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Emergence of multidrug-resistant *Bacillus* spp. derived from animal feed, food and human diarrhea in South-Eastern Bangladesh

Md Atiqul Haque^{1,2†}, Huilong Hu^{1†}, Jiaqi Liu¹, Md Aminul Islam³, Foysal Hossen³, Md Arifur Rahman³, Firoz Ahmed^{3*} and Cheng He^{1*}

Abstract

Background Antimicrobial resistance poses a huge risk to human health worldwide, while Bangladesh is confronting the most severe challenge between the food supply and the huge consumption of antibiotics annually. More importantly, probiotics containing *Bacillus* spp. are claimed to be an alternative to antimicrobial stewardship programs. However, their antibiotic resistance remains elusive. Thus, we employed the antimicrobial susceptibility test and PCR to assess the prevalence of resistance, including multidrug resistance (MDR) and resito-genotyping of isolated *Bacillus* spp.

Results The phenotypic profile showed that *Bacillus* spp. were 100% sensitive to gentamicin (2 µg/mL), whereas lowered sensitivity to levofloxacin (67.8%, 0.5–1 µg/mL), ciprofloxacin (62.3%, 0.5–1 µg/mL), clindamycin (52.2%, 0.25–0.5 µg/mL), amoxicillin-clavulanic acid (37.6%, 0.06 µg/mL), azithromycin (33.4%, 1–2 µg/mL), tetracycline (25.6%, 2–4 µg/mL), nitrofurantoin (21.1%, 16–32 µg/mL), co-trimoxazole (19.2%, 2 µg/mL), and erythromycin (18.8%, 0.25–0.5 µg/mL). The strains were completely resistant to penicillin, amoxicillin-clavulanic acid, cefixime, ceftriaxone, vancomycin, and co-trimoxazole, and a species-specific trend was seen in both phenotypic and genotypic resistance patterns. Genotypic resistance indicated prevalence of the *bla1* (71.5%), *tetA* (33%), *erm1* (27%), *bla*_{TEM} (13.1%), *bla*_{CTX-M-1}/*bla*_{CTX-M-2} /*sul1* (10.1%), *bla*_{SHV} (9.6%), and *qnrS* (4.1%) genes. The β-lactamase resistance gene *bla1* was found in all penicillin-resistant (MIC ≥ 32 µg/mL) *Bacillus* spp. One hundred ninety-one isolates (89.6%) were MDR, with 100% from diarrhea, 90.3% from food, and 88.7% from animal feed.

Conclusion Based on the MIC value and profile analysis of antibiotic resistance genes, this is the first study that *Bacillus* spp. antimicrobial susceptibilities have been identified in Bangladesh, and our study will shed light on the adverse effects of feed-borne *Bacillus* spp. emerging from animal feed to the food chain. A comprehensive investigation is urgently needed by policymakers on tolerance limits and harmful effects in the animal industry.

Keywords Antimicrobial resistance, *Bacillus* spp., Food chain, Food security, Multidrug resistant, Resistant gene

[†]Md Atiqul Haque and Huilong Hu contributed equally to this work.

*Correspondence:

Firoz Ahmed

firoz@nstu.edu.bd

Cheng He

hecheng@cau.edu.cn

Full list of author information is available at the end of the article



Background

Antimicrobial resistance (AMR) is a serious, multifaceted, and complicated healthcare concern worldwide that impacts people, animals, and the environment, resulting in harder-to-treat infections and even death. The “One Health” approach, which incorporates public health and veterinary regulators, the food and agriculture industry, financiers, environmentalists, and customers, is highlighted in the WHO-led Global Action Plan on Antimicrobial Resistance [1, 2]. AMR develops naturally over time, generally through genetic mutations that can transmit from one generation to another or between humans and animals via animal-sourced food. A variety of strategies, including target defence, target replacement, detoxification, and suppression of cellular antibiotic deposition, are used by bacteria to develop antimicrobial resistance (AMR) [2–4]. Although not all resistant bacteria produce diseases, they may initiate the manifestation of a disease or spread the gene encoding AMR to new bacterial pathogens in favorable environments [4]. Consequently, improper and abusive antibiotics might contribute to the development of different drug-resistant bacteria and can disperse antibiotic residues from various settings throughout the food supply chain, acting as a reservoir and propagation matrix for AMR with the potential for antibiotic-resistant gene (ARG) to cross the animal-to-human microbes due to bacterial contamination [5–7]. The transfer of ARGs is a common way that ABR spreads. Once resistant genes are transmitted by plasmids, transposons, or integrons, dispersion is quick, and horizontal gene transfer across bacteria is frequent. Hitherto, it was thought that this type of genetic exchange only occurred among the same bacterial species. Nevertheless, the transmission of ARGs among phylogenetically distinct bacterial clusters, particularly across gram-positive and gram-negative bacteria, has now been proven in natural habitats [8].

Bacillus spp. has long been used as probiotics in human, veterinary, aquaculture, plant, and environmental applications, either directly as microbial food or as food additives heavily contaminated in animal feed and food chains, making a major financial burden for livestock producers and a potential threat to public health [6, 9–11]. *B. cereus*-caused foodborne diseases are classified into diarrheal (toxico-infections) and emetic (intoxications) syndromes resulting from the formation of several toxins (enterotoxins such as *nhe*, *hbl*, *cytK*, *entFM*, *BceT*, *HlyII*; emetic toxins *ces*), which occur globally and are becoming a serious challenge [7, 12]. *B. cereus* exacerbates severe diarrhea and malnutrition in chickens and ducks by causing gizzard erosion and ulceration (GEU) and facilitating

recurrent bacterial infections in the lungs by disrupting the gastrointestinal tract and following lung hemorrhagic lesions [13–16]. More interestingly, *B. cereus* was reported to induce non-gastrointestinal diseases, including bacteremia, septicemia, endophthalmitis, meningitis, endocarditis, urinary tract infections, and lung infections. Furthermore, *B. cereus* may lead to serious health effects, especially in newborn infants and immunosuppressed individuals [7, 12].

Nonetheless, some *B. cereus* and other bacteria possess ARG, which may spread among bacteria and ultimately impact humans through the food supply chain or the surroundings [5, 6]. Probiotic strains of *B. cereus*, *B. clausii*, *B. subtilis*, and *B. licheniformis* have shown resistance markers for β -lactams (*bla*_{BCL-1}), chloramphenicol (*cat*_{Bcl}), aminoglycosides (*aadD2*), macrolides (*erm34*), tetracycline (*tetM* and *tetK*), and erythromycin (*ermD* and *ermK*) [6]. The existence of mobile genetic components in *B. cereus* enables the uptake and transmission of drug-resistance genes from the environment [12].

Bangladesh, with a significant level of AMR and multidrug-resistant (MDR) bacteria against drugs indicated for use in both animals and people, confronts a local and worldwide hazard [3, 17–19]. However, *B. cereus* is resistant to numerous antibiotics, posing a global issue [20]. To inhibit the spread of AMR, it is essential to evaluate *Bacillus* spp. and their AMR profile. Some strains of *Bacillus* spp. are becoming increasingly resistant to antibiotics, allowing for the acquisition and emergence of new AMR strains. In our prior report, 39% of *Bacillus* spp. from animal feed and animal-based foods at a contamination level > 10⁵ CFU/g carried 80%, 71%, 55%, and 33% of the *entFM*, *cytK*, *nheABC*, and *hblACD* enterotoxin genes, respectively, and foodborne *Bacillus* spp. caused 4.5% of human diarrhea cases in south-eastern Bangladesh [15]. There is a lack of scientific data on the AMR in the livestock sector in Bangladesh. According to available report, resistant bacteria such as *E. coli*, *Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., *Staphylococcus* spp., and *Vibrio* spp., were commonly detected in poultry, dairy cattle, raw milk, farm surroundings, and fish items [21, 22]. Nevertheless, there is a dearth of comprehensive data regarding the antibiotic resistance patterns of *Bacillus* spp. in the human, animal, and environmental sectors in Bangladesh. Thus, to fill in this knowledge gap, this work aimed to determine the prevalence of resistance, particularly MDR, and the correlated genetic factors in isolated *Bacillus* spp. In the present study, we focused on whether animal feed, food, and human stool could harbor MDR strains and disseminate them through the food supply chain.

Results

Bacterial isolates

The isolates tested in this investigation were chosen from our prior work [15] and identified to be *B. cereus*, *B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis*, *B. thuringiensis*, *B. megaterium*, and *B. coagulans*, with 152, 56, and 10 strains from animal feed, food, and human diarrheal cases, respectively (Table S1, Figure S1-S4, Table S2-S3). In all analyzed samples, *B. cereus* was the predominant isolate (49.3–70%), followed by *B. subtilis* (14.2–30%), *B. amyloliquefaciens* (5.2–21.4%), *B. thuringiensis* (3.9–8.9%), *B. licheniformis* (8.5%), *B. megaterium* (5.2%), and *B. coagulans* (1.7–2.6%). Particularly, 7 *B. cereus* and 3 *B. subtilis* were isolated and identified from human stool with diarrhea cases.

Phenotypic profile of antimicrobial resistance

MICs and MBCs of isolated bacteria were displayed in the Table 1. As for *B. cereus*, the MIC and MBC values were determined in our study: PG-NIT-CFM-CTR/VAN-TET/GEN/CMX-AZM-EM/CIP/LEV-CM-AMC and PG-NIT-CFM/CTR/VAN/CMX-TET/GEN-EM/CIP/LEV/CM-AZM-AMC respectively. While the MIC/MBC ratio for *B. subtilis* was as follows: NIT-CFM-VAN-CTR/TET/GEN/CMX-AZM-PG/CIP/LEV-EM/CM-AMC, *B. amyloliquefaciens* recorded a similar trend. In contrast, *B. licheniformis* and *B. thuringiensis* showed similar patterns: PG-NIT-CFM-VAN-CTR/TET/GEN/CMX-AZM/CIP-LEV/CM-EM-AMC. Interestingly, *B. megaterium* and *B. coagulans* followed the same pattern as NIT-TET/VAN-CTR/GEN/CMX-CFM/AZM-EM/CIP/LEV-PG/CM-AMC (Table 1).

The antibiogram profiles of 14 antibiotics revealed that all isolated *Bacillus* spp. were generally sensitive to GEN (100%), LEV (67.8%), and CIP (62.3%), 52.2% to CM, 37.6% to AMC, and 33.4% to AZM (Table S4, Supplementary file 1). Further, the *Bacillus* spp. were generally resistant to β -lactam, glycopeptide, and sulfonamide antibiotics, including CFM (97.2%), PG (95.8%), CMX (81.1%), CTR (72.9%), VAN (71.5%), AMC (62.3%), while 55.9% were resistant to E, 55.5% to NIT, and 47.2% to TET (Fig. 1A, Table S4). The antibiotic-resistant pattern of AMC and AZM in diarrheal cases was significantly higher ($p < 0.01$) compared to animal feed and food samples. In contrast, the antibiotic-resistant pattern of CIP in animal feed was substantially greater ($p < 0.05$) than in food and diarrheal cases (Table 2).

Regarding *B. cereus*, the isolates were generally sensitive to GEN (100%), CIP (61.6%), and LEV (57.1%) and generally resistant to PG/CFM (100%), CTR (97.4%), CMX (97.3%), VAN (98.2%), EM (85.7%), AMC (80.4%), NIT (79.4%), and TET (70.5%). In the instance of *B. subtilis*, the isolates were generally sensitive to GEN (100%),

LEV (85.7%), CM (65.3%), CIP (63.3%), and AZM (51%). Furthermore, the *B. subtilis* isolates showed a somewhat similar resistant pattern to that of *B. cereus*. Interestingly, all *B. megaterium* and *B. coagulans* were 100% sensitive to GEN, CM, NIT, CIP, LEV, and CMX (Fig. 1B, Table S5). The ABR profiles of PG, AMC, CFM, CTR, VAN, AZM, EM, TET, CM, NIT, CIP, LEV, and CMX differed significantly ($p < 0.01$), and species-specific resistance was observed among the seven isolated *Bacillus* spp. (Table 3).

Pearson correlation coefficients (ρ) for pairs of antibiotics to assess ABR *Bacillus* isolates

Bivariate analysis showed a highly significant association ($p = < 0.001$ – 0.000) between the resistance patterns of TET and PG, EM/NIT and CFM, AZM/CM and AMC, NIT/CFM/CTR/VAN/EM/CMX and PG, CTR/VAN/EM/TET/NIT/CIP/LEV/CMX and AMC, CTR/VAN/CMX and CFM, VAN/AZM/EM/TET/CM/NIT/CIP/LEV/CMX and CTR, AZM/EM/TET/CM/NIT/CIP/LEV/CMX and VAN, EM/TET/NIT/CIP/CMX and AZM, TET/CM/CIP/LEV/CMX and EM, CM/NIT/CIP/LEV/CMX and TET, NIT/CIP/LEV/CMX/ and CM, CIP/LEV/CMX and NIT, LEV/CMX and CIP, CMX and LEV, and NIT and EM, CM and AZM. A moderate association ($p = 0.035$ – 0.017) was found between AMC/CIP/LEV and PG, and TET and CFM. There was a weaker correlation ($p > 0.05$) between AZM/CM/AMC/CIP/LEV and CFM, and AMC/AZM and PG (Table 4).

