Original Research Article

Antimicrobial resistance (AMR) bacteria isolated from raw milk of dairy cows: a public health concern

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ABSTRACT

Milk is harboring both pathogenic and beneficial bacteria. This study aimed to investigate the emergence of antimicrobial resistance (AMR) food borne pathogens in raw milk. The 100 milk samples of cow were aseptically collected from 10 randomly selected dairy farms of Dhaka City of Bangladesh during the period from August, 2022 to July, 2023. The collected milk samples were then processed and cultured on to selective media. The isolated microorganisms were identified based on staining, motility, cultural and biochemical properties according to standard laboratory methods. The isolated bacteria were also subjected to characterize their antibiotic sensitivity. Among 100 milk samples, 56% milk samples were reported as positive for Staphylococcus species, followed by Escherichia coli (23%), Klebsiella spp. (19%), and Salmonella spp. (9%) (P <0.001). On the other hand, 143 pathogenic bacteria were isolated and identified from the milk samples inspected of which 49.65% were Staphylococcus species, 25.17% were Escherichia coli, 18.88% were Klebsiella spp., and 6.29% were Salmonella spp. Multidrug resistant (MDR) property was observed in around 90% of the isolates (P <0.001). Among them, 100% of the Escherichia coli and Klebsiella spp. showed MDR properties followed by Staphylococcus spp. (81.69%), and Salmonella spp. (77.78%). Most of the isolates showed their resistance against ampicillin, amoxicillin, and tetracycline. Therefore, care should be taken to use antibiotic in the livestock production judiciously.

Keywords: Antimicrobial resistance, raw milk, Escherichia coli, Klebsiella, Salmonella, Staphylococcus

1. INTRODUCTION

Milk is an essential source of diverse nutrients for human consumption due to its excellent nutritional value [1]. The consumption of milk and milk products is increasing day by day worldwide including in Bangladesh [2–4]. However, the microbial safety of milk and milk products is an increasing public health concern [5]. Raw milk offer themselves as an excellent medium for the growth of spoilage and pathogenic microbes [6]. Several factors can contaminate raw milk, including a dirty milking machine, an unclean milking area, faecal contamination, inadequate storage conditions, and water adulteration of milk [7]. The common milk borne pathogens are *Staphylococcus, Salmonella, Eschericia coli, Mycobacterium, Listeria*, *Klebsiella*, etc. [8]. Earlier, few studies have been conducted regarding milk products in Dhaka city, Bangladesh. For instance, Afroz et al. [9] isolated *E. coli* and *S. aureus* from powder milk samples collected from different area of Dhaka, Bangladesh. Azam [10] isolated and identified coliform bacteria from spicy yogurt milk and plain butter milk sold in different markets of Dhaka City. Likewise, Malek et al. [11] isolated and quantified microorganisms from some common milk products within Dhaka city, Bangladesh. However, microbial contaminations in the raw milk of dairy cows of Dhaka is less documented. Moreover, the number of multidrug-resistant (MDR) bacteria increasing day by day [12]. These antimicrobial resistance (AMR) pathogens that can spread to humans via the food chain have emerged as a result of the extensive use of antibiotics in food animal production systems. Antibiotic-resistant bacterial infections have a detrimental effect on public health because they increase the likelihood of treatment failure and the severity of the illness.

AMR in bacteria isolated from raw milk poses a significant public health concern, especially in dairy farming settings. Large ruminants, such as cattle and buffalo, significantly contribute to antibiotic resistance by serving as reservoirs for resistant strains [13]. Bacteria commonly associated with disease called mastitis, such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* spp., *Proteus* spp., *Klebsiella* spp. etc., and antibiotic residues in milk following therapy can be attributed as the major causes [14]. Lubna et al. [15] identified multidrug-resistant strains of *S. aureus* and *K. pneumoniae* in raw milk samples from dairy cows, with high resistance rates to commonly used antibiotics like penicillin, oxacillin, and tetracycline. Considering the above facts, it is needed to explore a clearer picture of milk borne AMR bacteria and their resistance pattern against the commercially available antibiotics in Bangladesh is needed. Therefore, considering the public health issue, the current study aimed to look into the emergence of AMR bacteria in raw milk in Dhaka, Bangladesh.

2. MATERIAL AND METHODS

The whole study was conducted in the laboratory of the department of Microbiology and Parasitology (MIPA), Sher-e-Bangla Agricultural University (SAU), Dhaka-1207 during the period from August, 2022 to July, 2023.

