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ORIGINAL RESEARCH

Prevalence and antibiotic susceptibility pattern of *Escherichia coli* in cattle on Bathan and intensive rearing system

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ABSTRACT

The aim of this longitudinal study was to verify the prevalence and antibiotic susceptibility pattern of *Escherichia coli* (*E. coli*) in cattle reared on Bathan and intensive farming system in Bangladesh. Fecal materials originated from recto anal junction (RAJ) of 100 cattle used for primary screening on MacConkey agar. The diversities among the pink color colony producing isolates on MacConkey agar were verified by conventional cultural methods and biochemical tests. Phenotypically positive *E. coli* isolates were further investigated for the variations in the antimicrobial resistance profiles to 10 selected antibiotics, by the disk-diffusion method. This study revealed that the overall prevalence of *E. coli* was 70% of in the rectal swab sample of cattle. However, the prevalence of *E. coli* was found significantly higher (p= 0.002) in cattle under intensive farming (84%) than cattle on Bathan (56%). Antibiotic susceptibility pattern shows that among the tested isolates 83%, 73%, 68% and 64% were sensitive to chloramphenicol, gentamicin, ciprofloxacin and ampicillin, respectively. On the other hand, all the 70 (100%) *E. coli* isolates were found resistant to tetracycline and sulfamethoxazole. A high antibiotic resistance profile was also found against amoxicillin (90%), ampicillin (87%), nalidixic acid (86%) and erythromycin (83%). In total, 24 (34%) isolates were resistant against ≥2 antimicrobials. The result clearly shows that antibiotic resistant *E. coli* isolates are commonly present in cattle of different management systems (intensive and Bathan). Therefore, careful selection of appropriate antibiotics with optimal doses might be ensured to prevent the emergence of antibiotic resistance bacteria.

Key Words: *E. coli*; Antibiotic; Resistance; Cattle; Bangladesh

Introduction

Escherichia coli (E. coli) is one of the highly diverse bacterial species. These bacteria primarily reside as commensal in the lower intestine of warm blooded animals. However, some strains of *E. coli* may emerge as harmful pathogens due to the presence of pathogenic properties and virulence genes which distinguished themselves from ordinary commensal strains (Ronsengren *et al.*, 2009). Based on the pathogenicity and difference in biochemical properties, pathogenic *E. coli* are divided into six major categories: enterohemorrhagic (EHEC), enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enteroaggregative (EAEC) and diffusely adherent (DAEC) (Nataro and Kaper, 1998). Strains of pathogenic *E. coli* may produce intestinal and extra intestinal infections, including gastroenteritis, neonatal meningitis, septicemia, urinary tract infection, hemolytic-uremic syndrome, kidney failure in human which can even lead to death (Todar, 2007).

Ruminant particularly, cattle are recognized as the main natural reservoir of pathogenic *E. coli* like STEC O157:H7 that are highly virulent to human (Caprioli *et al*., 2005). *E. coli* is genetically the most diverse group of bacteria and contains many plasmid and phage mediated genes (Saylers and Whitt, 2002). Previous reports showed that *E. coli* became highly resistant to antimicrobial agents that have been using for a long time in human and veterinary medicine (CDC, 2010). In most of the case, this antibiotic resistance mechanism is plasmid mediated while some are chromosomally mediated.

In present days, antibiotics are frequently incorporated in animal feeds and drinks as growth-promoters and for antibiotic prophylaxis. The sub-therapeutic use of these antibiotics may lead to an emergence of antibiotic-resistant bacteria (Ajayi*et al.*, 2011). The intensive use of antibiotics in food animals can lead to development of antibiotic resistant *E. coli*. Human might be infected by ingestion of contaminated food and drinking water with these bacteria that may lead to antibiotic treatment failure and other serious consequences in the patients (Anette and Ole, 2008). Recent studies in Bangladesh revealed that the presence of multidrug resistant sorbitol non-fermenting *E. coli* in smallholding cattle (Islam *et al*., 2013 and Black Bengal goat (Das Gupta *et al*., 2016 and Das Gupta *et al*., 2013). These previous findings encouraged us to investigate whether a population of dairy cattle under Bathan and intensive farming in Bangladesh is harboring antibiotic resistant *E. coli*, owing to its serious public health significance. Therefore, in this study *E. coli* from fecal samples were isolated and identified based on cultural and biochemical properties and the antibiotic resistance pattern against 10 common antibiotics was investigated using Bauer-Kirby disk-diffusion method.