Genotyping profile of antibiotic resistance

Antibiotic resistance genes (ARGs) of isolated *Bacillus* spp. showed unique amplified target genes (Fig. S5A-S5I). The ARG profiles exhibited by *Bacillus* spp. were categorized into ten discrete types of isolates, indicating a considerable degree of genetic heterogeneity (Table 5). Of the 9 ARGs, the *bla1* gene was the most frequent (71.5%), followed by *tetA* (33%), *erm1* (27%), *bla*_{TEM} (13.7%), *bla*_{SHV} (9.6%), and *qnrS* (4.1%). The *bla*_{CTX-M-1}, *bla*_{CTX-M-2}, and *sul1* genes were observed with an equivalent frequency of 10.1% (Fig. 2, Table S6). According to sample-wise distribution, the prevalence of *bla1* was highest (80.9%) and *qnrS* was lowest (1.3%) in animal feed, whereas *bla1* and *tetA* were highest (42.8%) and *bla*_{CTX-M-1} and *bla*_{CTX-M-2} were lowest (7.1%) in food, and *bla1* was highest (90.0%) and *bla*_{TEM} and *sul1* were lowest (10.0%) in diarrheal cases. The *bla1* and *erm1* genes were significantly higher in the diarrheal case ($p < 0.01$ and $p < 0.05$, respectively) compared to the animal feed and food samples. In contrast, the *qnrS* gene was significantly greater ($p < 0.01$) in animal feed compared to both food and diarrheal cases (Table S7). The distribution of ARGs among 7 *Bacillus* strains is shown in Fig. 3 and Table S8. Obviously, *bla1*

Table 1 Antibiotic MICs and MBC of *Bacillus* species strains isolated from animal feed, food and diarrhea

| Antibiotics | Bacterial strains → | <i>B. cereus</i> | | <i>B. subtilis</i> | | <i>B. amyloliquefaciens</i> | | <i>B. licheniformis</i> | | <i>B. thuringiensis</i> | | <i>B. megaterium</i> | | <i>B. coagulans</i> | |
|-------------|---------------------|------------------|-------------|--------------------|-------------|-----------------------------|-------------|-------------------------|-------------|-------------------------|-------------|----------------------|-------------|---------------------|-------------|
| | | MIC (µg/mL) | MBC (µg/mL) | MIC (µg/mL) | MBC (µg/mL) | MIC (µg/mL) | MBC (µg/mL) | MIC (µg/mL) | MBC (µg/mL) | MIC (µg/mL) | MBC (µg/mL) | MIC (µg/mL) | MBC (µg/mL) | MIC (µg/mL) | MBC (µg/mL) |
| PG | 0.25–32 | > 32 | > 32 | 0.5 | 1 | 0.5 | 1 | > 32 | > 32 | > 32 | > 32 | 0.25 | 0.5 | 0.25 | 0.5 |
| AMC | 0.01–0.5 | 0.06 | 0.12 | 0.06 | 0.12 | 0.06 | 0.12 | 0.06 | 0.12 | 0.06 | 0.12 | 0.06 | 0.12 | 0.06 | 0.12 |
| CFM | 0.5–8 | > 4 | > 4 | > 4 | 2 | > 4 | 2 | > 4 | > 4 | > 4 | > 4 | 1 | 2 | 1 | 2 |
| CTR | 0.5–8 | 4 | 8 | 2 | 2 | 2 | 2 | 4 | 4 | 2 | 4 | 2 | 4 | 1 | 2 |
| VAN | 0.5–64 | 4 | 8 | 4 | 8 | 4 | 8 | 4 | 8 | 4 | 8 | 4 | 8 | 4 | 8 |
| AZM | 0.5–8 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 2 | 4 | 1 | 2 | 1 | 2 |
| EM | 0.25–32 | 0.5 | 1 | 0.25 | 0.5 | 0.25 | 0.5 | 0.25 | 1 | 0.5 | 1 | 0.5 | 1 | 0.5 | 1 |
| TET | 0.25–32 | 2 | 4 | 2 | 4 | 2 | 4 | 2 | 4 | 4 | 8 | 4 | 8 | 2 | 4 |
| GEN | 0.5–32 | 2 | 4 | 2 | 4 | 2 | 4 | 2 | 4 | 2 | 4 | 2 | 4 | 2 | 4 |
| CM | 0.25–8 | 0.25 | 1 | 0.25 | 0.5 | 0.25 | 0.5 | 0.5 | 1 | 0.5 | 1 | 0.25 | 0.5 | 0.5 | 1 |
| NIT | 1–128 | 16 | 32 | 16 | 32 | 16 | 32 | 16 | 32 | 32 | 64 | 16 | 32 | 16 | 32 |
| CIP | 0.12–16 | 0.5 | 1 | 0.5 | 1 | 0.5 | 1 | 1 | 2 | 1 | 2 | 0.5 | 1 | 0.5 | 1 |
| LEV | 0.12–16 | 0.5 | 1 | 0.5 | 1 | 0.5 | 1 | 0.5 | 1 | 1 | 2 | 0.5 | 1 | 0.5 | 1 |
| CMX | 1–128 | 2 | 8 | 2 | 4 | 2 | 4 | 2 | 4 | 2 | 4 | 2 | 4 | 2 | 4 |

MIC Minimum inhibitory concentration, MBC Minimum bactericidal concentration, PG Penicillin G, AMC Amoxicillin-Clavulanic acid, CFM Cefixime, CTR Ceftriaxone, VAN Vancomycin, AZM Azithromycin, EM Erythromycin, TET Tetracycline, GEN Gentamicin, CM Clindamycin, NIT Nitrofurantoin, CIP Ciprofloxacin, LEV Levofloxacin, CMX Co-Trimoxazole

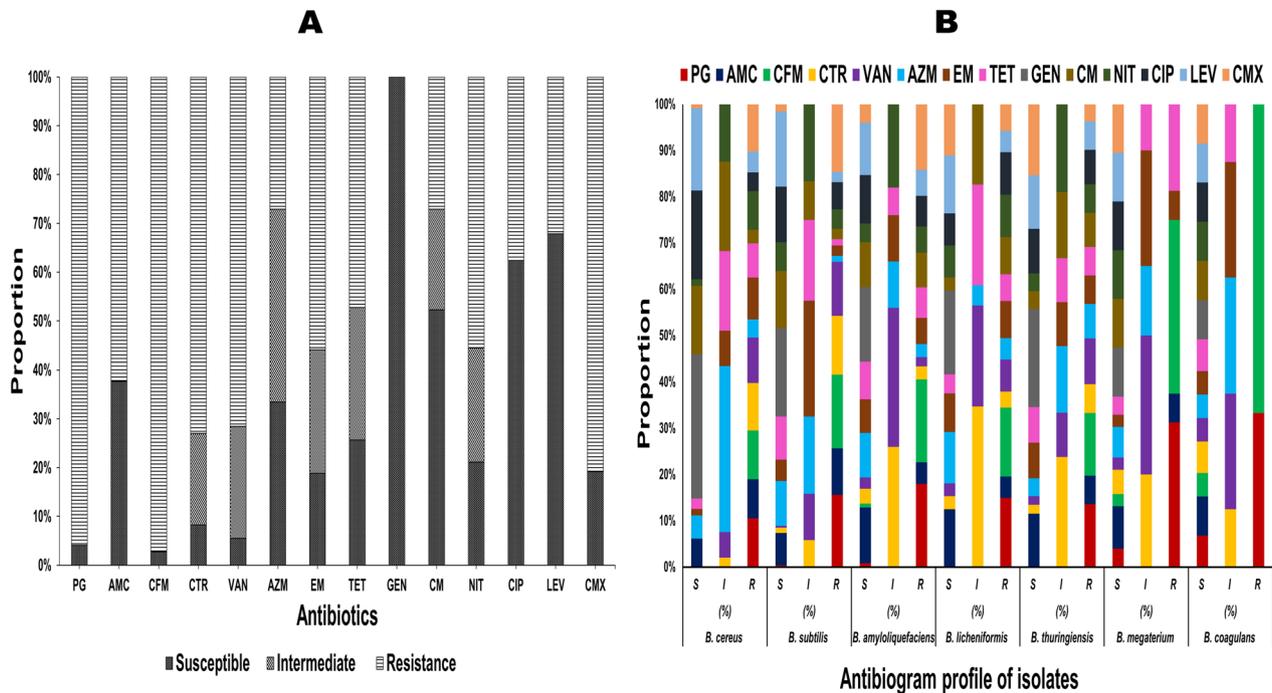


Fig. 1 **A** Overall antibiogram profile of the isolated *Bacillus* spp. The bar diagram displayed the proportions of susceptible, intermediate and resistant strains among 218 isolated *Bacillus* strains to 14 antibiotics. PG: Penicillin G, AMC: Amoxicillin-Clavulanic acid, CFM: Cefixime, CTR: Ceftriaxone, VAN: Vancomycin, AZM: Azithromycin, EM: Erythromycin, TET: Tetracycline, GEN: Gentamicin, CM: Clindamycin, NIT: Nitrofurantoin, CIP: Ciprofloxacin, LEV: Levofloxacin, CMX: Co-Trimoxazole. **B** Overall antibiogram profile of 7 *Bacillus* strains

Table 2 Antibiotic resistance pattern of *Bacillus* spp. in animal feed, food and diarrhea

| Antibiotic | Antibiotic resistance pattern (%) | | | Level of significance |
|------------|-----------------------------------|-----------------|---------------------|-----------------------|
| | Animal feed % (n/N) | Food % (n/N) | Diarrhea % (n/N) | |
| PG | 94.1 (143/152) | 98.2 (55/56) | 100 (10/10) | NS |
| AMC | 55.2 (84/152) | 87.5 (49/56) | 100 (10/10) | ** |
| CFM | 95.3 (145/152) | 100 (56/56) | 100 (10/10) | NS |
| CTR | 73.6 (112/152) | 67.8 (38/56) | 100 (10/10) | NS |
| VAN | 69 (105/152) | 73.2 (41/56) | 100 (10/10) | NS |
| AZM | 23 (35/152) | 30.3 (17/56) | 70 (7/10) | ** |
| EM | 53.9 (82/152) | 60.7 (34/56) | 70 (7/10) | NS |
| TET | 48.6 (74/152) | 39.2 (22/56) | 70 (7/10) | NS |
| GEN | 0 | 0 | 0 | - |
| CM | 23.6 (36/152) | 37.5 (21/56) | 20 (2/10) | NS |
| NIT | 57.2 (87/152) | 46.4 (26/56) | 70 (7/10) | NS |
| CIP | 44 (67/152) | 33.9 (19/56) | 0 | * |
| LEV | 33.5 (51/152) | 23.2 (13/56) | 50 (5/10) | NS |
| CMX | 80.2 (122/152) | 85.7 (48/56) | 100 (10/10) | NS |

PG Penicillin G, AMC Amoxicillin-Clavulanic acid, CFM Cefixime, CTR Ceftriaxone, VAN Vancomycin, AZM Azithromycin, EM Erythromycin, TET Tetracycline, GEN Gentamicin, CM Clindamycin, NIT Nitrofurantoin, CIP Ciprofloxacin, LEV Levofloxacin, CMX Co-Trimoxazole, n Number of resistant isolates, N Number of *Bacillus* isolates, * = significant ($p < 0.05$), ** = significant ($p < 0.01$), ns = non-significant

and *tetA* were predominantly distributed among *Bacillus* isolates, accounting for 40.1–100%. Furthermore, *B. cereus* and *B. licheniformis* harbored the remaining ARGs (*bla*_{TEM}, *bla*_{CTX-M-1}, *bla*_{CTX-M-2}, *bla*_{SHV}, *qnrS*, *sul1*, and *erm1*) at a rate of 6.2–38.4%. Interestingly, *B. coagulans* lacked almost all of the ARGs except for the *bla1* gene. The distribution of ARGs among animal feed, food and diarrheal cases is depicted in Fig. 4 and Table S9. Importantly, there were substantial variations in the prevalence of 6 ARGs among 7 isolated *Bacillus* spp., including *bla*_{TEM}/*bla*_{CTX-M-1}/*bla*_{CTX-M-2}/*tetA* ($p < 0.01$), *sul1/erm1* ($p < 0.05$), and species-specific occurrence was observed (Table S8).