2.1 Study areas and Sources of samples

A total of 100 milk samples of Cattle were collected from 10 randomly selected dairy farms of Dhaka city, Bangladesh. The collected samples were then transferred to the MIPA laboratory immediate after collection using ice box.

2.2 Isolation and identification of bacteria from milk samples

At first, milk samples were inoculated onto MacConkey (MC) agar (for the bacteria of Family *Enterobacteriacae*), and Mannitol salt (MS) agar for *Staphylococcus* species and incubated at 37°C for 24 hours. From the primary culture, individual single colony were sub-cultured onto the same media to obtain pure culture [16]. Immediate after isolation, all the isolates were preserved in agar slant and 20% buffered glycerin. All the isolated bacteria were identified based on morphological (gram staining), cultural and biochemical characteristics.

2.3 Antibiotic sensitivity tests of isolated milk borne bacteria

Antibiotic sensitivity tests were performed using disc diffusion test following the method described by Kirby-Bauer [17]. 1-2 ml of freshly growing broth culture were pour on Mueller Hinton (MH) agar media and spread uniformly. Antibiotic discs were place apart onto the surface of the inoculated plates aseptically with the help of a sterile forceps and were incubated at 37°C for 24 hours. After incubation, the plates were examined and the diameters of the zone of inhibition were measure. Then these diameters were interpreted with the standard diameters and were recorded as sensitive (S), intermediate (I) and resistant (R). Isolates those were shown resistant to more than two antibiotics were considered as multidrug resistant species [18]. The following antibiotics were used for disc diffusion test: ampicillin (AMP), 10 μg; amoxicillin (AMX), 30 μg; amoxicillin–clavulanic acid (AMC), 20/10 μg; ceftriaxone (CTR), 30μg; ciprofloxacin (CIP), 5 μg; nalidixic acid (NAL), 30 μg; erythromycin (E), 15 μg; gentamicin (GEN), 10 μg; tetracycline (TE), 30 μg; and meropenem (MEM), 10 μg.

2.4 Statistical Analysis

The data obtain from this study were analyzed using Minitab 17 program (Minitab Ltd., UK). Significant difference among the variables were calculated using Pearson's Chi-square test.

3. RESULTS AND DISCUSSION

3.1 Prevalence of the bacteria isolated from milk samples

In this study, all the isolates were identified according to their cultural (Figure 1), staining, motility, and biochemical (Table 1) properties following standard laboratory procedures.

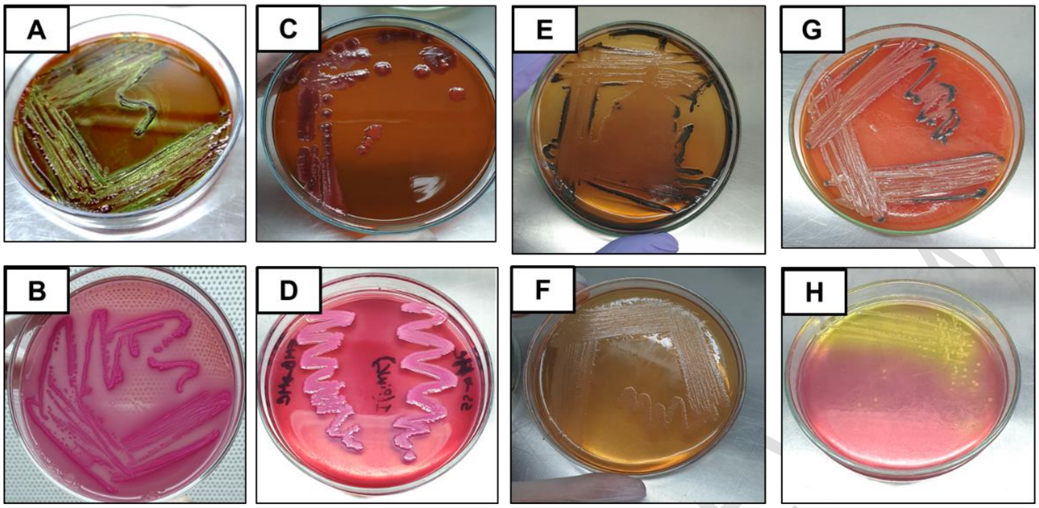

Fig. 1. Cultural characteristics of isolated bacteria on different selective agar media. *Escherichia coli produced greenish metallic sheen colonies on EMB agar (A) and pink lactose fermenter colonies on MacConkey (MC) agar (B); Klebsiella spp. produced large mucoid brownish colonies on EMB agar (C) and large pink mucoid lactose fermenter colonies on MC agar (D); Salmonella spp. produced colorless black centered colonies on SS agar (E), colorless lactose non-fermenter colonies on MC agar (F), and colorless black centered colonies with slight pinkish background on XLD agar (G).*