Materials and methods

Study population

The study population comprised apparently healthy cattle from the commercial dairy farms of Chittagong district and from a "Bathan" in Sirajganj district, Bangladesh. Bathan is a local term used for a semi-intensive system in Bangladesh where animals are kept in the pasture encircled by a bamboo fence.

Collection and processing of fecal samples

A total of 100 rectal swab samples (50 samples from the commercial dairy farms and 50 samples from Bathan) were collected during the period of this study (2013-2014) following a convenience sampling method without repetition of animals.

Sterile cotton buds were used for the collection of fecal swab samples by inserted into the Recto anal Junction of the cattle and the swab was transferred to Buffer Peptone Water (BPW). The swab samples were transported to the Laboratory at the Department of Microbiology and

Veterinary Public Health of Chittagong Veterinary and Animal Sciences University. A cool ice box was used to maintain the cold chain. During sample collection information regarding the age, sex, breed, and antibiotic use and management systems of the animals were also recorded.

Culture protocol for isolation and identification E. coli

Buffer peptone water (primary enrichment media) containing fecal sample was incubated overnight at 37 ^{o}C. Each faecal sample was then immediately inoculated onto freshly prepared MacConkey agar (Oxoid Ltd., P^{H} 7.4±0.2) and incubated at 37 °C for 24 hours. Isolates that produced large pink colored colony were considered as *E. coli*. Isolated pink colored colonies were taken into Brain Heart Infusion Broth and incubated at 37 °C for 24 hours. For confirmation, these isolates were again inoculated onto Eosine Methylene Blue agar (EMB, Oxoid, UK) and incubated at 37 °C for 24 hours. The production of characteristic metallic green sheen like colonies was confirmed to *E. coli* positive. All strains were further confirmed using standard biochemical techniques as previously described by Garcia and Isenberg (2007). *E. coli* positive isolates were then preserved at -80 °C with 15% glycerol until use.

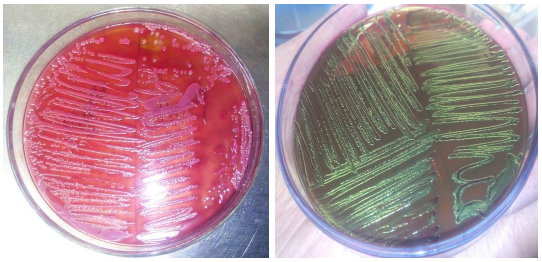

Figure 1: (a) *E. Coli* colonies on MacConkey agar. (b) *E. Coli* colonies on EMB agar

Antimicrobial Sensitivity testing

The isolates were tested against ten selected antimicrobials: Ciprofloxacin, Tetracycline, ampicillin, sulfamethoxazole, gentamicin, chloramphenicol, amoxicillin, cephalexin, nalidixic acid and erythromycin. Bauer-Kirby disk-diffusion procedure was used to perform the antimicrobial sensitivity testing. For that purpose, Mueller-Hinton (MH) agar, prepared according to the manufacturer's instructions (Oxoid) was used. The antimicrobial micro-disks from HIMEDIA Ltd (Mumbai, India) were used and the result was interpreted according to Clinical and Laboratory Standards Institute CLSI, 2007(Table 1). The isolates were considered as "resistant (R)", "intermediately resistant (I)" and "sensitive (S)" according to their zone of inhibition and group of antibiotics used for the assays, based on the performance standards from the CLSI (CLSI, 2007). Isolates showing resistance to at least two of the antimicrobials tested were considered as multi-drug resistant *E. coli*.

Data analysis

Collected data were entered into Microsoft office 2007 Excel worksheet. Statistical analyses of the data were performed using GraphPad software (http://www.graphpad.com/quickcalcs/contingency). Differences among the variables were calculated using Chi-square ($\chi 2$) test. *P* values less than 0.05 were considered as significant.

Results

Prevalence of *E. coli*

A total of 100 rectal swab samples were examined, 84 (84%) of the isolates produced large pink colored colony on MacConkey agar were primarily considered as positive for *E. coli* (Figure 1.a). These isolates were again inoculated onto EMB agar, where only 70 (70%) isolates produced metallic green sheen (Figure 1.b). All the EMB positive isolates were tested positive to methyl red (MR) and indole production but negative to Voges-Proskauer (VP) test which then finally confirmed as *E. coli*.