Pearson correlation coefficients (ρ) for pairs of ARGs of *Bacillus* isolates

A bivariate analysis conducted on ARGs of *Bacillus* isolates showed a highly significant correlation ($p = < 0.001-0.000$) between *bla1* and *bla*_{SHV}/*bla*_{CTX-M-1}/*bla*_{CTX-M-2}, *sul1* and *bla*_{TEM}, *sul1* and *bla*_{SHV}, *bla1* and *bla*_{TEM}/*tetA/erm1*, *bla*_{TEM} and *bla*_{CTX-M-1}/*bla*_{CTX-M-2}/*bla*_{SHV}/*tetA/erm1*, *bla*_{CTX-M-1} and *sul1/tetA/erm1*, *bla*_{CTX-M-2} and *bla*_{SHV}/*sul1/tetA/erm1*, *bla*_{SHV} and *tetA/erm1*, and *erm1* and *tetA*, *bla*_{CTX-M-1} and *bla*_{CTX-M-2}. A moderate association ($p = 0.027-0.015$) was observed

Table 3 Antibiotic resistant pattern of seven *Bacillus* species

| Antibiotics | Fractions of resistant isolates | | | | | | | Level of significance |
|-------------|---------------------------------|--------------------|-----------------------------|-------------------------|-------------------------|----------------------|---------------------|-----------------------|
| | <i>B. cereus</i> | <i>B. subtilis</i> | <i>B. amyloliquefaciens</i> | <i>B. licheniformis</i> | <i>B. thuringiensis</i> | <i>B. megaterium</i> | <i>B. coagulans</i> | |
| | % (n/N) | % (n/N) | % (n/N) | % (n/N) | % (n/N) | % (n/N) | % (n/N) | |
| PG | 100 (112/112) | 98 (48/49) | 95 (19/20) | 100 (13/13) | 100 (11/11) | 62.5 (5/8) | 20 (1/5) | ** |
| AMC | 80.3 (90/112) | 63.3 (31/49) | 25 (5/20) | 30.7 (4/13) | 45.5 (5/11) | 12.5 (1/8) | 0 | ** |
| CFM | 100 (112/112) | 100 (49/49) | 95 (19/20) | 100 (13/13) | 100 (11/11) | 75.0 (6/8) | 40 (2/5) | ** |
| CTR | 97.4 (109/112) | 79.6 (39/49) | 15 (3/20) | 23.1 (3/13) | 45.5 (5/11) | 0 | 0 | ** |
| VAN | 92.8 (104/112) | 73.5 (36/49) | 10 (2/20) | 46.1 (6/13) | 8/11 (72.7) | 0 | 0 | ** |
| AZM | 37.5 (42/112) | 8.2 (4/49) | 15 (3/20) | 30.8 (4/13) | 54.5 (6/11) | 0 | 0 | ** |
| EM | 85.7 (96/112) | 14.3 (7/49) | 30 (6/20) | 53.8 (7/13) | 45.5 (5/11) | 12.5 (1/8) | 0 | ** |
| TET | 70.5 (79/112) | 8.2 (4/49) | 35 (7/20) | 38.5 (5/13) | 45.5 (5/11) | 37.5 (3/8) | 0 | ** |
| GEN | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| CM | 27.7 (31/112) | 14.3 (7/49) | 40 (8/20) | 53.8 (7/13) | 54.5 (6/11) | 0 | 0 | ** |
| NIT | 79.4 (89/112) | 26.5 (13/49) | 30 (6/20) | 61.5 (8/13) | 45.5 (5/11) | 0 | 0 | ** |
| CIP | 38.4 (43/112) | 36.7 (18/49) | 35 (7/20) | 61.5 (8/13) | 54.5 (6/11) | 0 | 0 | * |
| LEV | 42.8 (48/112) | 14.3 (7/49) | 35 (7/20) | 30.8 (4/13) | 54.5 (6/11) | 0 | 0 | ** |
| CMX | 97.3 (109/112) | 91.8 (45/49) | 75 (15/20) | 61.5 (8/13) | 27.3 (3/11) | 0 | 0 | ** |

PG Penicillin G, AMC Amoxicillin-Clavulanic acid, CFM Cefixime, CTR Ceftriaxone, VAN Vancomycin, AZM Azithromycin, EM Erythromycin, TET Tetracycline, GEN Gentamicin, CM Clindamycin, NIT Nitrofurantoin, CIP Ciprofloxacin, LEV Levofloxacin, CMX Co-Trimoxazole. n Number of resistant isolates, N Number of *Bacillus* isolates, *significant ($p < 0.05$), **significant ($p < 0.01$)

between *qnrS/sul1* and *bla1*, *tetA/erm1*, and *sul1*. There were weaker correlations ($p > 0.05$) between *qnrS* and *sul1/bla_{TEM}/erm1/bla_{CTX-M-1}/bla_{CTX-M-2}/bla_{SHV}/tetA* (Table 6).

MDR and MAR resistance profiles of *Bacillus* spp.

Antibiogram typing revealed that 89.6% of isolated *Bacillus* strains were MDR (Fig. 5). *B. cereus* exhibited a higher MDR (96.4%), followed by *B. subtilis* (93.8%), *B. amyloliquefaciens* (90%), *B. licheniformis* (84.6%), *B. thuringiensis* (81.8%), and *B. megaterium* (16.6%), while *B. coagulans* had no MDR. Moreover, MDR was found in 100% of diarrheal isolates, followed by food (90.3%) and animal feed (88.7%) isolates. Interestingly, the species-wise MDR patterns differed significantly ($p < 0.01$); in contrast, the sample-wise distribution was not statistically different ($p > 0.05$) (Table 7, Table S10). Among the antibiogram types, pattern PG-AMC-CFM-CTR-VAN-CMX showed the highest prevalence (12 isolates) in animal feed. On the contrary, the PG-AMC-CFM-CTR-EM-VAN-NIT-CMX pattern revealed the highest prevalence (9 isolates) in food, whereas the PG-AMC-CFM-CTR-VAN-CMX and PG-AMC-CFM-CTR-AZM-EM-TET-LEV-VAN-NIT-CMX patterns revealed the highest prevalence (3 isolates) in diarrhea (Table 6).

Furthermore, the MAR index of *Bacillus* spp. was arranged from 0.071–0.928, while *B. cereus* yielded the highest MAR index, ranging from 0.285–0.928, followed by *B. thuringiensis* (0.142–0.857), *B. subtilis*

(0.142–0.642), *B. amyloliquefaciens/B. licheniformis* (0.142–0.571), *B. megaterium* (0.142–0.285), and *B. coagulans* (0.071–0.142). Regarding sample type, animal feed isolates had the highest MAR index (0.428–0.928), followed by diarrheal isolates (0.428–0.857) and food isolates (0.071–0.714) (Table S10, Table 6). In our study, 90.3% of isolates showed a MAR index > 0.2 , with 100, 94.6, and 88.1% from diarrhea, food, and animal feed, respectively. Furthermore, there was a substantial variation ($p < 0.05$) among the MAR index having > 0.2 of seven *Bacillus* strains, while no significant difference ($p > 0.05$) was observed among different sample types (Tables S11 and S12).

Discussion

In the current study, the isolated *Bacillus* strains were sensitive to GEN, CIP, LEV, CM, AMC, TET, EM, AZM, NIT, and CMX, which was consistent with the previous reports [4, 5, 7, 9, 12, 20, 23, 24]. However, there were species-specific sensitivities of the *Bacillus* strains to CIP, LEV, CM, TET, EM, AZM, and NIT at various doses. Regarding the resistant pattern, diarrheal strains were completely resistant to PG, AMC, CFM, CTR, VAN, and CMX, whereas animal feed-borne strains were generally resistant to PG, AMC, CFM, VAN, and CMX, and food-originated strains were generally resistant to PG, CFM, CTR, and CMX. As for different species, *B. cereus*, *B. thuringiensis*, and *B. licheniformis* strains were all completely resistant to the beta-lactam antibiotics of PG and

Table 4 Pearson correlation coefficients for pairs of antibiotics to assess antibiotic-resistant *Bacillus* isolates from animal feed, food and diarrhea

| Statistical analysis | | PG | AMC | CFM | CTR | VAN | AZM | EM | TET | GEN | CM | NIT | CIP | LEV | CMX |
|----------------------|---------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| PG | Pearson Correlation Coefficient | 1 | | | | | | | | | | | | | |
| | p-value (two tailed) | - | | | | | | | | | | | | | |
| AMC | Pearson Correlation Coefficient | 0.148* | 1 | | | | | | | | | | | | |
| | p-value (two tailed) | 0.029 | | | | | | | | | | | | | |
| CFM | Pearson Correlation Coefficient | 0.811** | 0.120 | 1 | | | | | | | | | | | |
| | p-value (two tailed) | <0.001 | 0.077 | | | | | | | | | | | | |
| CTR | Pearson Correlation Coefficient | 0.341** | 0.434** | 0.276** | 1 | | | | | | | | | | |
| | p-value (two tailed) | <0.001 | <0.001 | <0.001 | | | | | | | | | | | |
| VAN | Pearson Correlation Coefficient | 0.329** | 0.450** | 0.267** | 0.966** | 1 | | | | | | | | | |
| | p-value (two tailed) | <0.001 | <0.001 | <0.001 | <0.001 | | | | | | | | | | |
| AZM | Pearson Correlation Coefficient | 0.126 | 0.190** | 0.102 | 0.371** | 0.384** | 1 | | | | | | | | |
| | p-value (two tailed) | 0.062 | 0.005 | 0.131 | <0.001 | <0.001 | | | | | | | | | |
| EM | Pearson Correlation Coefficient | 0.234** | 0.473** | 0.190** | 0.687** | 0.711** | 0.540** | 1 | | | | | | | |
| | p-value (two tailed) | <0.001 | <0.001 | 0.005 | <0.001 | <0.001 | <0.001 | | | | | | | | |
| TET | Pearson Correlation Coefficient | 0.196** | 0.379** | 0.159* | 0.576** | 0.597** | 0.644** | 0.840** | 1 | | | | | | |
| | p-value (two tailed) | 0.004 | <0.001 | 0.019 | <0.001 | <0.001 | <0.001 | <0.001 | | | | | | | |
| GEN | Pearson Correlation Coefficient | - ^c |
| | p-value (two tailed) | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| CM | Pearson Correlation Coefficient | 0.126 | 0.190** | 0.102 | 0.371** | 0.384** | 1.000** | 0.540** | 0.644** | - ^c | 1 | | | | |
| | p-value (two tailed) | 0.062 | 0.005 | 0.131 | <0.001 | <0.001 | 0.000 | <0.001 | <0.001 | - ^c | | | | | |
| NIT | Pearson Correlation Coefficient | 0.232** | 0.468** | 0.188** | 0.680** | 0.704** | 0.545** | 0.991** | 0.847** | - ^c | 0.545** | 1 | | | |
| | p-value (two tailed) | <0.001 | <0.001 | 0.005 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | - ^c | <0.001 | | | | |
| CIP | Pearson Correlation Coefficient | 0.161* | 0.288** | 0.131 | 0.473** | 0.490** | 0.784** | 0.689** | 0.820** | - ^c | 0.784** | 0.695** | 1 | | |
| | p-value (two tailed) | 0.017 | <0.001 | 0.054 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | - ^c | <0.001 | <0.001 | | | |
| LEV | Pearson Correlation Coefficient | 0.143* | 0.237** | 0.166 | 0.419** | 0.434** | 0.886** | 0.610** | 0.727** | - ^c | 0.886** | 0.616** | 0.886** | 1 | |
| | p-value (two tailed) | 0.035 | <0.001 | 0.088 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | - ^c | <0.001 | <0.001 | <0.001 | | |
| CMX | Pearson Correlation Coefficient | 0.431** | 0.343** | 0.350** | 0.790** | 0.763** | 0.293** | 0.543** | 0.455** | - ^c | 0.293** | 0.538** | 0.374** | 0.331** | 1 |
| | p-value (two tailed) | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | - ^c | <0.001 | <0.001 | <0.001 | <0.001 | |

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed), ^cCannot be computed because at least one of the variables is constant, PG Penicillin G, AMC Amoxicillin-Clavulanic acid, CFM Cefixime, CTR Ceftriaxone, VAN Vancomycin, AZM Azithromycin, EM Erythromycin, TET Tetracycline, GEN Gentamicin, CM Clindamycin, NIT Nitrofurantoin, CIP Ciprofloxacin, LEV Levofloxacin, CMX Co-Trimoxazole

