Prevalence of Multiple Antibiotic Resistance Among *Salmonella* Isolates from Clinical Samples in Dhaka, Bangladesh

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ABSTRACT

Objectives: Antibiotic resistance in clinical isolates is often correlated to poor disease management or prolonged hospitalization. The study aimed to determine the prevalence of multidrug-resistant (MDR) *Salmonella* isolates in clinical samples collected from Dhaka, Bangladesh. *Methods*: We investigated 150 clinical samples for the presence of *Salmonella*. The *Salmonella* isolates were tested against 12 antibiotics categorizable into three groups based on action mechanism and nine classes based on chemical structure. The susceptibility patterns were analyzed using the Kirby-Bauer disk diffusion method on Mueller Hinton agar. The isolates were classified as MDR and extensively drug-resistant based on their resistance patterns against different antibiotics. *Results:* We used 100 *Salmonella* isolates for this study. The highest prevalence of resistance was observed against penicillin G (96.0%), cefuroxime (75.0%), and clindamycin (71.0%). The isolates exhibited 33.0% and 43.0% resistance against imipenem and meropenem, respectively. Almost all (98.0%) isolates showed MDR. A quarter of the isolates (23.0%) were resistant to five classes of antibiotics and 18.0% to six classes of antibiotics. Moreover, 20.0% of isolates exhibited extensive drug resistance. Among the cell wall synthesis inhibitors, the highest resistance was observed against penicillin G (31.0%). Resistance rates were in the range 20–30% against protein synthesis inhibitors, clindamycin (29.0%), tetracycline (21.0%), and chloramphenicol (21.0%). Among the *Salmonella* isolates, 90.0% showed multiple antibiotic resistance index scores of ≥ 0.3, while 65.0% scored ≥ 0.5. The carbapenem group accounted for the maximum sensitivity (62.0%), followed by 60.0% each for gentamicin and norfloxacin. *Conclusions:* The high prevalence of MDR *Salmonella* isolates in clinical samples from Bangladesh indicates a serious threat to public health. Our findings emphasize the urgent need to control MDR through promoting responsible use of suitable antibiotics and public awareness of the dangers of antibiotic misuse, increasing testing facilities, and promoting genetic research.

The global rise in antibiotic resistance in recent years has given rise to a proliferation of multidrug-resistant (MDR) bacteria capable of causing life-threatening infections. The challenge is particularly pronounced in developing countries such as Bangladesh, where antibiotics are overprescribed and often available from pharmacies without prescription.[1]

Many Bangladeshis do not have access to clean drinking water, leading to a high prevalence of waterborne diseases such as *Salmonella* infections.[2] Widespread use of antibiotics over the years has caused MDR *Salmonella* strains to proliferate, rendering several commonly used antibiotics ineffective.[3,4]

Salmonella is responsible for causing salmonellosis, typhoid fever, gastroenteritis, and bacteremia.[5] More than 2700 serotypes of *Salmonella* have been identified.[6] Enteric infections caused by *Salmonella* are responsible for causing approximately 4500 deaths each year in Bangladesh.[7] Chloramphenicol was used as a first-line therapy for the treatment of *Salmonella* infections till growing resistance limited its use.[4–6] A study in Chattogram, Bangladesh, found that typhoid patients were not responsive to ciprofloxacin, a second-line therapy.[4] Antibiotic resistance generally develops in *Salmonella* strains through mutations in chromosomal genes and plasmids. The transmission of the genes is promoted significantly by horizontal gene transfer.[8]

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A significant reason for the rapid emergence of MDR strains is the excessive use of antibiotics for prevention, treatment, and metaphylaxis.[9] A study in southern rural Bangladesh found that around 44% of doctors prescribe antibiotics without a clinical diagnosis.[4] Another reason is poor patient adherence, many of whom do not understand the impact of incomplete doses.[10] Also, the uncontrolled use of prophylactic antibiotics in farm animals vastly contributes to antibiotic resistance.[11]

The lack of comprehensive data on antibiotic resistance patterns in Bangladesh has led to suboptimal therapeutic choices for treating infections.[4] To ensure optimal treatment of *Salmonella* infections in Bangladesh, it is necessary to determine the prevalence of resistance against the commonly used antibiotics. Therefore, this study aimed to identify the prevalence of resistance of *Salmonella* spp. against commonly used antibiotics in the city of Dhaka.