Table 1. Staining, motility, and biochemical properties of the isolated bacteria

Parameters		**Properties**			
		***Staphylococcus* spp.**	***Escherichia coli***	***Klebsiella* spp.**	***Salmonella* spp.**
Gram staining		Gram positive cocci with cluster arrangement	Gram negative rod	Gram negative rod	Gram negative coccobacilli
Motility		Non-motile	Motile	Non-motile	Motile
Sugar Fermentation	Dextrose	A	AG	AG	AG
	Lactose	A	AG	AG	-ve
	Maltose	A	AG	AG	AG
	Sucrose	A	AG	AG	-ve
	Mannitol	A	AG	AG	AG
MR		+ve	+ve	-ve	+ve
VP		-ve	-ve	+ve	-ve
Indole		-ve	+ve	-ve	-ve
Catalse		+ve	+ve	+ve	+ve

+ve = positive; -ve = negative; A = acid production; AG = acid and gas production

According to the data in Table 2, among 100 milk samples, 56% milk samples were reported as positive for *Staphylococcus* spp., followed by *Escherichia coli* (23%), *Klebsiella spp.* (19%), and *Salmonella* spp. (9%) (*P* <0.001). On the other hand, 143 pathogenic bacteria were isolated and identified from the milk samples inspected of which 49.65% were *Staphylococcus* species, 25.17% were *Escherichia coli*, 18.88% were *Klebsiella* spp., and 6.29% were *Salmonella* spp. Previous studies reported the presence of *Staphylococcus* spp., *E. coli*, and *Klebsiella* spp. in milk samples [19,20]. The presence of *Staphylococcus* species in raw milk samples also reported in earlier studies conducted by Jahan et al. [21], Jamali et al. [22], McMillan et al. [23], and Deddefo et al. [24]. The findings of this investigation are consistent with those of Thaker et al. [25] and Rahman et al. [26] who stated that 38% and around 30% of the raw milk, respectively were contaminated with *E. coli.* In contrast, Islam et al. [27] reported 75% of the raw milk samples were contaminated with *E coli* isolated from local market of Bangladesh. Nahar et al. [28] reported that 30% of the milk samples collected from small-scale dairy farm in Mymensingh were showed positive for *Klebsiella* species. Rahman et al. [29] reported around 2% of the milk sample contaminated with *Salmonella* species. Likewise, Aliyo and Teklemariam [30] observed *Salmonella* contamination in milk samples. However, the prevalence of all the above-mentioned isolates were varies among different studies might be due to the demographic factors, management of dairy farm, and hygienic collection of milk. The contamination of raw milk with different pathogenic bacteria is alarming to human health.

Table 2. Prevalence of the bacteria isolated from milk samples

Name of isolates	No. of sample tested	Samples positive for bacteria No. (%)	***P* value (χ2 test)**	Prevalence of the isolates No. (%)	***P* value (χ2 test)**
Staphylococcus spp.	100	56 (56)	<0.001	71 (49.65)	<0.001
Escherichia coli		23 (23)		36 (25.17)	
Klebsiella spp.		19 (19)		27 (18.88)	
Salmonella spp.		07 (7)		09 (6.29)	
Total	**-**	**-**	**-**	**143 (100)**	

No. = Number and % = Percentage

3.2 Antibiotic resistant pattern of the isolated pathogenic bacteria in raw milk

Multidrug resistance was present in around 90% of the isolates (*P* <0.001) (Table 3). Among them, 100% of the *Escherichia coli* and *Klebsiella* spp. showed MDR properties followed by *Staphylococcus* spp. (81.69%), and *Salmonella* spp. (77.78%). Earlier studies also reported the presence of MDR pathogenic bacteria in the milk samples [22–24,26–31].

