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Prevalence of mastitis and antimicrobial resistance patterns of *Escherichia coli* and *Staphylococcus aureus* isolated from the infected udder of dairy cows in coastal regions

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Abstract

Prevalence of mastitis and their antimicrobial resistance in Amtali (sub-district) area of Barguna District were studied. Mastitis was diagnosed by examining the udder and milk of 300 dairy cows. The bacteria were cultured and biochemical tests and antimicrobial profiling were done. The overall prevalence of mastitis was 5.0%, and cross-bred and local cows had 6.4% and 3.8%, respectively. The prevalence was higher in animals 7 - 8 years old (5.9%) and in cows of 3rd - 4th parity (8.8%). Cows with peri-parturient diseases and 1st - 2nd lactation had prevalence of 6.8% ($P<0.05$) and 5.7%, respectively. The prevalence in dry and wet seasons was 33.3% and 66.7% ($P<0.10$), respectively, and 53.3% of cows were affected with mastitis when the floor was wet and soiled. The prevalence was 73.3% ($P<0.05$) in unhygienic conditions. The prevalence of *E. coli* and *S. aureus* in mastitis milk samples was 73.3% and 66.7%, respectively. *E. coli* was sensitive to amoxicillin (36.4%), ampicillin (36.4%), tetracycline (54.6%), streptomycin and co-trimoxazole (81.8%), gentamicin & ceftriaxone (90.9%), cefuroxime and cefixime (100%). *S. aureus* was sensitive to co-trimoxazole (60%), tetracycline (70%), amoxicillin, ampicillin, streptomycin (80%), gentamicin, ceftriaxone (90%), cefuroxime and cefixime (100%). (*Bang. vet.* 2024. Vol. 41, No. 1 – 2, 13 – 22)

Introduction

Mastitis is a major economic burden on the dairy industry, affecting milk production and quality of milk (Abebe *et al.,* 2016). Mastitis is recognized as one of the costliest diseases in the dairy industry (Rahman *et al.,* 2009). Many microbes cause mastitis (Jamali *et al.,* 2018). These include both contagious and environmental bacteria, in addition to fungi, algae, and viruses. There is significant variation in the distribution of mastitis and mastitis-causing pathogens among countries, regions, and farms

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(Verbeke *et al.,* 2014). The most frequently isolated pathogens associated with mastitis in China are *E. coli, Klebsiella spp.*, NAS, *Streptococcus dysgalactiae*, and *S. aureus* (Gao *et al.,* 2017). Antimicrobials are used in the dairy industry for the prevention and control of mastitis and other bacterial diseases (Oliver and Murinda, 2012). Dependence on antimicrobials has become widespread on dairy farms. Broad-spectrum antibiotics are commonly used for controlling mastitis, but chloramphenicol, ciprofloxacin, novobiocin, vancomycin, and tetracycline were reported to have poor effectiveness against *S. aureus* (Deb *et al.,* 2013). Beta-lactam antibiotics are frequently used in mastitis therapy, but resistance has developed (Olsen *et al.,* 2006). Antimicrobial resistance (AMR) happens when bacteria and fungi develop the ability to defeat the drugs designed to kill them. Multiple studies have demonstrated irrational use of antimicrobials by practitioners, and the indiscriminate use of antibiotics in agriculture (Sutradhar *et al.,* 2014). AMR remains a serious threat to public health in Europe (ECDC, 2017), and is one of the biggest threats to health, food security and development. South East Asia poses the greatest risk to AMR dissemination (Chereau *et al*., 2017). Bangladesh has recently approved a National Action Plan for containing AMR, in alignment with the WHO and GAP guidelines. With this in mind, this study was undertaken with the following objectives:

- To know the prevalence and risk factors of clinical mastitis in dairy cows in a coastal area;
- To investigate the antimicrobial resistance profile of *E. coli* and *S. aureus* isolated from mastitis-infected animals.

Materials and Methods

This study was conducted at Amtali Upazila (Sub-district) under Barguna district a coastal area of Bangladesh from 1st January to 31st December 2020.

Data collection: The data were collected directly from the farmer by interviewing and by observing the cows. A pre-test questionnaire was prepared before data collection. The complaints of affected animals were recorded carefully asking questions to the farmer. Month, date, age, sex, and breed were recorded. The diseases were diagnosed primarily based on clinical signs, the owner's statement, and physical examination of the udder and teats of infected animals. The risk factors of mastitis: peri-parturient diseases, floor condition, and hygienic management of the farm were considered.