METHODS

Clinical samples were collected from different diagnostics centers across Dhaka from February to July 2023. A total of 150 samples (135 urine, 13 sputum, and two tracheal aspirates), were collected in a sterile container, transported to the laboratory, and stored at 4 °C for further analysis.

Salmonella spp. was isolated from the clinical samples by streak plate method using solidified Xylose Lysine Deoxycholate agar and Salmonella-Shigella agar media. A single isolate per sample was subjected to antibiotic susceptibility analysis to avoid redundancy or incidence of duplication. After incubation at 37 °C, the bacterial growth was assessed. *Salmonella* species developed red colonies with a black center on Xylose Lysine Deoxycholate agar plate. In Salmonella-Shigella agar, non-lactose fermenting, colorless colonies with black centers, were identified as *Salmonella* isolates. The selected colonies were characterized by Gram staining. For standard biochemical assays, the Indole test, Triple Sugar Iron test, Urease test, Methyl Red/Voges-Proskauer test, Oxidase test, and Citrate Utilization test were carried out.[12,13]

The antibiotic susceptibility of the isolates was tested using the Kirby-Bauer Disc Diffusion Method.[13] We used 12 commercially available antibiotics which belonged to nine different classes as follows: (1) penicillins: penicillin G (30 μg) and piperacillin (100 μg), (2) cephalosporins: cefuroxime (30 μg), (3) tetracyclines: tetracycline (30 μg), (4) macrolides: azithromycin (30 μg) and clindamycin (10 μg), (5) fluoroquinolones: norfloxacin (10 μg), (6) sulphonamides: cotrimoxazole (25 μg), (7) aminoglycosides: gentamicin (10 μg), (8) chloramphenicol (30 μg), and (9) carbapenem: imipenem (10 μg) and meropenem (10 μg). Based on action mechanisms, the antibiotics used in this study belonged to three major groups: (a) bacterial cell wall synthesis inhibitors (piperacillin, penicillin G, cefuroxime, imipenem, and meropenem); (b) bacterial protein synthesis inhibitors (tetracycline, gentamicin, clindamycin, chloramphenicol, and azithromycin); and (c) bacterial nucleic acid synthesis inhibitors (norfloxacin and cotrimoxazole).

The culture samples were spread on Mueller-Hinton Agar media and the antibiotic discs were placed. After incubation at 37 °C, the diameter of the clear zones around the antibiotic discs was measured and the isolates were categorized as sensitive, intermediate, or resistant according to the Clinical Laboratory Standard Institute guidelines.[13,14] Standard bacterial strains were used as control to validate the results.

The isolates showing resistance to ≥ 3 classes of antibiotics were considered MDR. The isolates exhibiting susceptibility to only 1–2 classes of antibiotics were considered extensively drug-resistant (XDR). The multiple antibiotic resistance (MAR) index was determined using the formula, x/y, where 'x' is the number of isolates resistant to antibiotics and 'y' is the total number of antibiotics used on each isolate.

RESULTS

From a total of 150 clinical samples, 100 *Salmonella* isolates were obtained, representing a prevalence of 66.7%. The sources were urine (90.0%), sputum (9.0%), and tracheal aspirate (1.0%).

Gram staining test on the *Salmonella* isolates revealed rod-shaped, pink-colored colonies forming a chain. All the isolates showed a positive citrate test evidenced by the media turning an alkaline blue. A positive triple sugar iron test was confirmed by a reddish-black slant and yellow butt, indicating production of hydrogen sulfide gas. The indole test was negative for the isolates, indicated by the

Table 1: Zones of inhibition (mm) against different antibiotics for categorizing *Salmonella* isolates as resistant, intermediate, and sensitive (N = 100).

Antibiotic	Resistant mean ± SD	Intermediate mean ± SD	Sensitive mean ± SD
Tetracycline	9.4 ± 0.3	13.3 ± 0.3	18.3 ± 0.4
Penicillin G	9.7± 0.4	-	29.4 ± 0.5
Piperacillin	9.7 ± 0.4	-	16.0 ± 0.3
Clindamycin	10.0 ± 0.4	15.1 ± 0.3	21.7 ± 0.4
Meropenem	10.5 ± 0.5	17.2 ± 0.6	23.6 ± 0.5
Imipenem	9.9 ± 0.4	14.2 ± 0.4	19.9 ± 0.4
Azithromycin	10.2 ± 0.4	13.9 ± 0.3	18.6 ± 0.3
Cotrimoxazole	9.2 ± 0.5	13.1 ± 0.6	19.0 ± 0.6
Gentamicin	10.5 ± 0.5	13.5 ± 0.5	18.4 ± 0.5
Chloramphenicol	10.6 ± 0.5	11.8 ± 0.5	22.3 ± 0.5
Norfloxacin	11.4 ± 0.6	14.8 ± 0.8	20.8 ± 0.5
Cefuroxime	12.0 ± 0.5	18.7 ± 0.5	24.3 ± 0.4