Table 5 Antibiotic resistance gene profiling by PCR

| Profile | Antibiotic resistance gene | | | | | | | | | | Origin | | | | | Total (%) (n = 218) | | | |
|---------|----------------------------|---------------|-------------------|-------------------|---------------|-------------|-------------|-------------|-------------|---|-------------|-------------|-------------|-------------|-------------|------------------------|------------|------------|-------------|
| | Antibiotic resistance gene | | | | | | | | | | Animal feed | | | | | | | | |
| | <i>bla1</i> | <i>blaTEM</i> | <i>blaCTX-M-1</i> | <i>blaCTX-M-2</i> | <i>blaSHV</i> | <i>qnrS</i> | <i>sul1</i> | <i>tetA</i> | <i>erm1</i> | | LF (n = 42) | BF (n = 37) | DF (n = 26) | CF (n = 28) | FF (n = 19) | | E (n = 25) | M (n = 31) | HS (n = 10) |
| I | + | + | + | + | - | - | + | + | + | + | 1 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 10 (4.6) |
| II | + | - | - | + | + | - | - | + | - | - | 6 | 2 | 4 | 1 | 2 | 3 | 3 | | 21 (9.6) |
| III | + | - | + | - | - | - | - | + | - | - | 3 | 3 | 1 | 1 | 1 | 1 | 1 | 2 | 13 (5.9) |
| IV | + | - | - | - | - | - | - | - | + | - | 10 | 4 | 9 | 3 | 5 | 3 | 3 | 4 | 38 (17.4) |
| V | + | - | - | - | - | - | - | - | - | - | 21 | 19 | 10 | 8 | 5 | 2 | 9 | 2 | 76 (34.8) |
| VI | - | - | - | - | - | - | - | + | + | + | | | | | | 5 | 5 | 10 (4.5) | |
| VII | - | - | - | - | - | - | - | + | - | - | 1 | 1 | | 10 | 3 | 4 | 4 | 19 (8.7) | |
| VIII | - | - | - | - | - | + | - | - | - | - | 1 | 1 | | 1 | 5 | 2 | 2 | 9 (4.1) | |
| IX | - | - | - | - | - | - | - | - | + | - | 1 | 1 | | | 3 | 1 | 1 | 6 (2.7) | |
| X | - | - | - | - | - | - | - | - | - | - | 5 | 5 | 3 | 3 | 1 | 5 | 2 | 16 (7.3) | |

LF Layer feed, BF Broiler feed, DF Duck feed, CF Cattle feed, FF Fish feed, E Egg, M Milk, HS Human stool, + positive, -negative

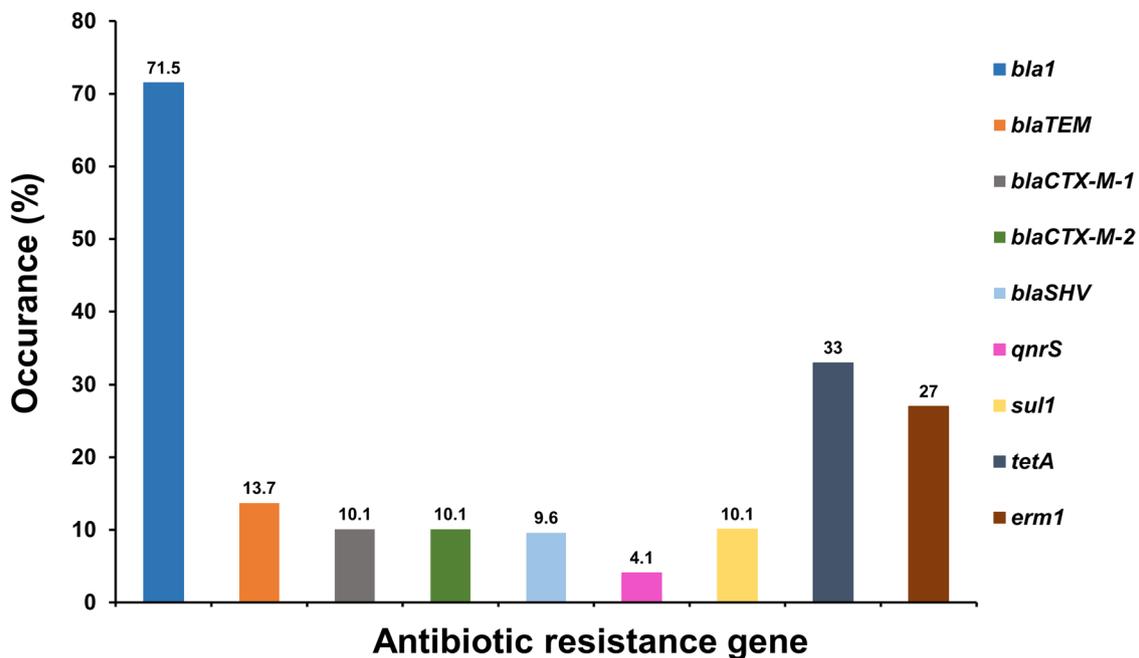


Fig. 2 Distribution of antibiotic resistance genes of *Bacillus* spp. isolated from animal feed, food and diarrheal cases in Bangladesh. The numerical value displayed above each bar shows the positive rate associated with the respective antibiotic resistance genes

CFM, while *B. subtilis* strains were completely resistant to CFM. Moreover, they were generally resistant to PG, AML, CFM, CTR, VAN, EM, TET, and NIT compared to AZM, CM, CIP, and LEV, which was compatible with the other studies [1, 3, 5, 7, 8, 20, 23–25].

The *B. cereus* strains were generally sensitive to GN, CIP, and LEV, while intermediately sensitive to AZM, CM, and TET. In contrast, they were generally resistant to PG, CFM, CTR, CFM, VAN, EM, AMC, NIT, and TET. This result agreed with earlier findings [4, 7, 20, 23, 24, 26–28], which were isolated from rice, cereals, chicken meat, fresh vegetables, edible fungi, powdered milk, foodstuffs, human stool, and clinical samples. The isolated *B. subtilis* was generally sensitive to GEN, LEV, CM, and CIP; intermediately sensitive to EM, TET, AZM, and NIT; and generally resistant to CFM, PG, CMX, CTR, VAN, and AMC. However, this bacteria strain detected in bread, powdered milk, soil, and shrimp culture ponds showed sensitivity to GEN, VAN, CM, EM, TET, and CMX while being resistant to PG, ampicillin, cefpodoxime, and cefepime [4, 9, 29, 30]. These two dominant species had species-specific responses to AMC, AZM, EM, TET, NIT, and LEV. The main factor might be large abuse, which prolongs time. The high sensitivity to GEN, CIP, and LEV could be attributed to the limited administration of CIP and LEV, while GEN is not absorbed via oral application. Consequently, CTR, CFM, AMC, CMX, and NIT were largely abused in the animal industry

and added frequently against infectious diseases. On the other hand, antibiotic regulation contributes to AMR. In several countries, CIP and LEV are prohibited for use on animals due to human drugs.

It is worth mentioning that 89.6% of *Bacillus* isolates were MDR, with 100% of isolates from diarrhea, 90.3% from food, and 88.7% from animal feed exhibiting the MDR pattern. These findings were consistent with other reports that found MDR *Bacillus* spp. in several sources, including food [4, 5, 7, 31, 32]. In our investigation, 100% *B. cereus* and 91.8% *B. subtilis* isolates yielded MAR indexes >0.2, indicating plasmid-mediated resistance and a significant risk of contamination. This implies a high inclination and trend for antibiotic resistance among the MDR bacterial isolates [33]. In Bangladesh, MDRs of *E. coli*, *Salmonella* spp., *Campylobacter* spp., and *Enterobacter* spp. were detected in livestock populations due to contamination of animal-derived food and food products [3]. However, MDR in *Bacillus* spp. has not been reported. Our data indicated *Bacillus* strains might produce extended-spectrum beta-lactamase (ESBL) and resistance to third-generation cephalosporins (CFM and CTR), macrolide (EM), tetracycline, second- and third-generation quinolones (CIP and LEV), and sulfonamides (CMX).

In this study, genes encoding β -lactamase (*bla1*, *blaTEM*, *blaCTX-M-1*, *blaCTX-M-2*, *blaSHV*), fluoroquinolone (*qnrS*), sulfonamide (*sul1*), tetracycline (*tetA*), and macrolide

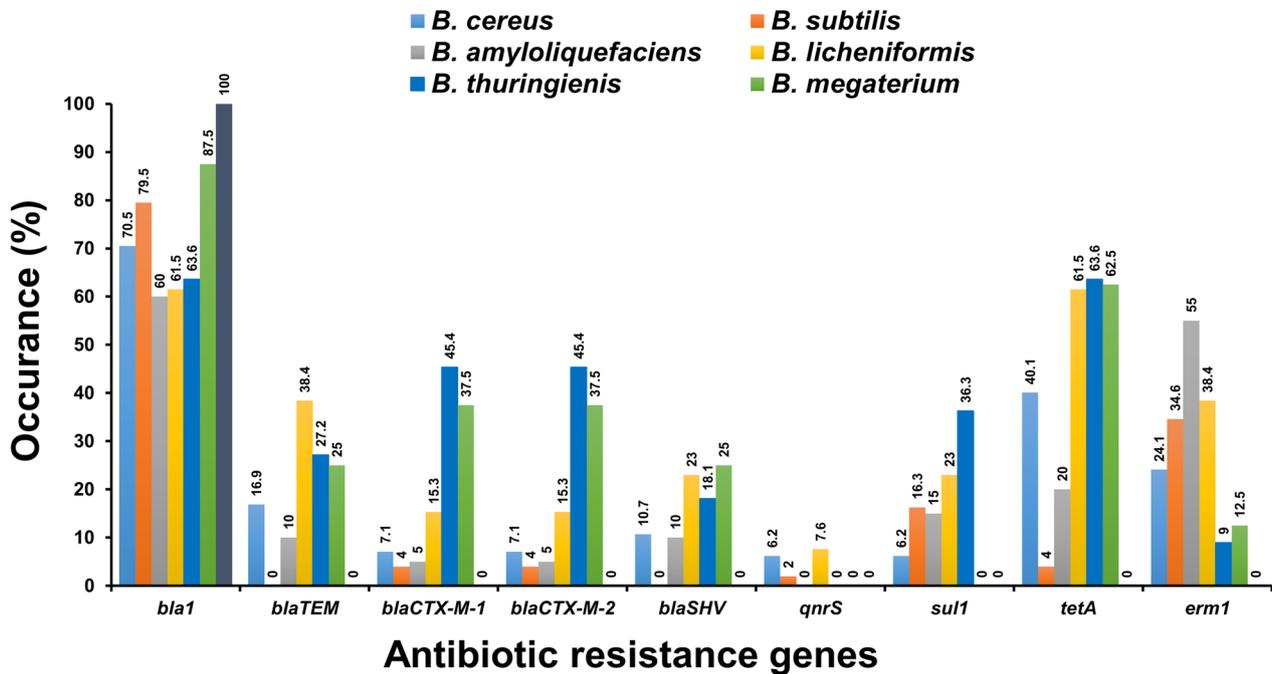


Fig. 3 Species wise distribution of antibiotic resistance gene of *Bacillus* spp. isolated from animal feed, food and diarrheal cases in Bangladesh. The numerical value displayed above each specific color bar shows the positive rate associated with antibiotic resistance genes of the respective *Bacillus* strains

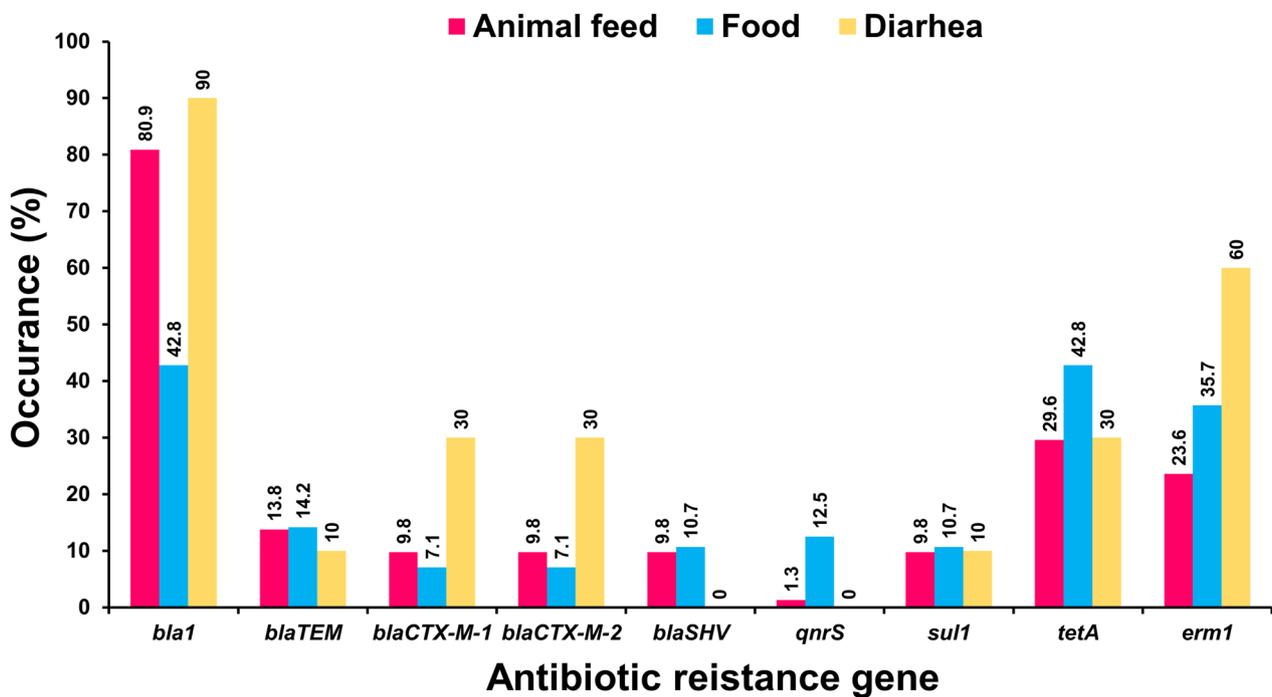


Fig. 4 Sample wise distribution of antibiotic resistance gene of *Bacillus* spp. isolated from animal feed, food and diarrheal cases in Bangladesh. The numerical value displayed above each specific color bar shows the positive rate associated with respective antibiotic resistance genes in animal feed, food and diarrhea