Sample Collection & preservation: A total of 300 milking cows were examined for clinical mastitis and 15 milk samples from cows with mastitis were collected. The samples were stored at-20^{o}C and transferred to the laboratory in a cool-box.

Isolation and Identification of *E. coli* & *Staphylococcus*: The samples were prepared for bacteriological culture as described by Ezatkhah *et al.* (2016). In brief, primary culture was done by mixing in PBS and culture in nutrient broth. Pure cultures of bacteria were done in EMB, Blood, Mannitol salt, and MacConkey agars where the colony characteristics were detected to identify *E. coli* and *Staphylococcus spp*. Catalase test and methyl red test were done to identify the bacteria.

Antimicrobial resistance pattern of isolated bacteria: The antimicrobial sensitivity testing of each isolate was carried out by the Kirby-Bauer disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) procedures (CLSI, 2020). Antimicrobial sensitivity discs used were gentamicin (GEN), amoxicillin (AMX), cefuroxime (CXM), tetracycline (TE), ampicillin (AMP), ceftriaxone (CTR) and cefixime (CFM), streptomycin (S), co-trimoxazole (COT). The zone diameters were translated into sensitive, intermediate, and resistant categories.

Statistical analysis: Data were entered into Microsoft Excel worksheets. Prevalence was defined as the number of cases of mastitis per 100 cows tested. SPSS software was used for conducting χ^2 test to compare the significance of prevalence of mastitis.

Results and Discussion

Prevalence of mastitis and factors influencing udder infection

Three hundred dairy cows were investigated for mastitis. Among these, 15 cases were recorded in one year with the prevalence at 5%. These findings were lower than Rahman *et al.* (2009) who found 19.9%, but Bari *et al.* (2014) reported overall prevalence of mastitis at 8.4%. Faruk *et al.* (2018) recorded the prevalence of clinical mastitis in cows at 11.0%. The difference was due to the smallholder farming system at Amtali Upazila (Sub-district) of Barguna district, where farm management system was good with small number of animals of 5 - 10.

Among 160 local cows, the prevalence rate was 3.8% (n = 6). Among 140 crossbred cows, the prevalence was 6.4% (n = 9) (Table 1). This finding was supported by Bari *et al.* (2014) who reported significantly higher prevalence rate of mastitis in crossbred cows (10.1%) than in indigenous cows (4.3%). Faruk *et al.* (2018) reported higher rate of mastitis in crossbred cows (15.2 %) than the local breed (6.7%). Hossain (2004) reported that high-yielding cows were more prone to udder infection than low-producing ones. It might be due to the larger udder and genetic predisposition. Cows are categorized into age groups 3 - 4, 5 - 6, 7 - 8 and 9-10 years and the prevalence rates were 3.1, 4.3, 5.9% and 6.3%, respectively (Table 1).

The prevalence of mastitis in cows having the peri-parturient disease (Abortion, retained placenta, milk fever, uterine prolapse, dystocia etc.) was 6.8% and in cows without a history of such disease was 1.8% which was significant at $P<0.10$ (Table 1). This result was supported by the report of Bari *et al.* (2014) where cows without a history of peri-parturient disease had a prevalence of 3.7% mastitis, but cows with a history of peri-parturient disease had a prevalence of 33.7%. The result was agreed by Rahman *et al.* (2009). The occurrence of 5.7% mastitis at the 1st - 2nd and 3rd - 4th months of lactation was evident, whereas 3.3% was at the 5th - 6th month of lactation (Table 1).

At the beginning of lactation and the 3rd month of the lactation, milk production was higher and had a greater chance of getting infection in udder.

Table 1: Prevalence of mastitis and animal-related factors

Parameters		Total No. of Animal	No. of Mastitis positive cases	Pre-valence (%)	χ^2 value	P-value
Total Prevalence		**300**	**15**	**5.0**	**-**	**-**
Breeds	Local	160	6	3.8	1.128	0.288
	Cross	140	9	6.4		
Age (Years)	3-4	65	2	3.1	0.984	0.805
	5-6	70	3	4.3		
	7-8	85	5	5.9		
	9-10	80	5	6.3		
Parity (No.)	1st-2nd	90	3	3.3	3.589	0.166
	3rd-4th	80	7	8.8		
	≥5th	130	5	3.8		
Peripartur ient diseases	Yes	110	2	1.8	3.702*	0.054
	No	190	13	6.8		
Lactation Period (Months)	1st-2nd	70	4	5.7	0.752	0.687
	3rd-4th	140	8	5.7		
	5th-6th	90	3	3.3		

