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Incidence and Antibiotic Susceptibility Profile of *Pasteurella maltocida* Isolates Isolated from Goats in Savar Area of Bangladesh

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ABSTRACT

In Bangladesh, goat farming is substantially hampered due to outbreaks of respiratory infectious diseases like pneumonia causing morbidity, mortality and economic losses in Black Bengal goats. Among the infectious agents, *Pasteurella maltocida* is more frequently associated with the outbreak of acute pneumonia and death of goats. Hence, the occurrence of *P. multocida* in the goat population should be regularly investigated to effectively control the disease. Furthermore, antibiotic resistance/sensitivity profiling of *P. maltocida* also needs to be regularly updated for designing or updating of efficient treatment strategy. In this investigation, 150 nasal swab samples from goats were collected from Savar region of Bangladesh and on the basis of colony, staining and biochemical characteristics *P. multocida* were isolated in 25 samples. Disc diffusion assay was used to determine the antimicrobial susceptibility of 20 isolates against 9 different antimicrobial agents. This study revealed that the rate of *P. multocida* isolated from goats in Savar area was about 16.67%. The incidence of *P. multocida* was higher in goats affected with acute pneumonia than apparently healthy goats. *P. multocida* was fully resistant to penicillin and amoxicillin whereas showed high sensitivity towards ciprofloxacin followed by streptomycin and neomycin. This study suggests that ciprofloxacin, streptomycin and neomycin are potent anti-*Pasteurella multocida* drugs.

Key words: Antibiotics, Goat, *Pasteurella multocida*, Pneumonia, Prevalence, Resistance.

INTRODUCTION

Goat rearing is one of the profitable sectors of livestock and it meets the increasing protein demand of gradually increasing population of Bangladesh (Husain, 1993). Black Bengal goat known as “poor man’s” cow has importance in rural economy and can be considered as a tool for poverty reduction in Bangladesh (Husain, 1993; Alam, 1993). However, goat farming is substantially hampered due to outbreaks of diseases such as pneumonia, goat pox, contagious ecthyma, enterotoxaemia, tetanus, foot and mouth disease, brucellosis, mastitis and metritis, mycotic diseases and ricketsial infections (Siddiky, 2017). Respiratory infectious disease like pneumonia is characterized by anorexia, painful coughing, dyspnea, mucopurulent nasal discharge and depression (Ackermann, 2000). In Bangladesh, pneumonia causes high mortality and poor production of goats incurring a significant national economic loss. Poor managemental condition, transportation stress, overcrowding pens, sudden environmental changes, poor housing conditions, concurrent viral infection (e.g. parainfluenza-3 virus), lung parasites and other stressful conditions increase goats’ susceptibility to pneumonia (Davies *et al.,* 1997). Pneumonia is caused by both infectious and noninfectious agents. Among the infectious agents, *Pasteurella maltocida* more frequently causes the outbreak of acute pneumonia and death of goats (Falade, 2002).

Pasteurella multocida is commonly found in the upper respiratory tract of healthy goats (Biberstein, 1978; Mutters, 1989), which can also be present as a commensal in the nasopharynx of apparently healthy animals and as a primary or secondary pathogen in several animal species (Rhimler and Rhoades, 1989). It is one of the most common pathogens of sheep and goats throughout the world where outbreaks usually lead to high mortality and great economic loss to the ruminant industry (FAO, 1991; Gilmour, 1991). A study in Bangladesh showed that economic losses resulting from haemorrhagic septicaemia, one of the most economically important pasteurelloses was $148 million annually (Ahmed, 1996). So, the prevalence of *P. multocida* in the goat population should be regularly investigated to control the disease. Furthermore, antibiotic resistance/ sensitivity profiling of *P. maltocida* also needs to be regularly updated for designing or updating of effective treatment strategy. Thus, the objectives of this research project were to isolate *P. multocida* from apparently healthy and diseased goats in selected areas in Bangladesh and to characterize them at the cultural, cellular and biochemical level, to assess the incidence of *P. multocida* in goats and to determine the antibiotic sensitivity profile of the isolated *P. multocida*.

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MATERIALS AND METHODS

Sample collection

In this study, a total of 150 nasal swab samples were collected from goats reared at Savar animal farm mainly and some of the samples were collected from the slaughter house of Savar bazar and Nabinagar bazar, Savar, Dhaka.