Table 6 Pearson correlation coefficients for pairs of ARGs of *Bacillus* isolates from animal feed, food and diarrhea

| Statistical analysis | | <i>bla1</i> | <i>bla_{TEM}</i> | <i>bla_{CTX-M-1}</i> | <i>bla_{CTX-M-2}</i> | <i>bla_{SHV}</i> | <i>qnrS</i> | <i>sul1</i> | <i>tetA</i> | <i>erm1</i> |
|------------------------------|---------------------------------|-------------|--------------------------|------------------------------|------------------------------|--------------------------|-------------|-------------|-------------|-------------|
| <i>bla1</i> | Pearson Correlation Coefficient | 1 | | | | | | | | |
| | <i>p</i> -value (two tailed) | - | | | | | | | | |
| <i>bla_{TEM}</i> | Pearson Correlation Coefficient | 0.252** | 1 | | | | | | | |
| | <i>p</i> -value (two tailed) | <0.001 | - | | | | | | | |
| <i>bla_{CTX-M-1}</i> | Pearson Correlation Coefficient | 0.211** | 0.839** | 1 | | | | | | |
| | <i>p</i> -value (two tailed) | 0.002 | <0.001 | - | | | | | | |
| <i>bla_{CTX-M-2}</i> | Pearson Correlation Coefficient | 0.211** | 0.839** | 1.000** | 1 | | | | | |
| | <i>p</i> -value (two tailed) | 0.002 | <0.001 | 0.000 | - | | | | | |
| <i>bla_{SHV}</i> | Pearson Correlation Coefficient | 0.206** | 0.817** | 0.975** | 0.975** | 1 | | | | |
| | <i>p</i> -value (two tailed) | 0.002 | <0.001 | <0.001 | <0.001 | - | | | | |
| <i>qnrS</i> | Pearson Correlation Coefficient | 0.165* | 0.092 | 0.096 | 0.096 | 0.097 | 1 | | | |
| | <i>p</i> -value (two tailed) | 0.015 | 0.176 | 0.157 | 0.157 | 0.153 | - | | | |
| <i>sul1</i> | Pearson Correlation Coefficient | 0.150* | 0.208** | 0.239** | 0.239** | 0.233** | 0.016 | 1 | | |
| | <i>p</i> -value (two tailed) | 0.026 | 0.002 | <0.001 | <0.001 | <0.001 | 0.815 | - | | |
| <i>tetA</i> | Pearson Correlation Coefficient | 0.443** | 0.569** | 0.477** | 0.477** | 0.465** | 0.098 | 0.150* | 1 | |
| | <i>p</i> -value (two tailed) | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.151 | 0.027 | - | |
| <i>erm1</i> | Pearson Correlation Coefficient | 0.384** | 0.656** | 0.550** | 0.550** | 0.536** | 0.093 | 0.159* | 0.867** | 1 |
| | <i>p</i> -value (two tailed) | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.169 | 0.019 | <0.001 | - |

ARGs Antibiotic resistance genes, *Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2 tailed)

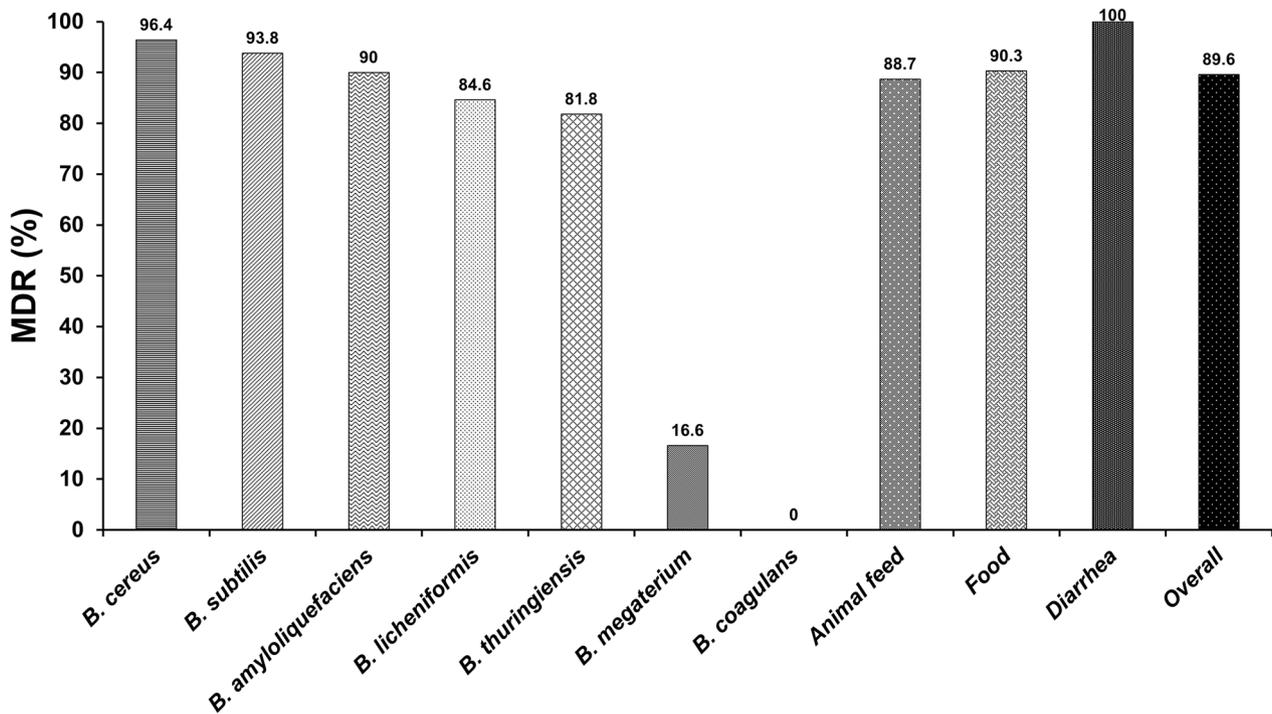


Fig. 5 Distributions of multidrug resistant pattern in isolated *Bacillus* spp., animal feed, food, diarrhea and overall. The numerical value displayed above each specific bar shows the positive rate of the respective multidrug resistance pattern

(*erm1*) were derived from animal feed, food, and diarrhea. The most commonly observed β -lactamase genes were *bla1* than *bla_{TEM}*, *bla_{CTX-M-1}*, *bla_{CTX-M-2}*, and *bla_{SHV}*

among *Bacillus* strains. Similar detection of the *bla1* gene in *Bacillus* strains was reported by other isolates from chicken meat, meat products, human stool, food, and the

Table 7 MDR profile of the isolated *Bacillus* spp

| Source | Pattern No | Antibiotic Resistance Pattern | No. of antibiotics (classes) | No of resistance isolates | MDR isolates (%) | MAR index | Level of significance |
|-------------|------------|--------------------------------|------------------------------|---------------------------|------------------|-----------|-----------------------|
| Animal feed | 1 | CFM | 1(1) | 1 | 0 (0) | 0.071 | NS ($p=0.517$) |
| | 2 | PG-CFM | 2 (1) | 9 | 0 (0) | 0.142 | |
| | 3 | PG-AMC-CFM | 3(1) | 1 | 0 (0) | 0.214 | |
| | 4 | PG-CFM-TET | 3(2) | 2 | 0 (0) | 0.214 | |
| | 5 | PG-CFM-CTR-CMX | 4 (1) | 2 | 0 (0) | 0.285 | |
| | 6 | PG-CFM-CTR-NIT | 4 (2) | 2 | 0 (0) | 0.285 | |
| | 7 | PG-CFM-TET-EM | 4(3) | 1 | 1 (0.6) | 0.285 | |
| | 8 | PG-CFM-CTR-TET-NIT | 5 (3) | 1 | 1 (0.6) | 0.357 | |
| | 9 | PG-CFM-CTR-TET-CMX | 5 (3) | 1 | 1 (0.6) | 0.357 | |
| | 10 | PG-CFM-CTR-NIT-CMX | 5 (3) | 3 | 3 (1.9) | 0.357 | |
| | 11 | PG-AMC-CFM-CTR-VAN-CMX | 6 (3) | 12 | 12 (7.7) | 0.428 | |
| | 12 | PG-CTR-AZM-NIT-CMX | 5 (4) | 1 | 1 (0.6) | 0.357 | |
| | 13 | PG-CFM-TET-CIP-LEV-VAN | 6 (4) | 3 | 3 (1.9) | 0.428 | |
| | 14 | PG-CFM-CTR-EM-VAN-CMX | 6 (4) | 2 | 2 (1.2) | 0.428 | |
| | 15 | PG-CFM-CTR-CIP-NIT-CMX | 6 (4) | 4 | 4 (2.5) | 0.428 | |
| | 16 | PG-CFM-EM-CIP-LFV-VAN | 6 (4) | 1 | 1 (0.6) | 0.357 | |
| | 17 | PG-CFM-LEV-VAN-NIT-CMX | 6 (5) | 1 | 1 (0.6) | 0.428 | |
| | 18 | PG-CFM-CIP-VAN-NIT-CMX | 6 (5) | 2 | 2 (1.2) | 0.428 | |
| | 19 | CFM-EM-CIP-CL-NIT-CMX | 6 (6) | 1 | 1 (0.6) | 0.428 | |
| | 20 | PG-AMC-CFM-CTR-EM-TET-CMX | 7 (4) | 1 | 1 (0.6) | 0.5 | |
| | 21 | PG-AMC-CFM-CTR-EM-VAN-CMX | 7 (4) | 2 | 2 (1.2) | 0.5 | |
| | 22 | PG-AMC-CFM-CTR-CIP-VAN-CMX | 7 (4) | 1 | 1 (0.6) | 0.5 | |
| | 23 | PG-CFM-CIP-LEV-VAN-NIT-CMX | 7 (5) | 1 | 1 (0.6) | 0.5 | |
| | 24 | PG-CFM-CTR-EM-VAN-NIT-CMX | 7 (5) | 1 | 1 (0.6) | 0.5 | |
| | 25 | PG-CFM-AZM-CIP-LEV-CM-VAN | 7 (5) | 2 | 2 (1.2) | 0.5 | |
| | 26 | PG-CFM-CTR-EM-TET-VAN-CMX | 7 (5) | 2 | 2 (1.2) | 0.5 | |
| | 27 | PG-CFM-TET-CIP-LEV-VAN-CMX | 7 (5) | 1 | 1 (0.6) | 0.5 | |
| | 28 | PG-CFM-CTR-EM-CIP-VAN-CMX | 7 (5) | 2 | 2 (1.2) | 0.5 | |
| | 29 | PG-CFM-AZM-EM-CIP-NIT-CMX | 7 (5) | 1 | 1 (0.6) | 0.5 | |
| | 30 | PG-CFM-AZM-EM-TET-CM-VAN | 7 (5) | 1 | 1 (0.6) | 0.5 | |
| | 31 | PG-CFM-EM-CIP-CM-NIT-CMX | 7 (6) | 2 | 2 (1.2) | 0.5 | |
| | 32 | PG-CFM-CTR-CIP-LEV-VAN-NIT-CMX | 8 (5) | 1 | 1 (0.6) | 0.571 | |
| | 33 | PG-CFM-CTR-TET-CIP-LEV-VAN-NIT | 8 (5) | 2 | 2 (1.2) | 0.571 | |