*Significant at $P<0.05$

The prevalence of mastitis was 3.1% in cows aged 3 - 4 years, which was the lowest prevalence. The prevalence of mastitis was higher at 6.3% in cows aged 9 – 10 years. Cows more than 9 - 10 years of age may have poor immunity, loose sphincter of teat canal that help bacteria to enter the udder. Faruk *et al. (*2018) found that mastitis was higher in cows above seven years old (16.9%), moderate in 5 - 7 years (9.5%) and lowest in cows less than four years old (8.2%). Sinha *et al.* (2011) reported that the prevalence of mastitis in cows age 3 - 4, 5 - 6, 7 - 8 and 9 - 10-years old were 33.3, 42.5, 45.3%, and 52.8%, respectively. Many studies agreed with the present findings of higher percentage of mastitis in older animals (Quaderi, 2005). Husain (2007) showed that older cows at about 14 years of age had 61% sub-clinical mastitis, in agreement with the present findings.

The occurrence of mastitis during different parity is shown in Table 1. Higher number of mastitis was 8.8% during the 3rd - 4th parity than 1st - 2nd parity 3.3% and ≥5th Parity 3.8%. This result was consistent with the observation of Sinha *et al.* (2011) who reported that the occurrence of mastitis in cows at parity 1 - 2, 3, 4 and 5 - 7 was 32.9, 50.6%, and 62.5%, respectively.

Season and management-related factors influence the outbreak of mastitis

Five cows in dry season (33.3%) and 10 cows in the wet season (66.7%) were significantly (P<0.01) affected (Table 2). The finding is supported by Bhuiyan *et al.* (2010) who reported that 347 cows in the dry and 388 cows in the wet seasons had prevalence of 19.9% and 44.8%, respectively. Rahman *et al.* (2009) explained that in wet season land was submerged and floor was muddy.

The rate of mastitis depending on floor condition is presented in Table 2. The occurrence of mastitis was 26.7% in cows living with brick-block floors and 20.0% in those with soil floors (Table 2). When the floor was wet and soiled 53.3% of cows were affected with mastitis. Kivaria *et al.* (2004) showed that water contamination as one of the potential risk factors for the occurrence of mastitis.

Table 2: Season and management factors influence the outbreak of Mastitis

Parameters		No. of infected case	Prevalence (%)	χ^2 value	P-value
Season	Dry (late October-mid June)	5	33.3	3.333**	0.068
	Wet (late June - mid October)	10	66.7		
Floor Condition	Brick	4	26.7	4.20	0.122
	Soiled	3	20		
	Partly or completely wet and soiled	8	53.3		
Cleanliness of Farm	Clean	4	26.7	6.533*	0.011
	Dirty	11	73.3		

*Significant at P<0.05, ** Significant at P<0.10

Table 2 showed that among 15 infected animals 73.3% cows were reared in dirty condition and 26.7% cows were reared in clean condition (P<0.05). This result was supported by the findings of Chishty *et al*. (2007) who reported that the prevalence of mastitis is higher in cows managed with poor drainage systems.

The overall prevalence of causal agents

The overall prevalence of *E. coli* and *Staphylococcus aureus* was 73.3% and 66.7%, respectively (Table 3). The prevalence of *E. coli* infection was higher compared to *Staphylococcus* infection.

Table 3: Prevalence of isolated & identified causal agent

Causal Agents	Total Sample	No. of Positive Sample	Prevalence (%)	χ^2 value	P-value
E. coli	15	11	73.3	0.159	0.690
Staphylococcus aureus	15	10	66.7		

This finding is closely similar to the findings of Kayesh *et al. (*2014) who reported that in Barishal, *Staphylococcus spp.* was most predominant isolate where prevalence was 73.3%, which was followed by *E. coli* (6.7%) and that was lower than the present finding. Bitew *et al.* (2010) reported a 72.2% prevalence of *Staphylococcus spp*. in clinical and subclinical mastitis, which was similar to this study. Chandrasekaran *et al. (*2014) reported that out of 401 clinical mastitis samples 184 (45.9%) were positive for *E. coli* and 162 (40.4%) were positive for *S. aureus.* This variation was due to environmental and ecology of Barguna coastal area in Bangladesh. Rahman *et al*. (2013*)* stated that *Staphylococcus spp.* (62.5%) and *E. coli* (31.3%) was identified as causal agents of mastitis. This study denoted that the major pathogen for mastitis infections was *E. coli* and *Staphylococcus spp.* and those pathogens were resistant to several antimicrobial drugs.