Table 3. Pathogenic bacteria showed MDR properties

Name of isolated	No. of the isolates	Isolates showed MDR	***P* value**

pathogenic bacteria		properties No. (%)	(χ2 test)
Staphylococcus spp.	71	58 (81.69)	<0.001
Escherichia coli	36	36 (100.00)	<0.001
Klebsiella spp.	27	27 (100.00)	<0.001
Salmonella spp.	09	07 (77.78)	0.096
Total	**143**	**128 (89.51)**	**<0.001**

No. = Number and % = Percentage

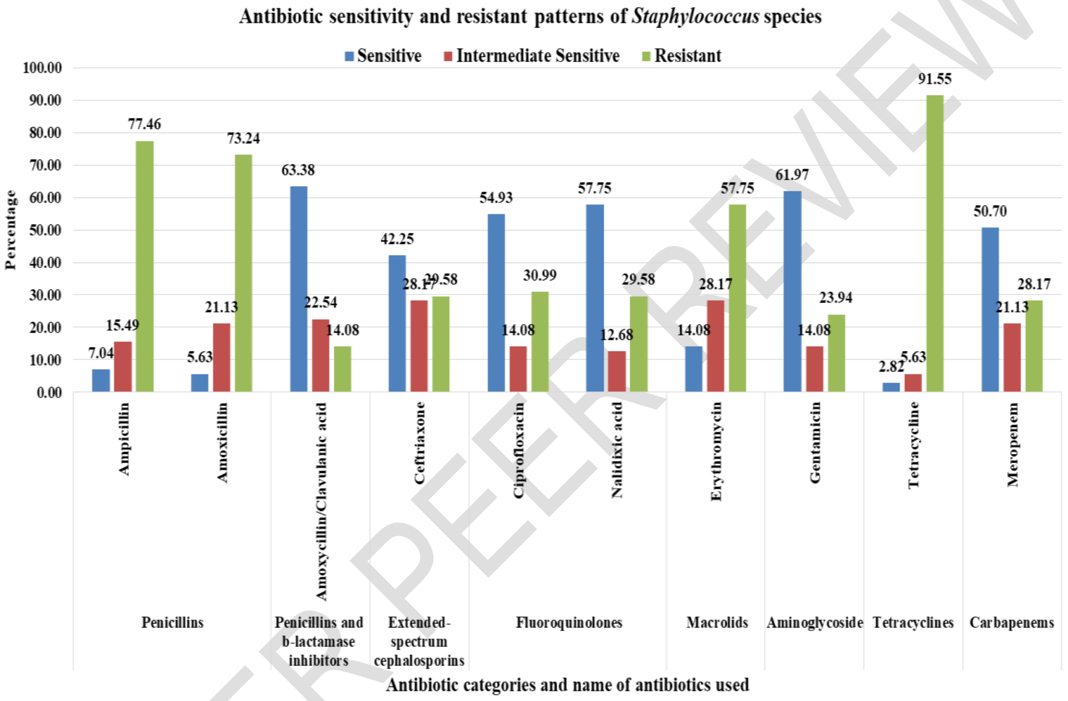

Fig. 2. Antibiotic sensitivity and resistant patterns of isolated *Staphylococcus* spp.

Around 92% of the isolated *Staphylococcus* species were phenotypically positive for resistance to tetracycline followed by ampicillin (77.46%), and amoxicillin (73.24%) while approximately 63% and 62% isolates showed their sensitivity to amoxycillin/clavulanic acid, and gentamycin, respectively (Figure 2). Likewise, Jamali et al. [22] reported that *Staphylococcus aureus* isolated form milk sample showed considerable degrees of resistance against various antibiotics such as tetracycline (56.1%), followed by penicillin G (47.3%). However, the variation of sensitivity percentage might be due the pattern of antibiotic uses between the study areas.

All the isolated *Escherichia coli* showed their phenotypic resistance to tetracycline, ampicillin, and amoxicillin, however, around 61%, 58%, and 50% of the isolates were sensitive to gentamycin, amoxycillin/clavulanic acid, and meropenem, respectively (Figure 3). This study was in close agreement with the study conducted by Bag et al. [31] who reported that the isolated *E coli* from milk developed resistance against all classes of antibiotics commonly used at the farm level in Bangladesh. They observed the highest resistance against amoxicillin (94.5%) followed by ampicillin (89.5%) and tetracycline (89.5%).

