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DETERMINATION OF ANTIBIOTIC RESISTANCE PATTERN OF BACTERIA ISOLATED FROM LIVESTOCK FECES

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Abstract

In Bangladesh, antibiotics are given to animals in order to improve their health and productivity. This can, however, lead to development of antibiotic resistance in the bacteria and subsequent transfer of the resistance property to human. Twenty one bacteria were isolated from five livestock fecal samples collected from Chankharpul, Sadarghat and Savar. Seven of the most commonly used antibiotics (Streptomycin, Chloramphenicol, Ciprofloxacin, Azithromycin, Oxytetracycline, Co-trimoxazole and Erythromycin) in animal feed in Bangladesh were tested. All (100%) the isolates were sensitive to Chloramphenicol and Tetracycline. Highest resistance was shown to Oxacillin (52.38%) followed by Erythromycin (33.33%) and Streptomycin (33.33%), Azithromycin (23.81%), Oxytetracycline (19.05%) and Ciprofloxacin (19.05%) and finally Co-trimomoxazole (14.29%). Four (19%, n = 21) of the bacteria were resistant to three or more antibiotics and were hence multidrug resistant. All isolates from Chankharpul were resistant to Streptomycin, Erythromycin and Oxacillin whereas all were sensitive to Chloramphenicol and Tetracycline. In case of Sadarghat, highest resistance (67%) was shown to Azithromycin and least (0 %) to Oxytetracycline, Streptomycin and Tetracycline. Isolates from Savar showed highest resistance (87.5%) to Oxacillin and all were sensitive to Chloramphenicol. Based on the findings of this study, we recommend the use of Chloramphenicol and Tetracycline for the treatment of livestock. High resistance to Streptomycin, Erythromycin, Oxacillin and Azithromycin suggests that the use of these antibiotics must be restricted in animal feed.

Introduction

Antimicrobial resistance is a global concern today. Indiscriminate use of antibiotics in livestock feed can give rise to antibiotic resistance. Antibiotics are frequently used as prophylactic drugs in livestock in low-middle-income countries[1-3]. In fact, the consumption of antibiotics in animal production is almost double that of human consumption[4]. The World Organization for Animal Health (OIE) has recommended a list of antibiotics for use in animals. It has also been recommended to avoid the use of antibiotics for prophylactic use in the absence of clinical signs in animals[5].

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In low-middle-income countries, the rise of antibiotic resistance is complex and requires multi-sectoral involvement in order to control the spread of antimicrobial resistance (AMR) bacteria in both humans and animals. A One Health approach is necessary to detect the rise of AMR, identify the causes of AMR, and develop interventions to mitigate the disease in humans and animals[6].

In Bangladesh, antibiotics are used in livestock feed as growth promoters. Many farmers in Bangladesh are not aware of the negative impact of excessive, irrational, and prophylactic use of antibiotics in animals and aquaculture. To reduce AMR, the government of Bangladesh has approved a National Strategy for AMR Containment: 2017-2021. The major objective of this strategy is to establish a multi-sectoral One Health approach to plan, coordinate and implement Antimicrobial Resistance containment[7].

In Bangladesh, very little data is available on the use of antibiotics in animal feed that can subsequently give rise to antibiotic resistance. The present study was undertaken to determine the antibiotic resistance patterns of bacteria isolated from animal feces.

Materials and Methods

Five fecal samples were collected from the mid portion of fresh droppings in a sterile bag and transported to the laboratory in an ice box. Upon arrival at the laboratory, samples were inoculated into nutrient broth. For every 180 ml of nutrient broth, 20 g of fecal matter was added and incubated at 37°C for 24 hours, one ml of 24 hours culture was inoculated in Nutrient Agar and incubated at 37°C for 24 hours. Colonies growing on Nutrient Agar plates were subcultured for obtaining pure culture and subsequently preserved in glycerol broth, kept at -20°C.

A total of 21 bacteria were isolated from the five fecal samples, 8 from Chankharpul, 3 from Sadarghat, and 10 from Savar. The isolates were designated NS 1-21. For the revival of bacterial samples from glycerol stocks, Tryptic Soy Broth (TSB) was used. Fifty microliters of the stock was transferred to 200 microliters of TSB in an eppendorf tube with a micropipette. The inoculated tubes were then incubated at 37°C for 24 hours. It is was carefully monitored to ensure that all the samples went to the correctly designated tubes. Tips were changed every time when working with a new sample.

To perform the test, bacterial samples were inoculated in nutrient broth and incubated in shaker incubator at 37ºC for 4 hours. Bacterial lawn was prepared by sterile cotton swabs on Mueller Hinton agar plate. Antibiotic disks were placed aseptically on the agar surface. All the plates were then incubated overnight at 37ºC. The seven antibiotics used in the experiment were Oxytetracycline, Azithromycin, Streptomycin, Oxacillin, Erythromycin, and Tetracycline.

All isolates were tested for their Gram reaction using standard procedure[8].