Antimicrobial Resistance Profiling of E. coli

The antimicrobial sensitivity of the isolates is shown in Figure 1. Amoxicillin showed high resistance 45.5% followed by ampicillin at 36.4%, tetracycline at 36.4%, streptomycin 18.2%, co-trimethaxole 18.2%, gentamicin 9.1% and ceftriaxone 9.1%.

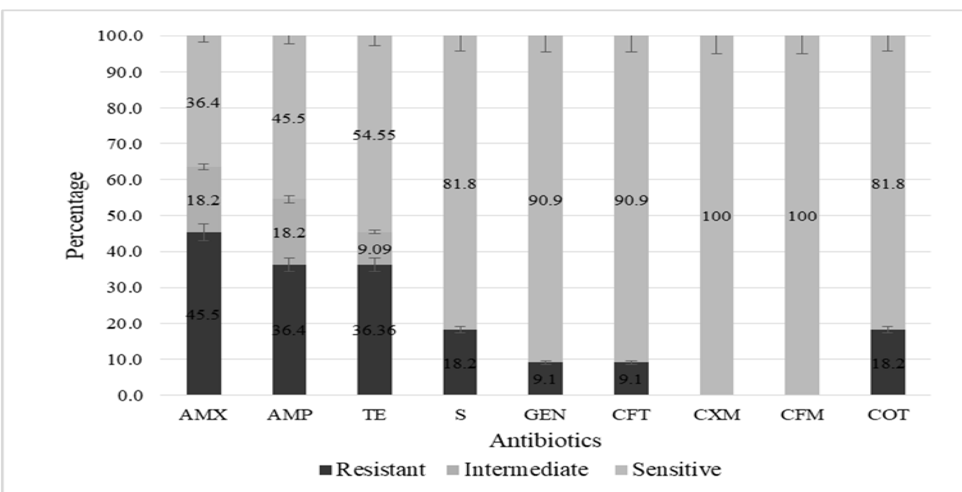

Fig. 1: Antimicrobial resistance profile of *E. coli.*

Resistance to amoxicillin was 18.2%, ampicillin 18.2% and tetracycline 9.2%. *E. coli* were 100% sensitive to cefuroxime and cefixime. This study was in agreement with

Moges *et al. (*2011) who reported that *E. coli* showed less sensitivity to ampicillin (40%), tetracycline (40%) and was highly sensitive to streptomycin (80%). Chandrasekaran *et al.* (2014) showed that *E. coli* was sensitive to gentamicin (73.1%) and ceftriaxone (69%). The isolates had the highest resistance to amoxicillin (52.1%) and oxytetracycline (48.0%). Gashe *et al.* (2018) reported that *E. coli* (73%) was resistant to ceftriaxone and 41(65%) ceftazidime. Moges *et al.* (2011) reported 76% resistance to ampicillin, 18% to tetracycline, 6% to streptomycin and amoxicillin, 24% to ampicillin, 12% to amoxicillin, 21% to tetracycline, 82% to amoxicillin, and tetracycline, but 72% were sensitive to streptomycin. Majumdar *et al.* (2021) reported that resistance among *E. coli* isolates was highest towards streptomycin (17.7 %) followed by tetracycline (15.9 %) and ampicillin (11.5 %), whereas less than 10 % resistance was seen towards the remaining antimicrobial. Alamin *et al. (*2020) reported that *E. coli* isolates were resistant to amoxicillin (60.3 - 100%), ampicillin (65.4-100%), tetracycline (89.4-100%), sulfamethoxazole (100%), and streptomycin (47.4-100%) and gentamicin (37.2%) had resistance genes among the antimicrobial multidrug resistance (AMR) *E. coli* were isolated from animal in Bangladesh. In the present study, *E. coli* was more or less resistant *to* all antimicrobials except cefixime and cefuroxime.