The prevalence of *E. coli* was significantly higher (p= 0.002) in cattle under intensive farming than cattle on Bathan (Table 2). Results show that *E. coli* were more prevalent in the calves, female, cross breed cattle and non-diarrheic animals in comparison with the adult, male, local and diarrheic animals, respectively; however, the differences were not statistically significant (Table 3).

Antimicrobial sensitivity

The antibiotic susceptibility pattern of *E. coli* against 10 antimicrobials is shown in Figure 2. All the 70 *E. coli* isolates were found resistant to tetracycline and sulfamethoxazole. The majority of isolates have shown resistance to amoxicillin (90%), ampicillin (87%), nalidixic acid (86%) and erythromycin(83%). Antibiotic susceptibility profiles show that among the tested isolates 83%, 73%, 68% and 64% were sensitive to chloramphenicol, gentamicin, ciprofloxacin and ampicillin, respectively. In total, 24(34%) isolates were resistant to ≥2 antimicrobials (Figure 3).

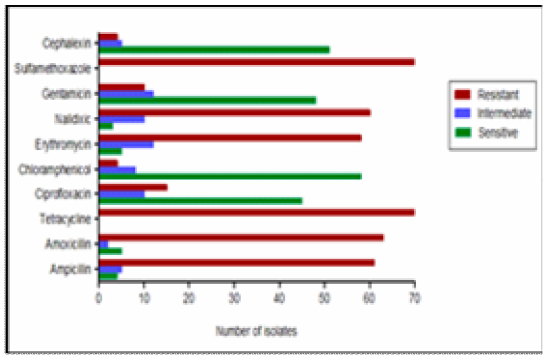

Figure 2. Antibiotic susceptibility pattern of *E. coli* in cattle

Discussion

In recent time, few studies were performed for isolation and characterization of *E. coli* from healthy cattle, small holding cattle and diarrheic calves in Bangladesh (Islam *et al*., 2015; Islam *et al.*, 2015; Hassan *et al.*, 2014). However, prevalence and characterization of *E. coli* from dairy cattle reared in different management systems e.g. Bathan (semi-intensive) vs. intensive was ignored. This study revealed that the overall prevalence of *E. coli* was 70% in cattle whereas 84% and 56% prevalence was observed in cattle of intensive and Bathan, respectively.

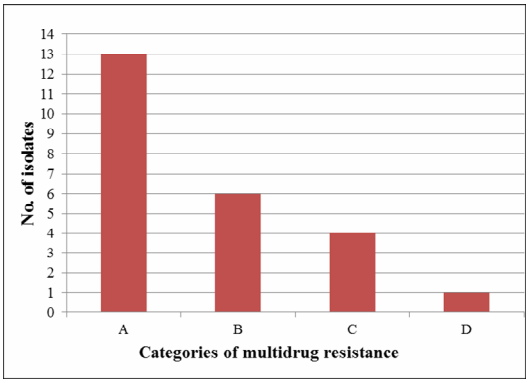

Figure 3. Distribution of multidrug resistance to 10 antimicrobials among *E. coli* isolates (n = 24). A = resistance to 2 - 3 antimicrobials; B = resistance to 4–5 antimicrobials; C = resistance to 6–7 antimicrobials; D = resistance to>7antimicrobials

Table 1. The concentrations and interpretative standard zone diameters of different antimicrobials (CLSI, 2007) used for this study to interpret the results

Group of Antimicrobial agent	Antimicrobial agent	Disk content	Zone diameter, nearest whole mm		
			R	I	S
Penicillin	Ampicillin	25 µg	≤ 13	14-16	≥ 17
B-lactamase inhibitor combination	Amoxicillin	30 µg	≤ 13	14-17	≥ 18
Cephalosporin	Cephalexin	30 µg	≤ 18	19-20	≥ 21
Amino glycosides	Gentamicin	10 µg	≤ 12	13-14	≥ 15
Tetracycline	Tetracycline	30 µg	≤ 11	12-14	≥ 15
Fluoroquinolones	Ciprofloxacin	5 µg	≤ 15	16-20	≥ 21
Macrolid	Erythromycin	15 µg	≤ 15	16-20	≥ 21
Quinolones	Nalidixic acid	30 µg	≤ 13	14-18	≥ 19
Folate pathway inhibitor	Co-Trimmoxazole	25 µg	≤10	11-15	≥ 16
Phenicoles	Chloramphenicol	30 µg	≤ 12	13-17	≥ 18

The prevalence of *E. coli* was significantly higher (p=0.002) in the intensive dairy cattle than semi-intensive cattle of Bathan. This might be attributable to the high density of animals in intensive system (Miller, 2000), which could elevate the risk of pathogenic *E. coli* infection among the dwellers and associated farm personnel. The differences in the prevalence of *E. coli* in adult animals compared to the calves, in female compared to males, cross-bred animals compared to the local animal and in diarrheic animals compared to the non-diarrheic animals could not be anticipated from this study, because of unrepresentative sample size.