Isolation and identification of bacteria

In the bacteriology lab, nutrient broth was inoculated with each sample and was incubated for 24 hours at 37°C with 5% CO_2 in the cabondioxide incubator. From the nutrient broth, subcultures were introduced into nutrient agar and incubated at 37°C for overnight in the presence of 5% CO_2 in the same incubator. The colony of *P. multocida* was picked up according to Shivachandra *et al.* (2006) and Tabatabai (2008). The selective medium such blood agar was used to subculture the *P. multocida* colony. The identification of the organisms was performed by the tests including oxidase, catalase, indole, methyl red-Voges-Proskauer, citrate utilization, H_2S production and carbohydrate fermentation as described by Ievy *et al.* (2013) and Christensen *et al.* (2014). On the basis of colony, staining characters and biochemical tests *P. multocida* strains were isolated in 25 samples. Representative samples from each group of bacteria were stained for identification.

Antibiotics sensitivity test

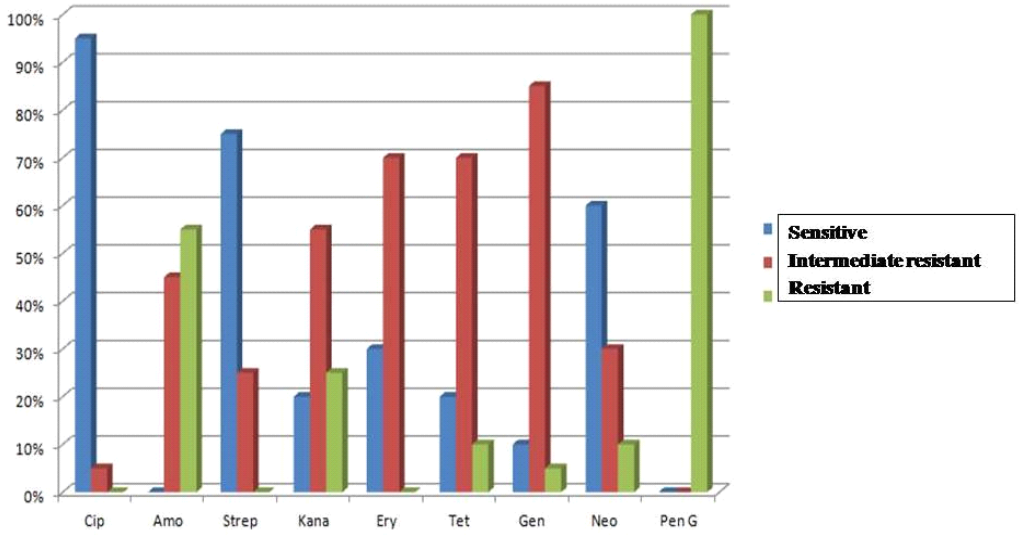
Disc diffusion assay was used to determine the antimicrobial susceptibility of 20 isolates against 9 different antimicrobial agents such as: Ciprofloxacin (CIP 5 µg), Amoxycillin (AMX 30 µg), Streptomycin (S 10 µg), Kanamycin (K 30 µg), Penicillin (P 10 µg), Erythromycin (E 15 µg), Tetracycline (TE 30 µg), Gentamicin (GEN 10 µg), Neomycin (N 30 µg), following the standard methods. The samples were inoculated on Muller-Hinton agar (MHA). Disks containing the antimicrobial agents were applied within 15 minutes of inoculating the MHA plate and the plates were incubated at 37°C for 24 hours in an incubator (Royalcare England. DNP 9022A). The diameters of the zones of inhibition were measured with a ruler or calipers (NCCLS, 2003).

RESULTS AND DISCUSSION

Respiratory infections are complex syndrome and its etiology involves many different factors including stress factors, environmental factors, bacterial and viral infections. Pneumonia occurring due to bacteria or others is generally regarded as the most frequent and serious cause of morbidity, mortality and economic losses associated with respiratory diseases in Black Bengal goats (Husain *et al.,* 1995). *P. multocida* was considered among the primary etiological agents that incriminated pneumonia in goats (FAO, 2003).

In this study, the isolates matched the expected result of the biochemical tests of *Pasteurella multocida*. The colonial morphology of the isolated isolates was smooth mucoid or rough colony round 1-2 mm in diameter, yellowish, glistening, translucent and non haemolytic. Typically, *P. multocida* is found in goats in association with other bacteria which are usually presented in large excess, so efficient isolation of *P. multocida* requires a selective medium. Consequently, we assessed the value of selective medium for *P. multocida* described by Ruoff (1999) and FAO (2003) which was used to isolate *P. multocida* from the nasal swabs of goats (Ruoff, 1999; FAO, 2003). There was no growth of the isolates on MacConkey agar (Wijewardana *et al*., 1986). The microscopical examination proved that it was Gram negative coccobacilli, occured singly, in pairs or less frequently in short chain. *P. multocida* showed bipolarity with Leishman's stain (Quinn *et al.*, 1994). Our study has shown that *P. multocida* colonized at the nasal passages of apparently healthy and clinically sick goats. These results are in agreement with Momin *et al.* (2011) where they isolated *P. multocida* from the pneumonic goats' nasal swabs.