Antimicrobial Resistance Profiling of Staphylococcus aureus

Figure 2 showed that *Staphlococcus aureus* was highly resistant to co-trimoxazole at 30% followed by ampicillin 20%, tetracycline 20%, amoxicillin 10%, streptomycin 10%, gentamicin 10%, and ceftriaxone 10%. *Staphlococcus aureus* was 100% sensitive to cefuroxime and cefixime. Moderate resistance was shown to amoxicillin; tetracycline, streptomycin and co-trimoxazole at 10%. Among these antimicrobials, cotrimethaxole/trimethoprim showed 60% sensitive, tetracycline showed 70% sensitivity, but amoxicillin, ampicillin, and streptomycin showed 80% sensitivity. Gentamicin, ceftriaxone showed 90% sensitivity, whereas cefuroxime and cefixime had shown 100% sensitivity.

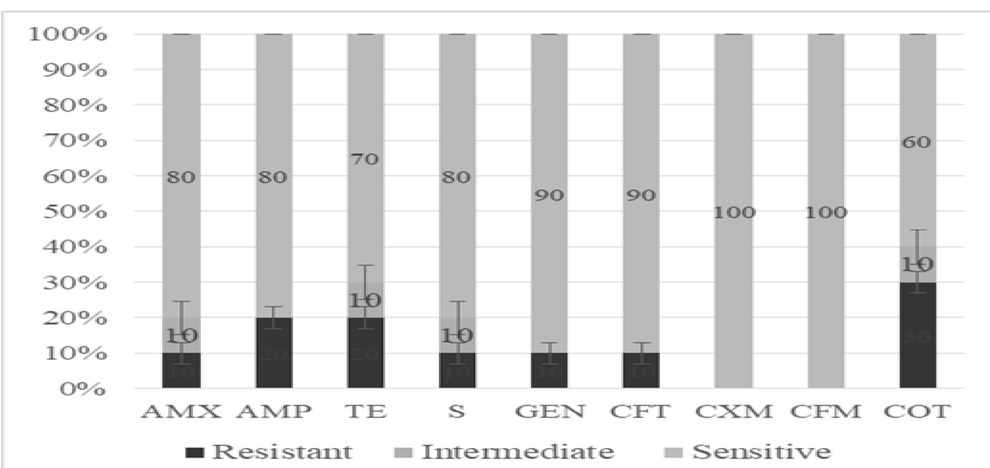

Fig. 2: Antimicrobial resistance profile of *Staphlococcus aureus.*

Those findings were consistent with Alamin *et al. (*2020) who reported that *Staphylococcus spp*. was resistant to amoxicillin (42-100%), ampicillin (73–100%), streptomycin (70-100%), tetracycline (30.8-88.0%), sulfamethoxazole-trimethoprim (30.8%) in dairy and other animals in Bangladesh. Schmidt (2011) reported that *Staphylococcus spp*. in Denmark, Brazil, and Argentina had 75.0, 55.1% and 40.0% antimicrobial resistance, respectively. Moges *et al*. (2011) reported that *S. aureus* was less sensitive to ampicillin (18.5%), streptomycin (51.8%), and highly sensitive to tetracycline (70.4%). *Staphylococcus aureus* was more sensitive to gentamicin (71.2%) and ceftriaxone (69.2%). The isolates had the highest resistance to amoxicillin (61.5%) and oxytetracycline (49%). Gashe *et al. (*2018) reported that *Staphylococcus aureus* accounted for 19% of the total bacterial isolates, and showed 23.4% and 34.0% resistance to ceftriaxone and ceftazidime, respectively. Unakal and Kaliwal (2010) reported that *Staphylococcus aureus* was susceptible to ceftriaxone 80.9% followed by cefotaxime 79.4%, gentamicin 52.9%, amoxicillin 36.8% and ampicillin 29.4%. Sharma *et al. (*2015) reported that *Staphylococcus aureus* was resistant to cefixime 66.7%, streptomycin 44.4%, ampicillin 33.3%, cefuroxime, gentamicin and tetracycline (22.2%). Resistance to antimicrobial agents is increasing.

Conclusions

The study showed a higher occurrence of clinical mastitis in cross-bred cattle, higher age and parity, dirty farms, and in the wet season. In case of drug resistance, *E. coli* and *Staphylococcus aureus* showed resistance to multiple antimicrobials. Awareness of practitioners and clients for rational use of antimicrobials can contribute positively to reduce it resistance rates and to be more conscious about the use of rational antimicrobials drugs.

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