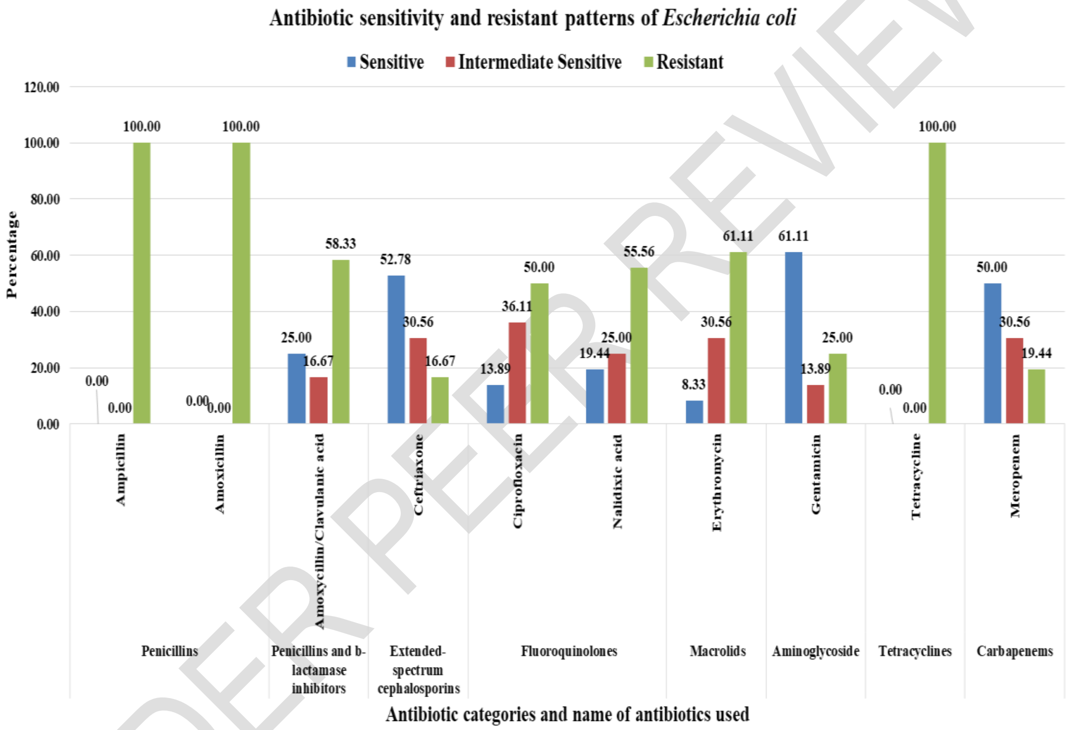

Fig. 3. Antibiotic sensitivity and resistant patterns of isolated *Escherichia coli.*

All the isolated *Klebsiella* showed their phenotypic resistance to tetracycline, ampicillin, and amoxicillin, however, both amoxycillin/clavulanic acid and meropenem showed their sensitivity for 62.96% of isolates (Figure 4). Previous study also reported that *Klebsiella* species showed 100% resistance against ampicillin, cefotaxime, and gentamicin [28]. They also showed resistance against ceftazidime (80%), cotrimoxazole/trimethoprim (40%), and oxytetracycline (30%). Likewise, Salauddin et al. [32] stated that *Klebsiella* species isolated from milk showed complete resistance against amoxicillin, erythromycin, and tetracycline.