absence of a red-violet ring on the surface of the liquid culture media. The isolates showed a negative result in the Voges-Proskauer test and a positive result in the Methyl Red test, as evidenced by the development of a brownish-yellow color and a cherry red color, respectively. All the isolates showed positive results for motility tests. The catalase, Oxidase, and Urease tests for the isolates yielded negative results.

The susceptibility of all 100 *Salmonella* isolates was evaluated against 12 different antibiotics and the diameters of the zones of inhibition were measured [Table 1]. The isolates were classified as resistant, intermediate, or sensitive to a particular antibiotic using a zone diameter interpretive standards chart. The isolates showed varying resistance against the 12 antibiotics [Table 2 and Figure 1].

As shown in Table 2, the highest prevalence of resistance was observed against penicillin G (96.0%), followed by cefuroxime (75.0%), clindamycin (71.0%), cotrimoxazole (68.0%), and piperacillin (61.0%). Against tetracycline and chloramphenicol, the isolates showed 50.0% resistance each.

A Significantly high degree of resistance was also observed against meropenem (43.0%), azithromycin (43.0%), imipenem (33.0%), and gentamicin (32.0%). The lowest resistance was observed against norfloxacin (27.0%).

Table 2: Resistance exhibited by *Salmonella* isolates against 12 different antibiotics (N = 100).

Antibiotics	Number of isolates, n		
	Resistant	Intermediate	Sensitive
Tetracycline	50	16	34
Penicillin G	96	0	4
Piperacillin	61	0	39
Clindamycin	71	12	17
Meropenem	43	8	49
Imipenem	33	11	56
Azithromycin	43	17	40
Cotrimoxazole	68	9	23
Gentamicin	32	8	60
Chloramphenicol	50	14	36
Norfloxacin	27	13	60
Cefuroxime	75	4	21
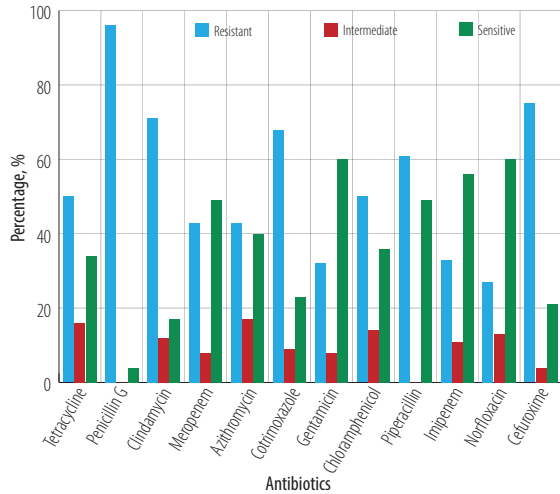
Figure 1: Antibiotic resistance pattern among *Salmonella* isolates (N = 100).

Figure 2: Resistance pattern among *Salmonella* isolates based on cell wall synthesis inhibitors (N = 100).

The majority of the isolates were sensitive to three antibiotics: gentamicin (60.0%), norfloxacin (60.0%), and imipenem (56.0%). The other antibiotics triggered sensitivities < 50%. Among the antibiotics, penicillin G was the least effective with only 4.0% sensitivity and 0.0% intermediate susceptibility. Seventeen percent of the isolates showed intermediate susceptibility to azithromycin.

The cell wall synthesis inhibitor antibiotics included penicillin G and piperacillin from the penicillin group, imipenem and meropenem from the carbapenems, and cefuroxime from the cephalosporins. Among these five antibiotics, *Salmonella* isolates exhibited the highest resistance against penicillin G (31.0%) and the lowest resistance against imipenem (11.0%) [Figure 2]. Among the cell wall inhibitory agents, the carbapenem group had the highest sensitivity (62.0%).

