Evaluation of *in vitro* antibacterial effect of garlic against poultry pathogens

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Abstract : *Garlic (Allium sativum) is well known for its antimicrobial activity and the poultry farmers in Namakkal (Tamilnadu) use it in feed to combat infections. However there are limited reports on the efficacy of garlic against poultry pathogens. Hence, in the present study antibacterial activity of fresh garlic extract was evaluated on eight pathogenic poultry isolates namely Bacillus, Staphylococcus, Clostridium, E.coli, Klebsiella, Pasteurella, Pseudomonas and Salmonella by agar well diffusion method and compared with enrofloxacin. Garlic extract was used at 100, 75, 50, 25 and 12.5% concentration. Among the organisms the sensitivity as measured by zone size for gram positive organism was identical for Staphylococcus (37 to 21mm) and Bacillus (37 to 20mm) while it was lowest for Clostridium (16 to 0mm). Similarly for gram negative organism the zone size was similar for Pasteurella (31 to 17mm) and Salmonella (30 to 19mm) followed by E. Coli (27 to 16mm) and then Pseudomonas (25 to 17mm) and Klebsiella (25 to 12mm). All the organisms were sensitive to enrofloxacin except E. coli and for Bacillus and Staphylococcus the activity of 25% garlic extract was identical to enrofloxacin. For Pasteurella both enrofloxacin and 100% extract recorded similar zone of inhibition and for other organisms the effect of garlic extract was comparable to enrofloxacin. The results of the study justify the use of garlic in poultry feed.*

Key words: *Garlic, Allium sativum, Antibacterial effect, Agar well diffusion, Enrofloxacin*

INTRODUCTION

Garlic (*Allium sativum*) belonging to *Liliaceae* is used as a spice in food and medicine (Ross *et al.*, 2001) and its antibacterial effect continue to be extensively studied. It is also proven to prevent spoilage of food and infection of wound (Arora and Kaur, 1999). Garlic is also found effective against some of the strains resistant to commonly used antibiotics (Jezowa *et al.*, 1966). This broad spectrum of activity has been attributed to the numerous phytochemicals present in varying concentrations in garlic (Alli *et* a*l.*, 2011). Poultry farmers in Namakkal district of Tamilnadu were found using garlic @ 5-20 kg / ton of feed for its antibacterial, antiviral and immunostimulant property. Despite numerous studies on antibacterial effect of garlic report against pathogenic poultry isolates are scanty. Hence the present study was undertaken to explore the effect of garlic against pathogenic poultry isolates so as to provide scientific evidence for its usage in poultry feed.

MATERIALS AND METHODS

Preparation of garlic extract

Garlic was purchased from the local market and identified with the help of a botanist. Fresh extract was prepared without adding water with the help of a blender and then filtered using a sterile gauze cloth. The extract was further filtered using Whatmann No.1 filter paper and this was considered as 100% concentration.

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It was further diluted with sterile distilled water to obtain 75, 50, 25 and 12.5% concentration. The fresh extracts were used immediately for antibacterial study.

Microorganisms

A total of eight pathogenic poultry isolates out of which three gram positive and five gram negative organisms were used in this study. The organisms namely Bacillus, Clostridium, Staphylococccus, E.coli, Klebsiella, Pseudomonas, Pasteurella and Salmonella were obtained from Department of Veterinary Microbiology, Veterinary College and Research Institute, Namakkal and used for the study.

Antibacterial assays

Antibacterial activity of garlic extract was evaluated by agar well diffusion method. The microorganisms grown in nutrient broth were matched with 0.5 Mc Farland Standard and swabbed on the surface of Muller Hinton agar plates. Agar wells of 6 mm diameter were punched with the help of sterile cork borer. Using micropipette 50µl of different concentrations of garlic extract was added to wells. The plates were incubated in upright position at 37° C for 24 hours. The diameter of the zone of inhibition was measured in mm and the results were recorded in triplicate and mean was calculated. The organisms were also tested against the standard antibacterial enrofloxacin (10 µg). All the media used in the study were obtained from Hi-media Laboratories Ltd., Mumbai, India.

RESULTS AND DISCUSSION

Antibacterial activity

The results revealed concentration dependent antibacterial activity against all the organisms tested (table 1). Among the organisms the sensitivity as measured by zone size was maximum and identical for the gram positive organisms, Staphylococcus (37 to 21 mm) and Bacillus (37 to 20mm) whereas, it was the lowest for Clostridium measuring 16 to 0 mm. Among the gram negative organisms the maximum zone size was recorded for Pasteurella (31 to 17mm) and Salmonella (30 to 19mm) followed by E. Coli (27 to 16mm) and then Pseudomonas (25 to 17mm) and Klebsiella (25 to 12mm).

Table 1. Diameter of zone of inhibition of fresh garlic extract and enrofloxacin against pathogenic isolates

Organism/ Conc.	Diameter of Zone of inhibition (mm)					
	100 %	75 %	50 %	25 %	12.5 %	Enrofloxacin
Bacillus	37	34	30	23	20	25 (S)
Staphylococcus	37	35	32	24	21	22 (S)
Clostridium	16	15	13	0	0	23 (S)
Pasteurella	31	29	24	21	17	30 (S)
Salmonella	30	28	25	21	19	47 (S)
E.coli	27	26	24	20	16	10 (R)
Pseudomonas	25	24	22	19	17	29 (S)
Klebsiella	25	23	20	16	12	33 (S)

All the organisms were sensitive to enrofloxacin except *E. coli* and for Bacillus and Staphylococcus inhibition was identical to 25% garlic extract. For Pasteurella both enrofloxain and 100% extract recorded similar zone of inhibition and for other organisms enrofloxacin was better than 100% garlic extract.

The results indicate that garlic has broad spectrum of activity against both gram positive and gram negative organisms. The antibacterial activity of garlic has been attributed to more than 100 phytotherapeutic sulphur compounds present in varying concentration. They include allicin and thiosulphinates which are formed by crushing induced metabolic activation of the enzyme allicinase on the odorless aminoacid allicin (Alli *et al.,* 2011).

Allicin interferes with the formation of phospholipid bilayer of cell wall, synthesis of cell membrane and also RNA synthesis in bacteria. Hence bacteria cannot grow in the presence of allicin and produces bactericidal effect (Srinivasan *et al.,* 2009).

E. coli, the most common infection in poultry is resistant to enrofloxacin whereas it is susceptible to garlic extract. Ziarlarimi *et al.* (2011) also reported that aqueous extract of garlic possessed growth inhibiting activity against *E.coli* isolated from infected chicken. The absence of resistance to garlic enhances its ability to act effectively against resistant bacterial strains. Hence the use of fresh garlic filtrate can reduce the use of antibiotics in poultry and cattle industries (Safithri *et al*., 2011). Garlic may also be combined with antibiotics to act synergistically.

CONCLUSION

The antibacterial effect of aqueous extract of garlic is comparable to standard antibacterial enrofloxacin that is widely used in poultry industry. It is also found effective against *E.coli* that is resistant to enrofloxacin. Hence it can be concluded that garlic can be added in poultry feed to combat bacterial infections and thereby reserve the usage of antibacterials so that development of resistance can be avoided.

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