Table 7 (continued)

| Source | Pattern No | Antibiotic Resistance Pattern | No. of antibiotics (classes) | No of resistance isolates | MDR isolates (%) | MAR index | Level of significance |
|--------|------------|---|------------------------------|---------------------------|------------------|-----------|-----------------------|
| | 34 | PG-AMC-CFM-CIP-LEV-CM-NIT-CMX | 8 (5) | 1 | 1 (0.6) | 0.571 | |
| | 35 | PG-AMC-CFM-CTR-EM-TET-VAN-CMX | 8 (5) | 2 | 2 (1.2) | 0.571 | |
| | 36 | PG-AMC-CFM-CTR-LEV-VAN-NIT-CMX | 8 (5) | 3 | 3 (1.9) | 0.571 | |
| | 37 | PG-AMC-CFM-CTR-TET-VAN-NIT-CMX | 8 (5) | 2 | 2 (1.2) | 0.571 | |
| | 38 | PG-AMC-CFM-CTR-TET-LEV-VAN-CMX | 8 (5) | 1 | 1 (0.6) | 0.571 | |
| | 39 | PG-AMC-CFM-CTR-EM-VAN-NIT-CMX | 8 (5) | 3 | 3 (1.9) | 0.571 | |
| | 40 | PG-CFM-AZM-EM-TET-CM-VAN-CMX | 8 (6) | 2 | 2 (1.2) | 0.571 | |
| | 41 | PG-AMC-CFM-EM-CIP-CM-NIT-CMX | 8 (6) | 3 | 3 (1.9) | 0.571 | |
| | 42 | PG-CFM-TET-CIP-LEV-VAN-NIT-CMX | 8 (6) | 2 | 2 (1.2) | 0.571 | |
| | 43 | PG-CFM-CTR-AZM-TET-CIP-NIT-CMX | 8 (6) | 2 | 2 (1.2) | 0.571 | |
| | 44 | PG-CFM-CTR-EM-TET-VAN-NIT-CMX | 8 (6) | 2 | 2 (1.2) | 0.571 | |
| | 45 | PG-CFM-EM-TET-CIP-VAN-NIT-CMX | 8 (7) | 1 | 1 (0.6) | 0.571 | |
| | 46 | PG-AMC-CFM-CTR-AZM-EM-CM-NIT-CMX | 9 (5) | 2 | 2 (1.2) | 0.642 | |
| | 47 | PG-AMC-CFM-CTX-AZM-EM-TET-NIT-CMX | 9 (5) | 1 | 1 (0.6) | 0.642 | |
| | 48 | PG-AMC-CFM-CTR-TET-CIP-LEV-VAN-CMX | 9 (5) | 1 | 1 (0.6) | 0.642 | |
| | 49 | PG-AMC-CFM-CTX-EM-CM-VAN-NIT-CMX | 9 (6) | 2 | 2 (1.2) | 0.642 | |
| | 50 | PG-AMC-CFM-CTR-EM-TET-VAN-NIT-CMX | 9 (6) | 4 | 4 (2.5) | 0.642 | |
| | 51 | PG-AMC-CFM-CTR-EM-TET-CIP-LEV-VAN-CMX | 9 (6) | 1 | 1 (0.6) | 0.642 | |
| | 52 | PG-AMC-CFM-CTR-TET-CIP-VAN-NIT-CMX | 9 (6) | 1 | 1 (0.6) | 0.642 | |
| | 53 | PG-AMC-CFM-TET-CIP-LEV-VAN-NIT-CMX | 9 (6) | 1 | 1 (0.6) | 0.642 | |
| | 54 | PG-CFM-CTR-EM-TET-LEV-VAN-NIT-CMX | 9 (7) | 1 | 1 (0.6) | 0.642 | |
| | 55 | PG-CFM-CTX-EM-TET-CIP-VAN-NIT-CMX | 9 (7) | 1 | 1 (0.6) | 0.642 | |
| | 56 | PG-AMC-CFM-CTR-AZM-EM-CM-VAN-NIT-CMX | 10 (6) | 4 | 4 (2.5) | 0.714 | |
| | 57 | PG-AMC-CFM-CTR-AZM-ER-TET-CIP-LEV-VAN-CMX | 11 (6) | 4 | 4 (2.5) | 0.785 | |
| | 58 | PG-AMC-CFM-CTR-EM-TET-CIP-LEV-VAN-NIT-CMX | 11 (7) | 5 | 5 (3.2) | 0.785 | |
| | 59 | PG-AMC-CFM-CTR-AZM-EM-TET-CIP-LEV-CM-VAN-NIT | 12 (7) | 4 | 4 (2.5) | 0.857 | |
| | 60 | PG-AMC-CFM-CTR-AZM-EM-TET-CIP-LEV-VAN-NIT-CMX | 12 (7) | 7 | 7 (4.5) | 0.857 | |

Table 7 (continued)

| Source | Pattern No | Antibiotic Resistance Pattern | No. of antibiotics (classes) | No of resistance isolates | MDR isolates (%) | MAR index | Level of significance |
|----------|------------|--|------------------------------|---------------------------|------------------|-----------|-----------------------|
| | 61 | PG-CFM-CTR-EM-TET-LEV-CM-VAN-NIT-CMX | 10 (8) | 2 | 2 (1.2) | 0.714 | |
| | 62 | PG-CFM-CTR-EM-TET-CIP-LEV-CM-VAN-NIT-CMX | 11 (8) | 1 | 1 (0.6) | 0.785 | |
| | 63 | PG-AMC-CFM-CTR-EM-TET-CIP-LEV-CM-VAN-NIT-CMX | 12 (8) | 5 | 5 (3.2) | 0.857 | |
| | 64 | PG-AMC-CFM-CTR-AZM-EM-TET-CIP-CM-VAN-NIT-CMX | 12 (8) | 3 | 3 (1.9) | 0.857 | |
| | 65 | PG-AMC-CFM-CTR-AZM-EM-TET-CIP-LEV-CM-VAN-NIT-CMX | 13 (8) | 7 | 7 (4.5) | 0.928 | |
| | | Total | | 151 | 134 (88.7) | | |
| Food | 1 | PG | 1 (1) | 1 | 0 (0) | 0.071 | |
| | 2 | PG-CFM | 2 (1) | 3 | 0 (0) | 0.142 | |
| | 3 | PG-AMC-CFM-LEV | 4 (2) | 1 | 0 (0) | 0.285 | |
| | 4 | PG-AMC-CFM-LEV-NIT | 5 (3) | 1 | 1 (0.6) | 0.357 | |
| | 5 | PG-AMC-CFM-LEV-CMX | 5 (3) | 2 | 2 (1.2) | 0.357 | |
| | 6 | PG-CFM-CIP-CM-CMX | 5 (4) | 1 | 1 (2.1) | 0.357 | |
| | 7 | PG-CFM-TET-CIP-LEV-VAN | 6 (4) | 3 | 3 (6.2) | 0.428 | |
| | 8 | PG-AMC-CFM-CIP-LEV-CM-CMX | 8 (4) | 1 | 1 (2.1) | 0.571 | |
| | 9 | CFM-EM-TET-CM-CMX | 5 (5) | 1 | 1 (2.1) | 0.357 | |
| | 10 | PG-CFM-EM-TET-CM-CMX | 6 (5) | 3 | 3 (6.2) | 0.428 | |
| | 11 | PG-CFM-EM-CIP-CM-CMX | 6 (5) | 2 | 2 (4.1) | 0.428 | |
| | 12 | PG-AMC-CFM-CTR-VAN-CMX | 6 (3) | 1 | 1 (2.1) | 0.428 | |
| | 13 | PG-AMC-CFM-CTR-VAN-NIT-CMX | 7 (4) | 2 | 2 (4.1) | 0.5 | |
| | 14 | PG-AMC-CFM-CTR-CIP-LEV-VAN-CMX | 8 (4) | 3 | 3 (6.2) | 0.571 | |
| | 15 | PG-AMC-CFM-CTR-AZM-CM-VAN-CMX | 8 (5) | 4 | 4 (8.3) | 0.571 | |
| | 16 | PG-AMC-CFM-CTR-EM-CM-VAN-CMX | 8 (5) | 1 | 1 (2.1) | 0.571 | |
| | 17 | PG-AMC-CFM-CTR-EM-VAN-NIT-CMX | 8 (5) | 9 | 9 (18.7) | 0.571 | |
| | 18 | PG-AMC-CFM-CTR-AZM-EM-CM-VAN-CMX | 9 (5) | 3 | 3 (6.2) | 0.642 | |
| | 19 | PG-AMC-CFM-CTR-EM-TET-VAN-NIT-CMX | 9 (6) | 5 | 5 (10.4) | 0.642 | |
| | 20 | PG-AMC-CFM-CTR-AZM-EM-TET-VAN-NIT-CMX | 10 (6) | 5 | 5 (10.4) | 0.714 | |
| | | Total | | 52 | 47 (90.3) | | |
| Diarrhea | 1 | PG-AMC-CFM-CTR-VAN-CMX | 6 (3) | 3 | 3 (30.0) | 0.428 | |
| | 2 | PG-AMC-CFM-CTR-AZM-EM-TET-VAN-NIT-CMX | 10 (6) | 2 | 2 (20.0) | 0.714 | |
| | 3 | PG-AMC-CFM-CTR-AZM-EM-TET-LEV-VAN-NIT-CMX | 11 (7) | 3 | 3 (30.0) | 0.785 | |
| | 4 | PG-AMC-CFM-CTR-AZM-EM-TET-LEV-CM-VAN-NIT-CMX | 12 (8) | 2 | 2 (20.0) | 0.857 | |

Table 7 (continued)

| Source | Pattern No | Antibiotic Resistance Pattern | No. of antibiotics (classes) | No of resistance isolates | MDR isolates (%) | MAR index | Level of significance |
|------------------------------------|------------|-------------------------------|------------------------------|---------------------------|------------------|-----------|-----------------------|
| | | Total | | 10 | 10 (100) | | |
| Grand Total (overall MDR isolates) | | | | 213 | 191 (89.6) | | |

MDR Multidrug resistance, PG Penicillin G, AMC Amoxicillin-Clavulanic acid, CFM Cefixime, CTR Ceftriaxone, VAN Vancomycin, AZM Azithromycin, EM Erythromycin, TET Tetracycline, CM Clindamycin, NIT Nitrofurantoin, CIP Ciprofloxacin, LEV Levofloxacin, CMX Co-Trimoxazole, NS Not significant

environment [27, 34, 35]. However, some of our findings were inconsistent with the previous studies [23, 36, 37]. They observed a higher prevalence of *bla*_{TEM}, *bla*_{CTX-M-1}, *bla*_{CTX-M-2}, and *bla*_{SHV} in waste water, clinical samples, and food samples. The second dominant gene was the *tetA* gene in our study, which was consistent with prior studies [8, 18, 25], but other authors reported a higher occurrence [28, 35]. The prevalence of the erythromycin-resistant gene, *erm1*, was low compared to earlier reports [9, 25, 35, 37]. Furthermore, our study revealed a low prevalence of sulfonamide, *sul1* and quinolone, and *qnrS* resistance genes. The *sul1* gene in *Bacillus* strains isolated from aquaculture ponds was lower than our report [1], whereas the *sul1* gene in wastewater was higher than our report [37]. *Bacillus* spp. are often used as food microbial additives and spread ARGs by horizontal transfer of plasmids, leading to the failure of antibiotic treatment and dramatically altering their phenotypes [4, 5, 38]. The *Bacillus anthracis*-associated plasmids pXO1 and pXO2 have been detected in certain *B. cereus* strains with pathogenic potential resembling *B. anthracis* [39]. For instance, atypical *B. cereus* strains such as *B. cereus* G9241, *B. cereus* biovar anthracis CA, Bcbva, and Bcbva-like strain BC-AK were linked to anthrax-like disease in mammals, livestock, and humans in the United States, China, and West Africa, implying that it may be widespread [40]. The highly efficient mobilization capacities and horizontal gene transfer may pose a serious threat to gene circulation, particularly ARGs. Probiotic *Bacillus* spp. have already been connected to clinical infections, as well as β -lactams, aminoglycosides, macrolides, chloramphenicol, tetracycline, and erythromycin resistance genes, which may contribute to the spread of ABR in animal microbiota and the possible transmission of ARG to humans [10]. Nevertheless, abusive antibiotic use can transmit antibiotic residues in foods derived from animals, like milk, meat, and eggs, as well as in the environment [6]. As a potential driver of both genes and bacteria resistant to antibiotics, the ARG may be further transmitted to people directly through the food chain [10].

The high resistance of *Bacillus* strains to CFM, CTR, AMC, and PG might be caused by β -lactamase and the

presence of ABC (ATP binding cassette) efflux transporters from *B. subtilis* that are tolerant to lincosamide [25, 26]. The different antibiotic mechanisms might be associated with inherent resistance, built-up resistance, gene modification, and DNA transfer that aid in bacterial survival by manipulating the penicillin binding protein (PBP), enzymatic blockage, porin mutations, and efflux pumps [6]. The current study revealed that *Bacillus* isolates primarily carried the β -lactamase resistance genes *bla1*, *bla*_{TEM}, *bla*_{CTX-M-1}, *bla*_{CTX-M-2}, *bla*_{SHV}, tetracycline resistance gene *tetA*, and erythromycin resistance gene *erm1*, respectively. It was confirmed that certain ARG classes could be acquired by the majority of antibiotics and evaluated in various *Bacillus* strains. According to resistant gene distribution, 10 distinct ARG patterns were detected in the isolates. The association of the above β -lactamase and other antibiotic genes within the same isolate has been reported [9, 23, 28, 36, 37]. However, the most common associations were *bla1* + *erm1*, *bla1* + *tetA*, *bla1* + *bla*_{TEM}, *bla*_{TEM} + *tetA*, and *bla*_{CTX-M-1} + *tetA* (Table S2). This occurrence indicates a greater spread of β -lactamase, tetracycline, and erythromycin genes, most likely owing to a genetic component in their mobilization as well as the horizontal transfer of ABR determinants between *Bacillus* strains or from other bacteria into *Bacillus* spp. [7, 8, 36].