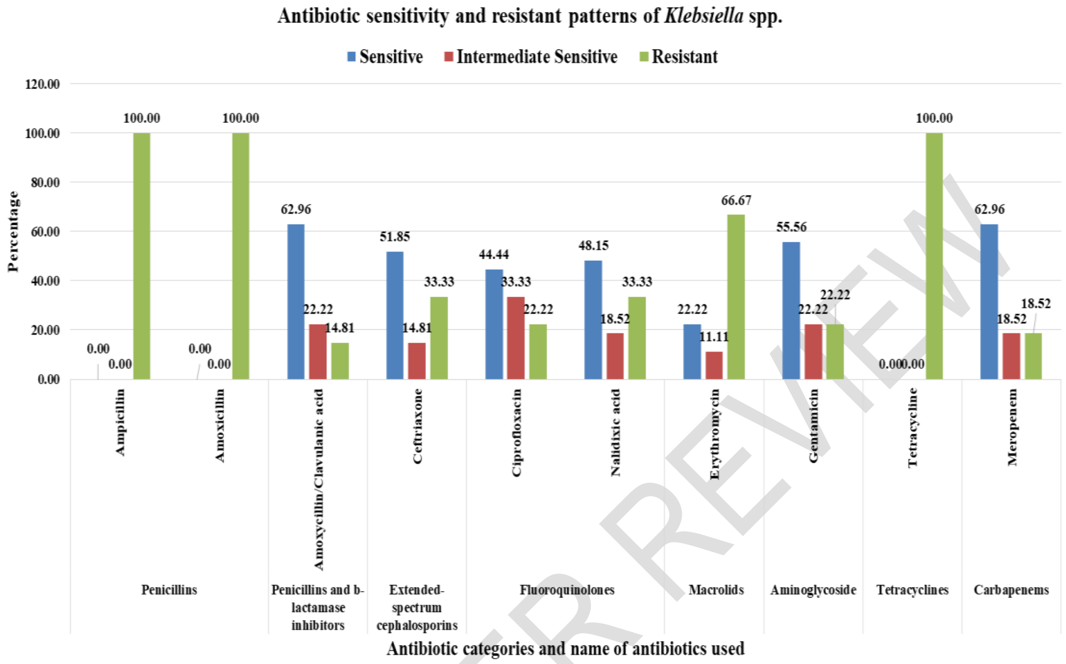

Fig. 4. Antibiotic sensitivity and resistant patterns of isolated *Klebsiella* spp.

All the isolated *Salmonella* species showed their phenotypic resistance to ampicillin and amoxicillin followed by tetracycline (77.78%). In contrast, both gentamycin and meropenem showed their sensitivity for 55.56% of isolates (Figure 5). Similarly, Gebeyehu et al. [33] reported that isolated Salmonella from raw milk showed 100% resistance against ampicillin. Likewise, Qamar et al. [34] reported that a number of multi-drug resistant *Salmonella* were reported, showing resistance various antibiotics. Numerous bacterial strains that are a threat to humans through the food chain have developed an increased resistance to antibiotics as a result of the widespread use of antibiotics in animals [35].

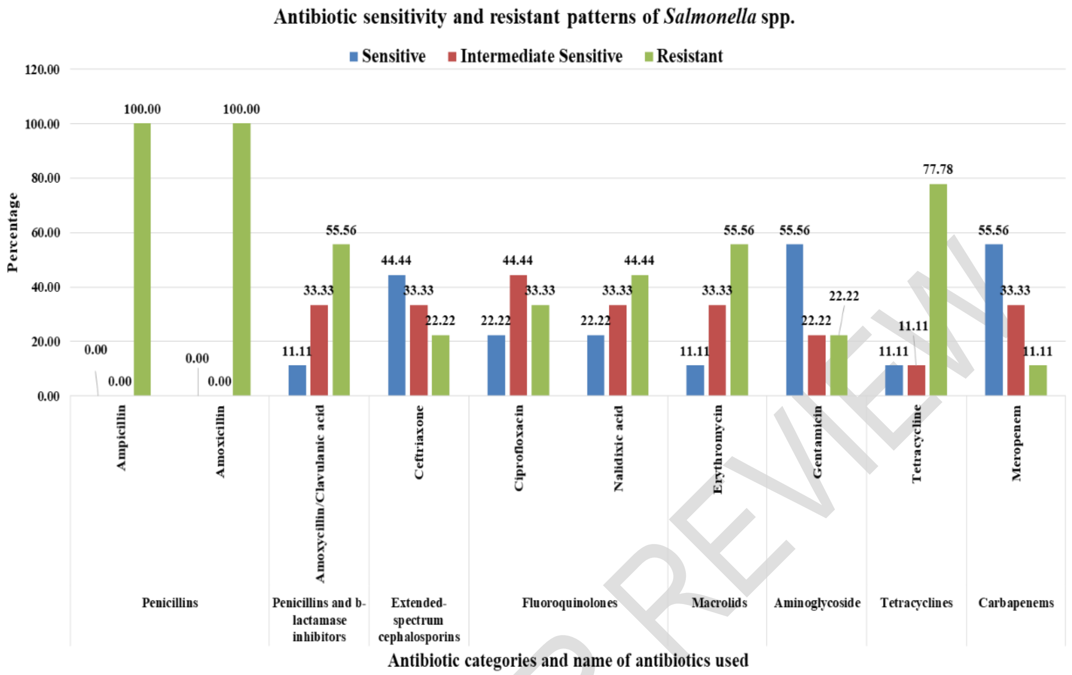

Fig. 5. Antibiotic sensitivity and resistant patterns of isolated *Salmonella* spp.

4. CONCLUSION

Food borne zoonotic *Staphylococcus* species, *Escherichia coli*, *Klebsiella spp.* and *Salmonella* spp. were isolated form raw milk samples in Dhaka city. Among them, all of the identified *Klebsiella* spp. and *Escherichia* spp. had MDR characteristics. Similarly, most of the isolated *Salmonella* spp. and Staphylococcus spp. exhibited MDR characteristics. The majority of the identified bacteria shown resistance to tetracycline, ampicillin, and amoxicillin. Therefore, care should be taken to use antibiotic in the livestock production judiciously.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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