In this study, the rate of *P. multocida* isolation varies among diseased dead or slaughter goats, diseased living



Cip = Ciprofloxacin, Amo = Amoxycillin, Step = Streptomycin,Kana = Kanamycin, Ery = Erythromycin, Tet = Tetramycin, Gen = Gentamycin, Neo = Neomycin, Pen G = Penicillin G.

Fig 1: Antibiotic resistance profile of *P. multocida* from Savar area samples.

Table 1: Incidence of *P. multocida* in different areas of Savar.

Area of sample collection	Apparently healthy			Diseased			Total		
	No. of Sample	Positive No.	% of sample	No. of Sample	Positive No.	% of sample	No. of Sample	Positive No.	% of sample
Savar animal farm	32	2	6.25	38	8	21.05	70	10	14.29
Savar slaughter house	19	1	5.26	21	7	18.18	40	8	20
Nabinagar slaughter house	17	2	11.76	23	5	24.39	40	7	17.50
Total	68	5	7.35	82	20	24.39	150	25	16.67

Table 2: Incidence of *P. multocida* in apparently healthy and disease (Slaughter or dead and living) goats.

Condition of the examined Sample	Apparently healthy			Diseased			Total		
	No. of Sample	Positive No.	% of sample	No. of Sample	Positive No.	% of sample	No. of Sample	Positive No.	% of sample
Slaughter or dead	28	3	10.71	38	12	31.58	66	15	22.72
Living	40	2	5	44	8	18.18	84	10	11.91
Total	68	5	7.35	82	20	24.39	150	25	16.67

goats, apparently healthy slaughter and living goats. The total number of *P. multocida* isolated from the occurred sample was 25 out of 150 (16.67%). The incidence of *P. multocida* in Savar animal farm, Savar slaughter house and Nabinagar slaughter house were 14.29%, 20% and 17.5% respectively indicating the highest incidence in Savar slaughter house (Table 1).

Concerning this study, the rate of *P. multocida* isolation from the diseased goats reached 24.30% (20 out of 82; a rate of 24.39%) and the rate of *P. multocida* isolation from the healthy goats reached 7.35% (5 out of 68; a rate of 7.35%) (Table 2). From this result, we can conclude that the occurance rate of *P. multocida* in diseased or dead goats with pneumonia is much higher than the apparently healthy goats. These results are nearly similar to that obtained by Momin *et al.* (2011) who recovered *P. multocida* from black Bengal goats with a rate of 20% and Rashid *et al.* (2013) who recovered *P. multocida* from goat lungs with a rate of 15% respectively. However, the overall prevalence of pasteurellosis in goats was 31.4% observed by Assefa *et al.* (2018) contrasting our result.

Antibiotics are extremely used in modern farm animal production. The use of these chemical agents should be based on an accurate diagnosis since there is an increased incidence of bacterial resistance to antibiotics in humans. This phenomenon was attributed to the use of anti-microbial drugs in food-producing animals. Also, there is a concern about possible residues in animal products (Refsdal, 2000). In this investigation, a total of 20 isolated *P. multocida* isolates were randomly selected among 25 isolates for antibiotic sensitivity tests against commonly used antibacterial agents of different groups. Antibiotic resistance profiles of the isolates were shown in Fig 1. This investigation revealed that potential sensitive antibiotics against *P. multocida* were ciprofloxacin (90%), streptomycin (75%) and neomycin (60%), whereas gentamycin (85%), tetracycline (70%), erythromycin (70%) were intermediate sensitive to *P. multocida.* Notabley, Pencillin G was totally resistant for *P. multocida.* This finding is supported by the reports of Kamruzzaman *et al.* (2016) and Albasha and Al-Sultan (2018) who added that ciprofloxacin, azithromycin and streptomycin were the best drugs of choice against infection in case of pneumonic goats whereas Penicillin G and amoxicillin were the less effective antibiotics.

CONCLUSION

This study states that the incidence of *P. multocida* is higher in diseased goats (24.39%) with acute pneumonia than apparently healthy goats (7.35%) and ciprofloxacin, streptomycin and neomycin should be considered as potent anti-*P. multocida* drugs.

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