimicrobial-resistant *Escherichia coli* in sewage treatment plants of South India. *J. Water Sanit. Hyg. Dev.* **10**, 48–55.

Qi C, Pilla V, Jessica HY, and Reed K. 2010. Changing prevalence of *Escherichia coli* with CTX-M–type extended-spectrum β-lactamases in outpatient urinary *E. coli* between 2003 and 2008. *Diagn. Microbiol. Infect. Dis.* **67**, 87–91.

Rabbani MAG, Howlader MZH, and Kabir Y. 2017. Detection of multidrug resistant (MDR) bacteria in untreated waste water disposals of hospitals in Dhaka city, Bangladesh. *J. Glob. Antimicrob. Resist.* **10**, 120–125.

Ranjbar R and Farahani O. 2017. The prevalence of plasmid-mediated quinolone resistance genes in *Escherichia coli* isolated from hospital wastewater sources in Tehran, Iran. *Iran. J. Public Health* **46**, 1285–1291.

Römling U, Rohde M, Olsén A, Normark S, and Reinköster J. 2000. *AgfD*, the checkpoint of multicellular and aggregative behaviour in *Salmonella typhimurium* regulates at least two independent pathways. *Mol. Microbiol.* **36**, 10–23.

Šeputienė V, Povilonis J, Ružauskas M, Pavilonis A, and Sužiedėlienė E. 2010. Prevalence of trimethoprim resistance genes in *Escherichia coli* isolates of human and animal origin in Lithuania. *J. Med. Microbiol.* **59**, 315–322.

Stepanović S, Vuković D, Dakić I, Savić B, and Švabić-Vlahović M. 2000. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *J. Microbiol. Methods* **40**, 175–179.

Tacão M, Moura A, Correia A, and Henriques I. 2014. Co-resistance to different classes of antibiotics among ESBL-producers from aquatic systems. *Water Res.* **48**, 100–107.

Talukdar PK, Rahman M, Rahman M, Nabi A, Islam Z, Hoque MM, Endtz HP, and Islam MA. 2013. Antimicrobial resistance, virulence factors and genetic diversity of *Escherichia coli* isolates from household water supply in Dhaka, Bangladesh. *PLoS ONE* **8**, e61090.

Uhlich GA, Cooke PH, and Solomon EB. 2006. Analyses of the red-dry-rough phenotype of an *Escherichia coli* O157: H7 strain and its role in biofilm formation and resistance to antibacterial agents. *Appl. Environ. Microbiol.* **72**, 2564–2572.

Vaiyapuri M, Raveendran K, George I, Gundubilli D, Sivam V, Krishnan SG, George JC, Mothadaka MP, Nagarajarao RC, and Badireddy MR. 2021. Comparison of single and multi-host enrichment approach for harnessing lytic phages against antimicrobial-resistant *E. coli*: repurposing the enrichment step. *Biologia* **76**, 1041–1052.

Wang H, Hu R, Gao Y, Yang Z, Deng X, Zhang J, Wu R, and Huhe B. 2014. Study on molecular characterization of class I integron and integron-associated antimicrobial resistance in *Escherichia coli* from beef cattle. *Chin. Anim. Husb. Vet. Med.* **41**, 63–67.

World Health Organization, WHO. 2014 . Antimicrobial resistance: global report on surveillance. World Health Organization. https://apps.who.int/iris/handle/10665/112642.

Yang L, Lin Y, Lu L, Xue M, Ma H, Guo X, Wang K, Li P, Du X, and Qi K. 2020. Coexistence of two bla_{NDM-5} genes carried on IncX3 and IncFII plasmids in an *Escherichia coli* isolate revealed by illumina and nanopore sequencing. *Front. Microbiol.* **11**, 195.

Zaman M, Pasha M, and Akhter M. 2010. Plasmid curing of *Escherichia coli* cells with ethidium bromide, sodium dodecyl sulfate and acridine orange. *Bangladesh J. Microbiol.* **27**, 28–31.