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Characterization and antibiotic sensitivity of urease positive pathogens from poultry droppings

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ABSTRACT

In the present study the faecal samples were collected from different poultry farms including both deep litter and cage system. Two government and eight poultry farms located in and around Jabalpur city were included in the study. The ammonia concentration [ppm] in individual farms was adjudged on the basis of pH. The ammonia concentration at all the levels was lower in the deep litter system compared to the cage system. A total of 145 isolates were identified out of 120 samples of poultry droppings. Among the 9 genera of microbes identified, urease positive bacteria were present in the genera Pseudomonas, Proteus, Klebsiella and Staphylococcus. The overall percentage of urease positive bacteria was 35.17%. With an increase in ammonia concentration an increase in the count was noted. The pathogenicity testing of representative enteric and urease producing bacteria was conducted in albino mice. Gross changes were observed in the carcass and in vital organs incase of all the test bacteria. Microscopically lesions were noted in the vital organs especially in the Liver, in case of all the bacteria. The inhibitory powder of eight antimicrobials was tested on bacteria of three predominant isolated genera viz. Pseudomonas, Proteus and Klebsiella. The overall percent inhibition was maximum with Amikacin followed by Ciprofloxacin, Enrofloxacin, Nalidixic acid, Gentamicin Cephalexin, Oxytetracycline and Sulfamethoxazole.

Keywords: Ammonia, Poultry, Urease, Antimicrobial.

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INTRODUCTION

For the poultry industry, concerns about ammonia are multifaceted and include issues of live production performance, animal health, and welfare, and environmental impact. In the case of poultry, N is excreted as uric acid and as undigested protein in fecal waste. The quantity and quality of litter flora that gets accumulated from the droppings of birds, plays a significant role in ammonia release. Microbial degradation of uric acid in the litter is the primary source of NH3 formation [8]. Both concentration and exposure time influence the effect that ammonia can have on poultry health. The gas could cause snicking, tracheal irritation, air sac inflammation, conjunctivitis and dyspnoea [2]. Following inhalation, on coming in contact with moisture present in the mucus membranes of the respiratory tract, ammonium hydroxide is formed, which causes damage and destroys cilia of the respiratory tracts of birds. The destruction predisposes to diseases Faeces of apparently healthy as well as diseased birds are prominent source of urease positive bacteria and enteric pathogens. The load of such microbes in feces can be greatly reduced by appropriate choice of drugs. Keeping in view the above facts, the problem was selected to characterize the urease positive bacteria from fresh and old droppings and drug sensitivity testing was conducted to find out the appropriate drugs to reduce urease producing microflora for reduction of ammonia.

MATERIAL AND METHODS

The present study was conducted in the Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, M.P.P.C.V.V., Jabalpur, M.P. Fresh and old poultry droppings were collected from two government and eight private poultry farms located in and around Jabalpur city. In five poultry farms the birds were maintained in deep litter system and in the rest they were kept in cages. In each farm the level of atmospheric ammonia was measured following the method of [10]. The concentration of ammonia was adjudged by colour change in the pH paper. For the isolation of microbes a total of 120 faecal samples, 60 fresh and 60 old were collected from 10 farms and processed as per method described by [6]. The simple media used were nutrient broth and nutrient agar. Blood agar was used as an enriched and differential medium, selective media like MacConkey medium, Brilliant green agar and Eosin methylene blue agar and Pseudomonas agar were used for isolating and differentiating enterobacteria. Identification of microbial genera was done on the basis of morphology, staining reaction, motility, cultural characteristics on various media and biochemical tests. The growth characteristics of well separated colonies on different media were noted. Bacteria were identified by different biochemical tests like sugar fermentation, indole, methyl red, voges prokauer, citrate utilization, desulfurase and nitrate tests. Sensitivity of microbes towards the antimicrobial drugs was determined using the single disc diffusion method described by Bauer *et al*. [1966]. A total of 8 antimicrobial drugs viz Gentamicin [G], Enrofloxacin [Ex], Amikacin [Ak], Ciprofloxacin [Cf], Cephalexin [Cp], Nalidixic acid [Na], Oxytetracycline[O] and Sulfamethoxazole [Sz]. Pathogenicity of the representative isolates of urease producing bacteria were conducted by inoculating 0.2 ml inoculums having $2.3x10^9$ cells /ml Intraperitoneally in albino mice weighing 18-20g. One week post- infection they were necropsied and observed for gross lesions. Histopathology of the affected organs was done. The

tissues were run in ascending grade of alcohol and finally embedded in paraffin. The paraffin sections were stained with haematoxylin and 1% aqueous solution of eosin [7].