In our prior study, we revealed a high *Bacillus* spp. contamination level with significant toxigenic potential in several resources, including animal feed, animal-derived goods, and regular food items [15], where 90.3% of isolates displayed >0.2 MAR index, indicating a high-risk source of contamination [41]. The presence of MDR and MAR *Bacillus* in animal feed, food, and diarrhea indicated that the abuse of antibiotics poses a severe public health hazard by transmitting AMR to people through the food supply chain.

There is a dearth of research data on the transmission of ARGs and the AMR of *Bacillus* strains in Bangladesh. According to a review report, the emergence of AMR is mainly attributed to antibiotic misuse or overuse by broiler (>60%) and layer (94.6%) farmers as over-the-counter medication and failure to maintain

the drug withdrawal period [3]. Empirical data suggests that antibiotic residues against *Bacillus* spp. exist in the liver and kidney as well as in commercially available feed in Bangladesh, acting as a subtherapeutic dose that hastens the emergence of AMR [15, 42]. Unhygienic livestock and poultry farming in Bangladesh is a significant risk indicator for spreading zoonotic bacteria and antibiotic resistance to people and the environment [3]. Our data confirmed that AMR- *B. cereus* strains prevailed in all analyzed samples. The significance of this finding is underlined by earlier research [4, 5, 7, 20, 24, 25] that showed *Bacillus* spp. can transfer ARGs. Bangladesh urgently requires the development of effective surveillance and control plans for the identification and prevention of ABR bacteria utilizing standard antibiotic susceptibility tests in regular animal and human microbiological laboratory settings.

Conclusion

It is the first investigation of the presence of ARGs of *Bacillus* spp. with public health significance in animal feed, food, and human stool in Bangladesh. The feed- and food-borne *Bacillus* spp. exhibited species-specific trends in both phenotypic and genotypic resistance patterns with respect to antibiotic resistance. The associations of various antibiotic-resistant genes indicated a greater spread of β -lactamase, tetracycline, and erythromycin genes across the food chain. Animal feed and animal-derived products might serve as a channel for *B. cereus* propagation regarding their potential pathogenicity and the development of AMR in humans. This work validates the sources examined as major outlets for the spread of MDR bacteria and ARGs in the food chain of Bangladesh and once again highlights the urgency of a global campaign to combat AMR.

Materials and methods

Sampling, selection, isolation, storage, and molecular characterization of *Bacillus* spp. isolates

A total of 218 *Bacillus* spp. isolates were examined and retrieved in our previous study (Table S1), including animal feed ($n=90$), food ($n=40$), and human stool ($n=50$) in southeast Bangladesh [15]. These *Bacillus* spp. strains were initially detected and isolated through cultivation on MYPA (HiMedia, Mumbai, Maharashtra, India) plates, grams staining, biochemical assays, and PCR targeting *16srDNA*, *nheABC*, *hblACD*, *cytK*, and *entFM* genes [15, 43, 44]. The strains were preserved in TSB medium (HiMedia, Mumbai, Maharashtra, India) containing 15% glycerol at -80°C . The bacterial strains were cultivated aerobically in TSB at 37°C with agitation at 225 rpm. The Ethical Reviewing Board on Institutional Animal Care and Use Committee at Noakhali Science and Technology

University, Bangladesh, granted approval for the experimental protocols. An Informed Consent Form (ICF) was obtained prior to initiating research activities and collecting human stool samples.

Antibiotic susceptibility test of the *Bacillus* isolates

Minimum inhibitory concentration (MIC)

To assess any link between antimicrobial resistance patterns and the chosen antibiotic category, the antibiogram profile of isolates was tested by estimating the MIC in appropriate broth using sterile U bottom 96-well plates with lids (SPL Life Science, Pochon, Kyonggi-do, South Korea) employing the microtiter broth dilution method [45]. The MIC represented the lowest level of antimicrobial that completely inhibited the growth of the organism. According to 2020 Clinical and Laboratory Standards Institute (CLSI) criteria, susceptible, intermediate, and resistant MIC ($\mu\text{g/ml}$) interpretation was done ([45], Table S13). Briefly, 2–3 single pure fresh colonies of *Bacillus* spp. grown on NA (24 h old) were inoculated into 5 ml of MHB (HiMedia, Mumbai, Maharashtra, India) and kept at 37°C for up to 8 h. To standardize the turbidity of bacterial suspension, MHB was used to achieve a turbidity of 0.5 McFarland concentration (1×10^5 CFU/mL) through a two-fold serial dilution of antibiotics through visual evaluation with a card featuring a white backdrop and distinct black lines. The microtiter plates were incubated in a shaking incubator at 37°C . The lowest concentration that inhibited indicator strains growth was noted. All MIC tests were done in triplicate. *Staphylococcus aureus* ATCC 29213 was used as a positive control.

Minimum Bactericidal Concentration (MBC)

Five microliters of inoculum from the MIC experiment's well that had no bacterial growth after 24 h were spotted on NA (HiMedia, Mumbai, Maharashtra, India). The plates were incubated at 37°C for 16 to 24 h in order to evaluate the MBC described earlier [46]. MBC was set as the lowest level on a NA plate where there was no visual growth. *Bacillus* spp. was detected, and it was determined that the growth of bacteria was bacteriostatic, while the absence of growth indicated bactericidal effects. The NA plate was cultivated with the indicated inoculum dilution to test for contamination and cell viability [45]. All analyses were performed in triplicate.

Determination of multidrug resistant (MDR) and multiple antibiotic resistance (MAR) index

MDR was considered to have at least one agent that was resistant to three or more types of antibiotics [47]. The following 14 antibiotics procured in powdered form from Sisco Research Laboratories Pvt. Ltd. (SRL, E,

Mumbai, Maharashtra 400,099, India) were used: Penicillin G, PG (0.25–32 µg/mL); Amoxicillin + Clavulanic acid, AMC (0.01–0.5 µg/mL); Cefixime, CFM (0.5–4 µg/mL); Ceftriaxone, CTR (1–8 µg/mL); Azithromycin, AZM (0.5–8 µg/mL); Erythromycin, EM (0.25–32 µg/mL); Tetracycline, TET (0.25–32 µg/mL); Ciprofloxacin, CIP (0.12–16 µg/mL); Levofloxacin, LEV (0.12–16 µg/mL); Clindamycin, CM (0.25–8 µg/mL); Vancomycin, VAN (0.5–32 µg/mL); Gentamicin, GEN (0.5–32 µg/mL); Nitrofurantoin, NIT (32–128 µg/mL), and Co-Trimoxazole, CMX (1–128 µg/mL). The MAR index of isolated *Bacillus* spp. was determined as a/b, where “a” is the number of antibiotics to which a strain is resistant and “b” is the total number tested [41].

Resistance genotyping

The genomic DNA of various *Bacillus* species isolated from animal feed, food, and stool samples was extracted utilizing the TaKaRa MiniBEST Bacteria Genomic DNA Extraction Kit Ver.3.0 (GW Vitek, Seoul, Korea). Following the manufacturer’s instructions, pure colonies of *Bacillus* spp. isolates were prepared for DNA extraction from Tryptic Soy Broth (TSB) and Luria Bertani (LB) broth. A NanoDrop™ 8000 spectrophotometer (Thermo Scientific, California, USA) was utilized to measure the concentration and purity of the eluted DNA.

Phenotypically resistant *Bacillus* spp. were screened by PCR for 9 ARGs, including 5 β-lactamase (*bla1*,

*bla*_{TEM}, *bla*_{CTX-M-1}, *bla*_{CTX-M-2}, and *bla*_{SHV}), single fluoroquinolone (*qnrS*), sulfonamide (*sul1*), tetracycline (*tetA*), and macrolide (*erm1*). PCR protocols were followed exactly as reported earlier [1, 8, 23, 36, 48–50] (Table 8, Table S14). *B. cereus* ATCC 14579 and *E. coli* ATCC 25922 served as positive controls, while sterile Milli-Q water (Sigma Aldrich, Bengaluru, Karnataka 560,099, India) served as a negative control. The PCR reaction was performed in a 25 µl volume with OneTaqQuick-Load 2×Master Mix (New England Biolabs Inc., United States), 0.2 µmol L⁻¹ final concentration of each primer, and 2.5 µl of ready DNA template. PCR was conducted on a T100 Thermal cycler (Bio-Rad, United States). The PCR products were analyzed on 1.5% agarose gel (MP Biomedicals LLC, United States) with a Mini-Sub Cell GT Horizontal Electrophoresis System (Bio-Rad, United States), stained with Ethidium bromide (EtBr), displayed with a UV transilluminator (Gel Doc EZ), and imaged using a gel documentation system.

Statistical analysis

The antibiotic susceptibility results were provided in MS-2016 Excel sheets and analyzed using IBM SPSS Statistics version 24 (SPSS Inc., Chicago, IL, USA). The prevalence was computed by a descriptive study and the Chi-square test, and the degree of significance was established using Pearson correlation coefficients. The statistical significance was calculated as **p* < 0.05 and ***p* < 0.01.

Table 8 Primer used in this study

| Primer | Sequence (5′ → 3′) | Annealing temperature (°C) | Product size (bp) | Reference |
|-------------------------------|--|----------------------------|-------------------|-----------|
| <i>bla1</i> | F=CATTGCAAGTTGAAGCGAAA R=TGTCCTCGTAACTTCCAGCTC | 50 | 680 | [27] |
| <i>bla</i> _{TEM} | F=ATGAGTATTCAACATTTCCG R=CCAATGCTTAATCAGTGAGG | 55 | 850 | [25] |
| <i>bla</i> _{CTX-M-1} | F=AAAAATCACTGCGCCAGTTC R=AGCTTATTCATCGCCACGTT | 52 | 415 | [25] |
| <i>bla</i> _{CTX-M-2} | F=CGACGCTACCCCTGCTATT R=CCAGCGTCAGATTTTTTCAGG | 52 | 552 | [25] |
| <i>bla</i> _{SHV} | F=GCGAAAGCCAGCTGTCGGGC R=GATTGGCGGCGCTGTTATCGC | 62 | 304 | [26] |
| <i>qnrS</i> | F=GCAAGTTCATTGAACAGGGT R-TCTAAACCGTCGAGTTCGGCG | 54 | 428 | [28] |
| <i>sul1</i> | F=CGGCGTGGGCTACCTGAACG R=GCCGATCGCGTGAAGTTCCG | 57 | 433 | [1] |
| <i>tetA</i> | F=GGCGGTCTTCTTCATCATGC R=CGGCAGGCAGAGCAAGTAGA | 58 | 502 | [8] |
| <i>ermA</i> | F=TCTAAAAAGCATGTAAAGAA R=CTTCGATAGTTTATTAATATTAGT | 52 | 645 | [50] |

Abbreviations

| | |
|------|---|
| AMR | Antimicrobial resistance |
| ARG | Antibiotic resistant gene |
| CDC | Center for Disease Control and Prevention |
| CFU | Colony-forming unit |
| CLSI | Clinical and Laboratory Standards Institute |
| DNA | Deoxyribonucleic acid |
| EtBr | Ethidium bromide |
| LB | Luria-Bertani |
| MAR | Multiple antibiotic resistance |
| MBC | Minimum bactericidal concentration |
| MDR | Multidrug-resistant |
| MHB | Muller Hinton Broth |
| MIC | Minimum inhibitory concentration |
| MYPA | Mannitol-Egg-Yolk-Polymyxin-Agar |
| NA | Nutrient Agar |
| PCR | Polymerase chain reaction |
| TSB | Tryptic soy broth |
| WHO | World Health Organization |

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12866-024-03199-3>.

Additional file 1: Figure S1. Cultural morphology and Grams staining of isolated *Bacillus* spp. **Figure S2.** *16srDNA* gene of the isolated *Bacillus* spp. by PCR test. **Figure S3.** Toxin genes (*nheA*, *nheB*, *nheC*, *cytK*) of isolated *Bacillus* spp. by PCR test. **Figure S4.** Toxin genes (*hblA*, *hblC*, *hblD*, *entFM*) of isolated *Bacillus* spp. by PCR test. **Figure S5.** Antibiotic resistance genes of isolated *Bacillus* spp. by PCR test. **Table S1.** Strains and source of selected *Bacillus* spp. **Table S2.** Biochemical characteristics of *Bacillus* spp. **Table S3.** Primers of toxin gene and *16srRNA* gene and PCR protocol used in this study. **Table S4.** Overall antimicrobial susceptibility profile of *Bacillus* spp. **Table S5.** Overall antimicrobial susceptibility profiles of 7 *Bacillus* species. **Table S6.** Distributions of antibiotic genes among animal feed, food and diarrhea. **Table S7.** Prevalence of ARGs of *Bacillus* spp. in animal feed, food and diarrhea. **Table S8.** Prevalence of ARGs of 7 *Bacillus* species. **Table S9.** Distributions of ARGs among 7 *Bacillus* species. **Table S10.** MDR profiles of *B. cereus*, *B. subtilis*, *B. amyloliquefaciens*, *B. licheniformis*, *B. thuringiensis*, *B. megaterium* and *B. coagulans*. **Table S11.** Species wise percentage of MAR index >0.2. **Table S12.** Sample wise percentage of MAR index >0.2. **Table S13.** MIC break point of Antibiotic used. **Table S14.** PCR protocol of antibiotic resistant gene primer used in this study.