We used five bacterial protein synthesis inhibitors: azithromycin, chloramphenicol, clindamycin, gentamicin, and tetracycline. The highest resistance was observed against clindamycin (29.0%), followed by tetracycline (21.0%), chloramphenicol (21.0%), azithromycin (18.0%), and gentamicin (11.0%) [Figure 3]. On the other hand, the *Salmonella* isolates showed the highest sensitivity towards gentamicin (32.0%), followed by azithromycin (22.0%), and chloramphenicol (19.0%) among the bacterial protein synthesis inhibitors.

Between the two nucleic acid synthesis inhibitor antibiotics, the resistance was higher against norfloxacin (72.0%) than against cotrimoxazole (28.0%).
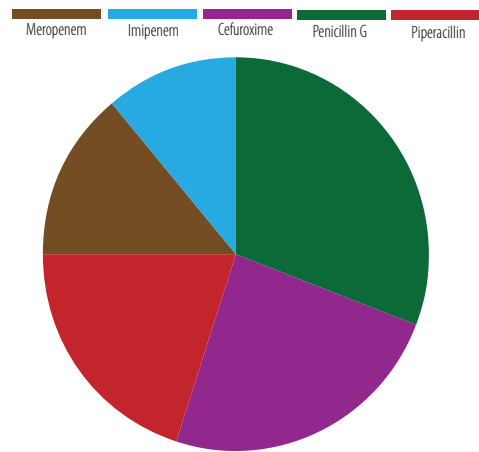
Among the *Salmonella* isolates from urine samples (n = 90), resistance was highest against penicillin G (96.0%), followed by cefuroxime (77.0%), clindamycin (70.0%), and cotrimoxazole (66.0%). Resistance to piperacillin, tetracycline, chloramphenicol, and azithromycin ranged 40–60%. Carbapenem antibiotics also encountered significant resistance; imipenem (32.0%) and meropenem (38.0%). Gentamicin was effective against 65.0% urine isolates and 78.0% sputum isolates. Among the 13 sputum samples, nine *Salmonella* strains were isolated. Their resistance against various antibiotics were: penicillin G (89.0%), meropenem (89.0%), clindamycin (78.0%), cotrimoxazole (78.0%), tetracycline (52.0%), imipenem (45.0%), piperacillin (55.0%), and chloramphenicol (48.0%).
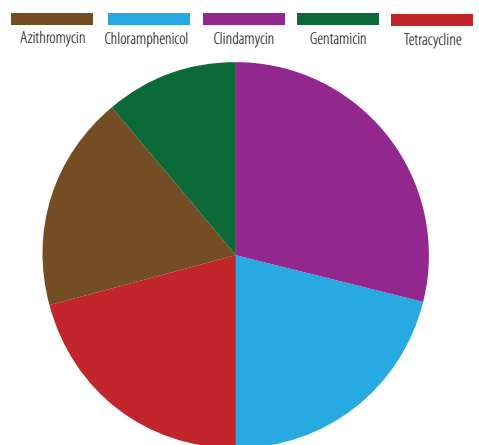

Figure 3: Resistance pattern among the *Salmonella* isolates based on protein synthesis inhibitors (N = 100).
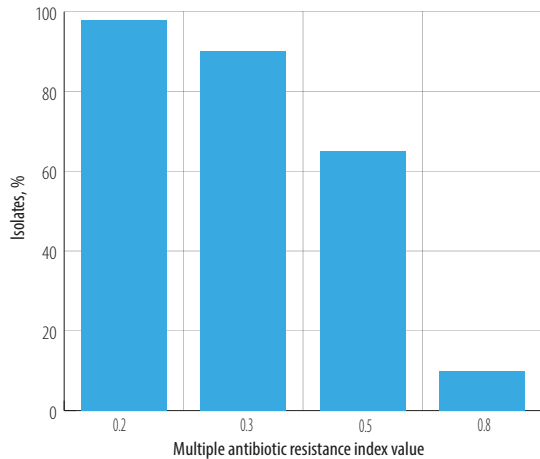
Figure 4: Percentage of isolates with varying Multiple Antibiotic Resistance index values (N = 100).

Figure 5: Resistance of *Salmonella* isolates against multiple classes of antibiotics (N = 100).