Results and Discussion

In Bangladesh, many studies reported antibiotic residues and antibiotic-resistant bacteria in animal-origin foods. In livestock, antibiotics are mainly used to treat diseases, which promotes antibiotic resistance. Two hospital-based studies reported that antimicrobials were prescribed to treat 56-66% of sick animals[9,10]. The most commonly prescribed antibiotics were streptomycin**,** penicillin (31%), followed by sulfadimidine (14%), amoxicillin (11%), gentamicin–sulfadiazine–trimethoprim combination (9%), and tylosin (1%)[11]. Shiga toxin-producing *E. coli* (STEC) and enterotoxigenic *E. coli* (ETEC) showed multidrug-resistant against erythromycin, trimethoprim–sulfamethoxazole, azithromycin, cephalothin, ciprofloxacin, and nalidixic acid[12]. *Salmonella* strains isolated from diarrheic cattle showed resistance to azithromycin, tetracycline, and erythromycin[13]. *Staphylococcus aureus* isolated from dairy cows showed resistance to oxytetracycline[14]. *Staphylococcus* spp., and *Bacillus* spp. showed a high level of resistance to ampicillin, amoxicillin, and streptomycin in goats[15]. *Listeria monocytogenes* isolates from cattle showed 100% resistance to penicillin, imipenem, and amoxicillin[16].

In this study, seven of the most commonly used antibiotics (Streptomycin, Chloramphenicol, Ciprofloxacin, Azithromycin, Oxytetracycline, Co-trimoxazole and Erythromycin) used in animal feed in Bangladesh were tested. The results are represented in Figure 1. The pattern of resistance varied among the farms. In spite of this, it was found that all (100 %) isolates were sensitive to Chloramphenicol and Tetracycline. Overall, the highest resistance was shown to Oxacillin (52.38%), followed by Erythromycin (33.33%) and Streptomycin (33.33%), Azithromycin (23.81%), Oxytetracycline (19.05%), Ciprofloxacin (19.05%), and finally Co-trimoxazole (14.29%). Four (19%, n = 21) of the bacteria were resistant to three or more antibiotics and were therefore multidrug resistant.

In the case of Sadarghat Farm, the highest resistance (67%) was demonstrated to Azithromycin and the least (0%) to Oxytetracycline, Streptomycin and Tetracycline. In this farm, both natural and artificial feed was provided to the animals. Isolates from Savar showed the highest resistance (87.5%) to Oxacillin, and all were sensitive to Chloramphenicol.

All isolates from Chankharpul farm were resistant to Streptomycin, Erythromycin and Oxacillin whereas all were sensitive to Chloramphenicol and Tetracycline. In this farm, a visual survey indicated that animals were fed artificial feed as not much provision for natural feed was evident.

In Chankharpul farm, all isolates were sensitive to Chloramphenicol and Tetracycline and resistant to Erythromycin and Oxacillin. For the remaining antibiotics, isolates demonstrated high resistance to Streptomycin followed by Ciprofloxacin (25%), Co-trimoxazole (20%), and finally Oxytetracycline (12.5%) and Azithromycin (12.5%). In case of Sadarghat farm, isolates were mostly sensitive to the four antibiotics tested, with all isolates being sensitive to Oxytetracycline, Streptomycin and Tetracycline and 66.7%

being resistant to Azithromycin. In case of isolates from Savar farm, all isolates were sensitive to Chloramphenicol and Tetracycline. In this farm, the highest resistance was shown to Oxacillin (87.5%), followed by Erythromycin (37.5%), Ciprofloxacin (37.5%), Oxytetracycline (30%), Streptomycin (30%), Co-trimoxazole (22.2%), and Azithromycin (20%).

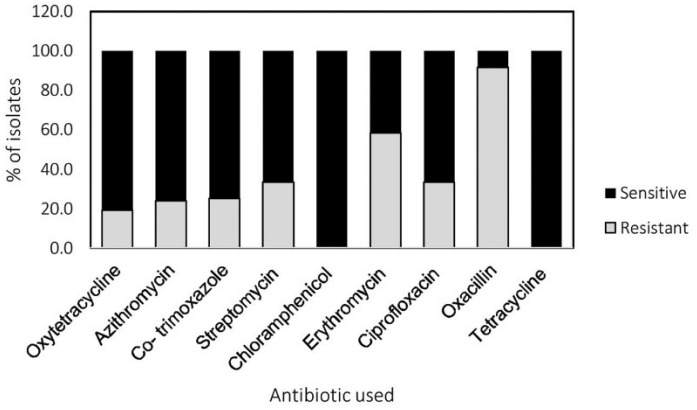

Fig. 1. Overall antibiotic resistance pattern of the isolates from the three farms combined

From the overall antibiotic sensitivity pattern, it was observed that isolates were generally sensitive to Chloramphenicol and Tetracycline, with the exception of isolates from Sadarghat, where Chloramphenicol was not tested. The isolates showed variable sensitivity to different antibiotics. Based on the findings of this study, it can be concluded that the two antibiotics that can be used for the treatment of cattle with bacterial infections are Chloramphenicol and Tetracycline. Also, there is a high risk of the transfer of resistance to other commonly used antibiotics from the resistant bacteria to other closely related bacteria. The present study, therefore, needs to be continued, and the determination of the horizontal gene transfer potential of the isolates and their pathogenicity needs to be demonstrated. This will give a clearer picture of the risk to animal and human health and contribute to the One health management of the environments involved.

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