Table 2. Prevalence of *E coli* in cattle on Bathan and intensive farming

Raring system	Number of sample	Positive	Negative	Prevalence (%)	$\chi 2$ value	P value
Bathan (Semi-intensive)	50	28	22	56	9.33	0.002
Intensive farm	50	42	08	84		

The overall prevalence of *E. coli* in this study supports the finding of Hassan *et al*. (2014), who stated a prevalence of 75% in healthy cattle in Mymensingh, Bangladesh. Our results corroborated with the findings of Ogunleye *et al*. (2013), but much lower than the findings of Masud *et al*. (2012). Differences in geographical locations might be the reason for this as Islam *et al*. (2014) reported a geographical variation in the prevalence of *E. coli* in cattle.

Table 3. Prevalence of *E. coli* in different groups of animal

Variables	Category	N	Positive	Negative	Prevalence (%)	$\chi 2$ value	P value
Age	Calf	25	18	7	72	0.0635	0.801
	Adult	75	52	23	64		
Sex	Male	31	19	12	61	1.62	0.203
	Female	69	51	18	74		
Breed	Cross	68	49	19	72	0.43	0.513
	Local	32	21	11	66		
Antibiotic used	Yes	29	17	12	59	2.52	0.113
	No	71	53	18	75		
Presence of diarrhoea	Yes	29	18	11	62	1.22	0.269
	No	71	52	19	73		

The antimicrobial susceptibility patterns observed in the isolates from cattle of both management systems (intensive and Bathan) towards 10 antimicrobials summarized that the isolates were diverse in their antimicrobial resistance spectrum. In the present study, 100% E. *coli* isolates showed resistance to tetracycline and sulfamethoxazole that agrees with the findings of some previous reports (Zhao *et al*., 2001). This resistance pattern might have relation with the wide use of sulfamethoxazole and tetracycline in treating enteric bacterial in Bangladesh. Tetracycline has been used for many years in treating the infectious disease of food animals due to their low cost, broad antimicrobial activity, ease of administration, and effectiveness (Prescott *et al*., 2000). Tetracycline has also been using as a growth promoter in animal feed for long time (Walsh, 2003). Taken together these observations support the high resistance of *E. coli* against tetracycline found in this study. Remarkable resistance also observed against ampicillin and erythromycin which supports the findings of Parveen *et al*., (2005) who reported that *E. coli* isolated from the manure of cattle had high levels of resistance to commonly used antibiotics such as ampicillin, erythromycin and tetracycline. The sensitivity against chloramphenicol (83%) was in line with the finding of Sarada *et al*., (2008). In Bangladesh, although chloramphenicol is not approved for use in food animals except some eye drop and topical cream, however, illegal use of chloramphenicol was reported in livestock particularly in poultry (Bakar *et al*., 2013). The illegal uses might be the reason for some resistance against chloramphenicol. The sensitivity of the *E. coli* isolates to gentamycin was 73 %, which justify the susceptibility pattern reported by Islam *et al*., (2013) and Das Gupta *et al*., (2013).These two studies also showed that multi-drug resistant *E. coli* isolates are prevalent in livestock of Bangladesh which supports our findings (Figure 3). It is recommended that the use of antimicrobial agents in food animals should follow an effective guideline and maintain proper slaughter hygiene to reduce the transmission of resistant bacteria to humans.

Conclusion

The result clearly showed that antibiotic resistant *E. coli* isolates are commonly present in cattle of different management systems (intensive and Bathan). This might have relation with the inappropriate use of antibiotics for therapeutic purpose. The sub therapeutic use of antibiotics for growth promotion and veterinary prophylaxis has probably contributed to an increased pressure in the prevalence of antimicrobial-resistant bacteria in food animals and which in turn; contribute to increased prevalence of antibiotic-resistant bacteria in humans that can produce serious and life-threatening antibiotic-resistant infections. Therefore, careful selection of appropriate antibiotics with optimal doses might be ensured to prevent the emergence of antibiotic resistance bacteria.

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Conflict of interest

The authors have declared no conflict of interest

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