As shown in Figure 4, 98.0% of isolates showed a MAR index value of ≥ 0.2, while 90.0% exhibited MAR index value of ≥ 0.3, and 65.0% had MAR index value of ≥ 0.5. The results revealed a high prevalence of multidrug resistance as 98.0% of the isolates showed MDR, while 23.0% were resistant to five classes of antibiotics [Figure 5]. As shown in Figure 5, 18.0% of the isolates showed resistance towards six classes of antibiotics, and 16.0% of the isolates exhibited resistance against four classes of antibiotics. Two of the isolates were resistant to all the nine classes of antibiotics used in this study. Moreover, in the current study, 20.0% of the isolates showed XDR.

DISCUSSION

This study was conducted to identify the antibiotic resistance pattern among *Salmonella* spp. isolated from clinical samples in Dhaka. Nearly all (98.0%) of the 100 *Salmonella* isolates were MDR, showing resistance against at least three different classes of antibiotics. The rising incidence of antimicrobial resistance caused by MDR pathogens raises serious concerns regarding the therapeutic usability of the available antibiotics in the near future.[15]

Seventeen percent of the isolates were resistant to seven classes of antibiotics, 18.0% to six classes of antibiotics, and 23.0% to five classes of antibiotics. The results revealed that the most effective antibiotics were gentamicin and norfloxacin, against which 60.0% of the isolates showed sensitivity. B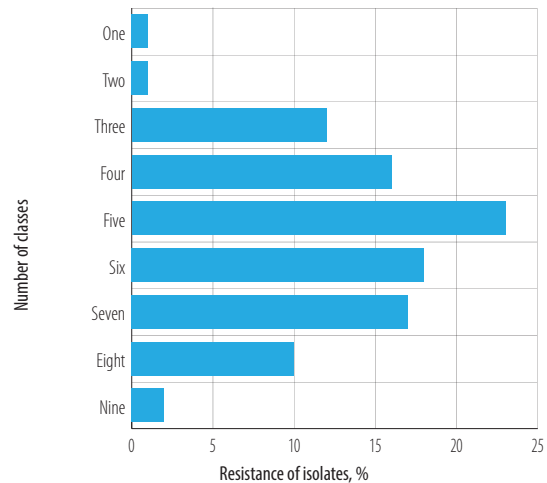road-spectrum antibiotics like imipenem or meropenem are increasingly prescribed due to the rising incidence of antimicrobial resistance, though they are reported to be more damaging to human microbiota. In a study carried out in the USA, the KPC-2 β- lactamase-producing strain of *Salmonella* was found to be resistant to most beta-lactam antibiotics, including oxyimino-cephalosporins and imipenem.[16]

The current study also revealed the significant prevalence of resistance to carbapenems, which is similar to a finding in Pakistan,[17] which also reported high (48%) resistance of *Salmonella* isolates to meropenem, a carbapenem class antibiotic. The diminishing efficacy of carbapenems against MDR pathogens indicates the urgent need for new alternatives.[18]

In the current study, 43.0% of the isolates were resistant to azithromycin, similar to a previous study in Bangladesh by Uddin et al,[19] which found 40% prevalence of resistance. However, regarding tetracyclines, the resistance found in the current study (50.0%) was nearly double of the 28% reported by Uddin et al,[19] An Iranian study in 2016 reported 100% resistance against tetracyclines among *Salmonella* spp.[20] The variation in the resistance could be attributed to the regional differences in disease patterns and MDR pathogens.[21]

Different studies have reported varying degrees of resistance against chloramphenicol among clinical *Salmonella* isolates across different countries such as 100% in Saudi Arabia,[22] 44% in Burkina Faso,[23] 50% in Kenya,[24] and 60% in Pakistan.[25] In our study, 50.0% of the isolates showed resistance to chloramphenicol.

A previous study (2018) in Bangladesh had reported 32.5% of chloramphenicol resistance, suggesting a significant increase in resistance over a relatively short period.[26]

In the 1970s, the rise of MDR *Salmonella* strains against chloramphenicol and others triggered a shift to fluoroquinolones as the preferred therapy in the South and South East Asia.[27] Within decades, MDR strains proliferated against fluoroquinolones as shown by the 2009–2011 *Salmonella* infection data from Pakistan,[28] which showed 91.7% resistance to ciprofloxacin, a fluoroquinolone derivative. A subsequent Pakistani study found that the resistance of ciprofloxacin rose marginally to 95%.[17] Southeast Asia also reported a significant rise in resistance to fluoroquinolones.[29] A study in India focused on the long-term genomic profiling of the resistant genes in *Salmonella typhi* reported 97% resistance against ciprofloxacin.[30] However, other studies reported a much lower prevalence of resistance to fluoroquinolones. From Bangladesh, Mannan et al,[31] and Habib et al,[32] reported 51% and 20% ciprofloxacin-resistant isolates, respectively. A study conducted in China documented that 20% of clinical *Salmonella* isolates were resistant to ciprofloxacin.[33] In the current study also, only 27.0% of the isolates showed resistance to norfloxacin, a fluoroquinolone molecule.