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Authors' contributions

M.A.H. acquisition of data, formal analysis, wrote the main manuscript text and prepared figure; H.H.L. and L.J.Q. acquisition of data and writing the original draft. M.A.L, F.H, M.A.R. interpretation of data, writing-review and editing; F.A. and C.H. concept and design. All authors reviewed the manuscript.

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Availability of data and materials

The dataset can be accessed upon a reasonable request made to the Corresponding author.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹National Key Laboratory of Veterinary Public Health Security, College of Veterinary Medicine, China Agricultural University, Beijing 100019, China.

²Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur 5200, Bangladesh.

³Department of Microbiology, Faculty of Science, Noakhali Science and Technology University, Noakhali 3814, Bangladesh.

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References

- Adekanmbi AO, Adejoba AT, Banjo OA, Saki M. Detection of sul1 and sul2 genes in sulfonamide-resistant bacteria (SRB) from sewage, aquaculture sources, animal wastes and hospital wastewater in South-West Nigeria. *Gene Rep.* 2020;20:100742.
- World Health Organization (WHO), Antimicrobial resistance. <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>. Accessed on 25 May 2022.
- Al Amin M, Hoque MN, Siddiki AZ, Saha S, Kamal MM. Antimicrobial resistance situation in animal health of Bangladesh. *Vet World.* 2020;13:2713–27.
- Alanber MN, Alharbi NS, Khaled JM. Evaluation of multidrug-resistant *Bacillus* strains causing public health risks in powdered infant milk formulas. *J Infect Public Health.* 2020;13:1462–8.
- György É, Laslo É, Antal M, András CD. Antibiotic resistance pattern of the allochthonous bacteria isolated from commercially available spices. *Food Sci Nutr.* 2021;9:4551–61.
- Haque MA, Khan A, He C. Feed-borne *Bacillus cereus*: an emerging threat to food chain related hazard, safety and pathogenic potentiality. In: Abbas RZ, Khan A, editors. *Veterinary Pathobiology and Public Health*. 1st ed. Faisalabad: Unique Scientific Publishers®; 2021. p. 251–69.
- Sornchuer P, Tiengtip R. Prevalence, virulence genes, and antimicrobial resistance of *Bacillus cereus* isolated from foodstuffs in Pathum Thani Province. *Thailand Pharm Sci Asia.* 2021;48:194–203.
- Rather MA, Aulakh RS, Gill JPS, Mir AQ, Hassan MN. Detection and sequencing of plasmid encoded tetracycline resistance determinants (*tetA* and *tetB*) from food-borne *Bacillus cereus* isolates. *Asian Pac J Trop Med.* 2012;5:709–12.
- Adimpong DB, Sorensen KI, Thorsen L, Stuer-Lauridsen B, Abdelgadir WS, Nielsen DS, et al. Antimicrobial susceptibility of *Bacillus* strains isolated from primary starters for African traditional bread production and characterization of the bacitracin operon and bacitracin biosynthesis. *Appl Environ Microbiol.* 2012;78:7903–14.
- Haque MA, Quan H, Zuo Z, Khan A, Siddique N, He C. Pathogenicity of feed-borne *Bacillus cereus* and its implication on food safety. *Agrobiol Records.* 2021;3:1–16.
- Sultana N, Haque MA, Rahman MM, Akter MR, Begum MD, Fakhruzzaman M, et al. Microbiological quality of commercially available poultry feeds sold in Bangladesh. *Asian J Med Biol Res.* 2017;3:52–60.
- Navaneethan Y, Esah EM. Prevalence, toxigenic profiles, multidrug resistance, and biofilm formation of *Bacillus cereus* isolated from ready-to eat cooked rice in Penang, Malaysia. *Food Control.* 2020;121:107553.

13. Zhang Q, Zuo Z, Guo Y, Zhang T, Han Z, Huang S, et al. Contaminated feed-borne *Bacillus cereus* aggravates respiratory distress post avian influenza virus H9N2 infection by inducing pneumonia. *Sci Rep*. 2019;9:7231.
14. Zuo Z, Li Q, Guo Y, Li X, Huang S, Hegemann JH, et al. Feed-borne *Bacillus cereus* exacerbates respiratory distress in birds infected with *Chlamydia psittaci* by inducing hemorrhagic pneumonia. *Avian Pathol*. 2020;49:251–60.
15. Haque MA, Fei W, Chen Y, Ahmed F, Hossen F, Islam MA, et al. *Bacillus* spp. contamination: a novel risk originated from animal feed to human food chains in south-eastern Bangladesh. *Front Microbiol*. 2022;12:783103.
16. Haque MA, Zuo Z, He C. Pathogenesis of gizzard erosion and ulcerations and comprehensive control strategy in poultry. Vol. 2. In: Abbas RZ, Saeed NM, Younus M, Aguilar-Marcelino L, Khan A, editors. *One Health Triad*. Unique Scientific Publishers: Faisalabad; 2023. p. 129–13.
17. Akter S, Haque MA, Jahan I. Epidemiological investigation, prevalence & antibiogram study of potential zoonotic bacterial pathogens of household pets at Dinajpur district of Bangladesh. *Int J Innov Sci Res Technol*. 2020;5:1630–41.
18. Habib A, Afroz F, Rahman MM, Begum MD, Kamruzzaman M, Haque MA. Molecular characterization and antimicrobial resistance of *Escherichia coli* isolated from retail chicken meat. *South Asian J Biol Res*. 2021;4:38–47.
19. Nupur MN, Afroz F, Hossain MK, Harun-ur-Rashid SM, Rahman MG, Kamruzzaman M, et al. Prevalence of potential zoonotic bacterial pathogens isolated from household pet birds and their antimicrobial profile in northern Bangladesh. *Agrobiological Records*. 2023;11:28–38.
20. Liu C, Yu P, Yu S, Wang J, Guo H, Zhang Y, et al. Assessment and molecular characterization of *Bacillus cereus* isolated from edible fungi in China. *BMC Microbiol*. 2020;20:310.
21. Ahmed I, Rabbi MB, Sultana S. Antibiotic resistance in Bangladesh: a systematic review. *Int J Infect Dis*. 2019;80:54–61.
22. Islam MM. Bacterial resistance to antibiotics: access, excess, and awareness in Bangladesh. *Expert Rev Anti Infect Ther*. 2021;19:973–81.
23. Torkar KG, Bedenić B. Antimicrobial susceptibility and characterization of metallo- β -lactamases, extended-spectrum β -lactamases, and carbapenemases of *Bacillus cereus* isolates. *Microb Pathog*. 2018;118:140–5.
24. Fiedler G, Schneider C, Igbinsola EO, Kabisch J, Brinks E, Becker B, et al. Antibiotics resistance and toxin profiles of *Bacillus cereus*-group isolates from fresh vegetables from German retail markets. *BMC Microbiol*. 2019;19:250.
25. Hu Q, Fang Y, Zhu J, Xu W, Zhu K. Characterization of *Bacillus* species from market foods in Beijing. *China Processes*. 2021;9:866.
26. Park YB, Kim JB, Shin SW, Kim JC, Cho SH, Lee BK, et al. Prevalence, genetic diversity, and antibiotic susceptibility of *Bacillus cereus* strains isolated from rice and cereals collected in Korea. *J Food Prot*. 2009;72:612–7.
27. Tahmasebi H, Talebi RB, Zarif RB. Isolated of *Bacillus cereus* in chicken meat and investigation β -lactamase antibiotic-resistant in *Bacillus cereus* from chicken meat. *Adv life sci*. 2014;4:200–6.
28. Avcı C, Civek S, Aras ES. Phenotypic and genotypic characterization of foodborne bacteria isolated from Sinop Province, Turkey. *Food Biotechnol*. 2017;31:141–61.
29. Aslim B, Saglam N, Beyatlı Y. Determination of some properties of *Bacillus* isolated from soil. *Turk J Biol*. 2002;26:41–8.
30. Balakrishnan S, John KR, George MR. Antibiotic susceptibility of *Bacillus* spp. isolated from shrimp (*Penaeus monodon*) culture ponds. *Indian J Mar Sci*. 2003;32:81–4.
31. Meena BS, Kapoor KN, Agarwal RK. Occurrence of multi-drug resistant *Bacillus cereus* in foods. *J Food Sci Technol*. 2000;37:289–91.
32. Khasnabis J, Adhikari P, Chowdhury D, Rai C, Roy A. Incidence of multiple drug resistant *Bacillus cereus* in some popular snacks and sweets sold in Kolkata city India. *Indian J Microbiol Res*. 2017;4:14–9.
33. Bayode MT, Olalemi AO, Babayemi Olawale Oladejo BO. Multiple antibiotic resistant index and detection of *qnrS* and *qnrB* genes in bacterial consortium of urine samples from clinical settings. *Eur J Biol Res*. 2021;11:45–56.
34. Savić D, Miljković-Selimović B, Lepšanić Z, Tambur Z, Konstantinović S, Stanković N, et al. Antimicrobial susceptibility and β -lactamase production in *Bacillus cereus* isolates from stool of patients, food and environment samples. *Vojnosanit Pregl*. 2016;73:904–9.
35. Mousa D, Abd El Tawab A, El Hofy F, Maarouf A. Molecular studies on antibiotic resistant *Bacillus cereus* isolated from meat products and human in Kaliobia, Egypt. *Benha Vet Med J*. 2020;38:125–30.
36. Adesoji AT, Ogunjobi AA. Detection of extended spectrum beta-lactamases resistance genes among bacteria isolated from selected drinking water distribution channels in southwestern Nigeria. *BioMed Res Int*. 2016;2016:7149295.
37. Obayiwana A, Ibekwe A. Antibiotic resistance genes occurrence in wastewaters from selected pharmaceutical facilities in Nigeria. *Water*. 2020;12:1897.
38. Helgason E, Okstad OA, Caugant DA, Johansen HA, Fouet A, Mock M, et al. *Bacillus anthracis*, *Bacillus cereus*, and *Bacillus thuringiensis*—one species on the basis of genetic evidence. *Appl Environ Microbiol*. 2000;66:2627–30.
39. Böhm ME, Huptas C, Krey VM, Scherer S. Massive horizontal gene transfer, strictly vertical inheritance and ancient duplications differentially shape the evolution of *Bacillus cereus* enterotoxin operons *hbl*, *cytK* and *nhe*. *BMC Evol Biol*. 2015;15:246.
40. Baldwin VM. You can't *B. cereus* – a review of *Bacillus cereus* strains that cause anthrax-like disease. *Front Microbiol*. 2020;11:1731.
41. Krumperman PH. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. *Appl Environ Microbiol*. 1983;46:165–70.
42. Molla MAM. 2019. Poultry feed laced with antibiotics. *The Daily Star*, 13 April 2019. Available online: <https://www.thedailystar.net/frontpage/news/poultry-feed-laced-antibiotics-1729231> (Accessed on 27 July 2022).
43. Microbiology Laboratory Guidebook [MLG]. United States Department of Agriculture, Food Safety and Inspection Service, MLG 3.02. 2015
44. Saeed ZK, Abbas BA, Othman RM. Molecular identification and phylogenetic analysis of lactic acid bacteria isolated from goat raw milk. *Iraqi J Vet Sci*. 2020;34:259–63.
45. CLSI. Performance standards for antimicrobial susceptibility testing, 30th ed. CLSI supplement M100. Wayne: Clinical and Laboratory Standards Institute. 2020.
46. Poh-Hwa T, Yoke-Kqueen C, Indu Bala J, Son R. Bioprotective properties of three Malaysia *Phyllanthus* species: an investigation of the antioxidant and antimicrobial activities. *Int Food Res J*. 2011;18:887–93.
47. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18:268–81.
48. Chen Y, Tenover FC, Koehler TM. Beta-lactamase gene expression in a penicillin-resistant *Bacillus anthracis* strain. *Antimicrob Agents Chemother*. 2004;48:4873–7.
49. Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. *J Antimicrob Chemother*. 2007;60:394–7.
50. Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L. Detection of erythromycin-resistant determinants by PCR. *Antimicrob Agents Chemother*. 1996;40:2562–6.

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