RESULTS AND DISCUSSION

In the present study the ammonia concentration was found higher in the cage system than that of in the deep litter system at the level of birds. The reason could be due to a high amount of faecal material accumulating in the cage system, resulting in a high concentration of urea. Results of the present study differ from the findings of [5] who found lesser ammonia concentration in cage system of management compared to deep litter. Among the bacteria identified from poultry droppings, urease positive bacteria belonged to the genera Pseudomonas, Proteus, Klebsiella and Staphylococcus. All the 28 isolates of P. Aeruginosa and 12 isolates of Proteus spp. were urease positive; while 9 out of 10 K. pneumonia and 2 out of 6 Staphylococcus Aureus were urease positive. The overall percentage of urease positive microorganisms was 35.17% out of 145 isolates. From both fresh and old droppings 23.33% P. Aeruginosa was isolated which was similar to the incidence reported by [4] in poultry droppings but differ from findings of [5]. 12 isolates [10%] of Proteus which included seven P. mirabilis and 5 P. vulgaris were isolated from poultry droppings while 10 isolates [8.33%] of K. pneumoniae were identified findings showed similarity to the incidence reported by [5].

On drug sensitivity testing P. aeruginosa showed high degree of resistance towards Gentamicin and Sulfamethoxazole, which is contrary to the report by [1]. In the present study maximum sensitivity was noted towards Amikacin [89.28 %] and Ciprofloxacin [82.14%].The sensitivity of 12 isolates of Proteus spp towards Ciprofloxacin [83.3]. Among the 10 isolates of K. pneumoniae all were found sensitive to Amikacin followed by Gentamicin [12].The intraperitoneal inoculation of Pseudomonas aeruginosa in mice produced hepatomegaly and haemorrhagic changes in lungs grossly. The histological changes revealed ballooning of the hepatocytes with sparse chromatin and centrilobular necrosis in liver [3, 11]. The experimental inoculation of P. mirabilis in mice resulted in anaemia hemorrhagic liver grossly and haemorrhage with fatty vacuolation histologically [9]. Experimental inoculation of K. pneumoniae in mice by intraperitoneal route resulted in haemorrhagic and hyperemic changes in lungs and liver grossly and histologically [13].

Table 1.Urease positive and negative microorganisms isolated from fresh and old droppings

Name of isolates	Total numbers	Urease positive	Urease negative
Escherichia coli	60	-	60
Pseudomonas aeruginosa	28	28	-
Salmonella spp	25	-	25
Proteus mirabilis	7	7	-
Proteus vulgaris	5	5	-
Klebsiella pneumoniae	10	09	01
Staphylococcus aureus	6	2	4
Bacillus subtilis	4	-	4
Total	145	51[35.17]	94[64.83]

Figures in parentheses indicate total percentage of urease positive out of the total isolates

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CONCLUSION

The ammonia concentration at all the levels was lower in the deep litter system compared to the cage system. The overall percentage of urease positive bacteria was 35.17%. With an increase in ammonia concentration an increase in the number of urease positive bacteria was noted. Amikacin was the most effective inhibitor of predominant urease producing bacteria while Sulfamethoxazole was the least effective inhibitor. Both gross and microscopic changes were noted in the carcass and vital organs of albino mice injected with representative urease producing bacteria.

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REFERENCES

[1] Bapat D A, Kulkarni V B and Nimje D V. Indian J Animal Sci 1985; 55[7]: 538-539.
[2] Carlile F S. World Poultry Science Journal 1984; 40: 99-112.
[3] Cash H A, Woods D E, McCullough B, Johanson W G J and Bass J A. The American Review of Respiratory Diseases. 1979;119[3]: 453-459.
[4] Chakraborty M and Roy J P. Indian Veterinary Journal 2001;78: 1079-1081.
[5] Ghadge R. The significance of urease positive enterobacteria present in poultry droppings with poultry welfare. M.V.Sc. Thesis, Deemed University, IGAU, Anjora. 2002.
[6] Gibson G R, Kelly S M, Macfarlane S and Macfarlane G T. J Microbiol Methods 1995; 23[3]: 321-328.
[7] Gridley M F. Manual of histologic special staining techniques. 2nd edn. McGraw Hill Book Company, Inc. New York. 1960.
[8] Koerkamp P W G. J Agr Eng Res 1994;59: 73-87.
[9] Maronpot R R and Peterson L G. Laboratory Manual of Animal Science 1981;31[6]: 697-700.
[10] Moum S G, Seltzer W and Goldhaft T M. Poultry Science 1969;48: 347-348.
[11] Olgert R P, Frank A V and Arligues H S. J Inf Dis 1976 ; 133 [3]: 253-259.
[12] Ramaswamy S V, Ferrieri P, Madoff LC, Flores A E, Kumar N, Tettelin H and Paoletti LC. J Med Microbiol 1997;55: 775-783.
[13] Yoshida K, Matsumoto T, Tateda K, Uchida K, Tsujimoto S, Iwakura Y and Yamaguchi K. J Med Microbiol 2001;50: 959-964.