Against cotrimoxazole (a sulfonamide drug), the *Salmonella* isolates in the current study exhibited a high resistance of 68.0%. An indication that cotrimoxazole-resistant strains have been proliferating over the years was revealed by a study from Pakistan,[34] which reported a resistance prevalence of only 38.5%. A recent Pakistani study reported 66.7% resistance,[17] similar to ours. Meanwhile, a study from Bangladesh found 48.4% resistance,[26] against cotrimoxazole.

In developing countries, the evolution of MDR bacteria is a significant public health concern.[1,10] The problem is mainly related to overusing antibiotics without proper diagnosis.[4,13] In the current study, 65.0% of the isolates had MAR index values of ≥ 0.5, indicating a high prevalence of resistance in the samples from Dhaka. Moreover, 20.0% of our isolates showed XDR. However, a large South Asian survey of 8705 enteric fever cases during 2016–2019 reported no XDR isolates from Bangladesh.[35] A study in Lahore, Pakistan by Zahid et al,[25] reported the presence of 67% MDR along with 34% XDR

among *Salmonella* isolates from clinical samples. More studies from Pakistan revealed strong XDR presence including 35% by Klemm et al,[36] and 41.6% by Ambreen et al,[37] High prevalence of XDR *Salmonella* isolates have also been reported from more distant developing countries, such as a 33.3% prevalence in Egypt in 2021.[38]

Our results demonstrate an alarming rise in the prevalence of MDR and XDR pathogens in Bangladesh, which could be attributed to the irrational use of antimicrobials combined with inadequate AMR surveillance.[13] In resource-limited countries such as Bangladesh, the habit of self-medication among patients, and the indiscriminate use of antibiotics in agriculture and farming are a major contributor to the emergence of resistant bacterial strains along with poor healthcare standards.[39] Moreover, data to monitor trends and susceptibility patterns against antibiotics are infrequently produced.[4,13] Hence, the implementation of strong antibiotic stewardship programs is crucial to track the spread of MDR pathogens in Bangladesh. As the problem of AMR is multi-sectoral, a multi-disciplinary and holistic approach is necessary to ensure the best use of antibiotics, prescribe the right antibiotics, and preserve the 'last resort' antibiotics.[1,39] The overall findings of the present study will provide useful information to the local healthcare authorities towards the development of a strong antimicrobial stewardship program.

The major limitation of this study was that the clinical samples were collected from the highly urbanized Dhaka region only, which limited the generalizability of our results. For tracking the resistance patterns of *Salmonella* spp., a more detailed study with a larger sample size, covering both rural and urban areas, is recommended. More facilities providing affordable microbiological tests should be provided nationwide to enable physicians to optimize antibiotic prescriptions. Simultaneously, the prohibition against dispensing antibiotics without a prescription should be strictly enforced and monitored.

Finally, identifying the constantly evolving genes responsible for resistance to pathogens is essential. Continuous genetic research is essential to stay a step ahead of the pathogens. Several past studies have revealed intriguing commonalities in the resistance trends of pathogens in the South Asian region. This is an opportunity for strong regional cooperation

regarding genetic research on resistant pathogens. The altruistic cooperation among the international healthcare industry during the COVID-19 pandemic has shown us the way. Continuing in the same spirit, we can turn the tide against the formidable challenge of antibiotic resistance.

CONCLUSION

The current study reveals a significant and growing prevalence of MDR *Salmonella* isolates in Dhaka, Bangladesh. This calls for urgent and effective measures to monitor and regulate the prescribing, sales, and consumption of antibiotics. Countrywide expansion of screening facilities for microbial infections and causative agents using standardized approaches is highly desirable. It is crucial to conduct systematic and focused studies on the prevalence and incidence of antibiotic resistance including MDR and XDR patterns. We also recommend stronger cooperation between South Asian countries to initiate joint research on the genetic aspects of antibiotic resistance, and to reach targeted remedies.

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