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Antibacterial Activity of Marine Fungi Against Veterinary Based Clinical Pathogens

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ABSTRACT

Marine fungal metabolites are bioactive compounds develop and inhibit the growth of microbial and clinical pathogens. So, the Present study was focussed on potentiality of gaps-1 and gaps-2 against standard antibiotic tetracycline by their bioactivity to fetch out the veterinary medicine that are used by the bovine and poultry farmers. Bioactive metabolites were extracted from marine fungal isolates (GAPS-1 and GAPS-2) through solvent extraction. Potency of metabolite was determined by antibacterial assay and MIC (minimum inhibitory concentration) against Clinical pathogens like Escherichia coli, Klebsiella, Pasteurella multocida using standard antibiotic tetracycline as a standard. Fungal extracts of (GAPS-1 and GAPS-2) are effectively extracted in Ethyl acetate, when compared to chloroform, hexane and petroleum either respectively, both extracts had showed a prominence antibacterial activity on all test organisms and MIC of both extracts starts from 500µg where as standard antibiotic tetracycline starts from 250µg. Since both fungal isolates revealed significant effect on clinical pathogens indicating the potentiality of marine environment in producing alternatives of chemically synthesizing standard antibiotics.

Keywords: Marine fungi, antibacterial assay, Clinical pathogens, MIC, Antibiotics.

I. INTRODUCTION

Present studies on marine environments are vigorously continuing to conquer various compounds like anticancer, antimicrobial, anti-inflammatory and other potential sources of new therapeutic agents from marine organisms (bacteria, fungi and actinomycetes). Due to the ecological pressure, Marine microorganisms like fungi are associated with other competitive and hostile environments to exist, and also produce complex secondary metabolites. Fungal metabolites from marine environments are biologically active against the development and growth of other microorganisms as well as Clinical pathogens like Escherichia coli, Klebsiella, Pasteurella multocida etc in bovine and poultry fowls, some of these bacteria leads to chronic infections.

Escherichia coli is a commensal of human, animals and avian, with virulent characteristics and distinct identifying groups which is able to cause intestinal and extra intestinal illnesses [1] which is characterized by the virulence and identifying distinct groups. E.coli transmits different enteric infections by inter-human contacts like Entero-invasive E.coli (EIEC), Entero-

pathogenic E. coli (EPEC) or Entero aggregative E.coli (EAggEC) [2], while those ascribed to Entero toxigenic E. coli are primarily transmitted to humans through contaminated food and water [3] which leads to life-threatening haemolytic uremic syndrome (HUS) [4]. As E.coli is usual contaminant in human and coli infections are most common in poultry birds. Till date mortality of the fowl and bovine are common due to the contaminant bacteria like E.coli, though there are many antibiotics in veterinary sciences. The virulent factors of E. coli comprise adhesions and toxins, adhere and colonize mucosal surfaces, which has an ability to disturb normal function of the host cell and cross the epithelial barrier and to invade the tissue [5] of host. Pathogensepticity ranges from mild signs to severe disease. In such severe cases bovine endure with an acute tissue damage and loss of milk or even the death. The severity of an infection depends on factors like age and early stages of lactation, initial phases of lactation in bovine is very susceptible to infection [6]. Respiratory diseases are common in 5 to 7 weeks of old calves majorly recorded in the autumn seasons [7] associated with poor ventilation and hygiene conditions at the shelters [8] resulting in huge economic impact due to increasing mortality and costly treatments.

In poultry fowl diverse respiratory syndromes occurs associated with Escherichia coli like acute coli septicaemia, hyperaemic and swollen viscera in young fowls and sub-acute fibrin purulent serositis involving air sacs and pericardium in old fowl. Pasteurella multocida may cause fowl cholera, diseases of economically important in commercial production such as peracute, acute, and chronic infections [9, 10]. P. multocida is a pathogen that also infects many animals and opportunistically human [11, 12].

Pathogenic bacteria are becoming resistant to the antibiotics and multiple drugs that are availing in the market; in this situation the researcher is targeting to explore the cheaper and effective, potential and less toxic antimicrobials [13]. The present study is focussed on bioactive metabolites of marine fungi on antimicrobial effects against clinical pathogens in bovine and poultry associated with various skin infections, urinary tract, gastrointestinal infections and waterborne diseases. The aim of the present study was to identify biologically potent secondary metabolites from marine fungi based on anti- bacterial assay.

II. MATERIALS AND METHODS

Collection of bovine and poultry pathogens: Test organisms from infected bovine and poultry samples are provided by Department of Epidemiology, Animal Husbandry, Vijayawada and Srinivasa hatcheries, Vijayawada.

Extraction of fungal metabolites: Potent fungal isolates were inoculated in 1000ml sterile conical flask with 500ml potato dextrose broth incubated at 25°C for 7 days. The biomass were removed aseptically through filtration and filtrate is mixed with equal volumes of different solvents. The organic solvent layer was collected and concentrated by evaporation. The concentrated crude extract was collected and stored for antibacterial assay [13].

Antibacterial activity: By using ethyl acetate extracts of marine fungal isolates, antibacterial test was conducted by agar cup plate technique in nutrient agar medium (Nutrient agar medium consisting of 2% (g/L agar), 5% (g/L) peptone, 5% (g/L) NaCl,, 10% (g/L glucose and: 3% (g/L beef extract and pH 7.0.) using tetracycline as a positive control and ethyl acetate as negative control against the test organisms viz. Escherichia coli, Pasteurella multocida and klebsiella from bovine, Escherichia coli and Pasteurella multocida from poultry. The zone of inhibition was measured after 24 hours of incubation [14-16].

Minimum inhibitory concentration: MIC of secondary metabolite was performed by agar-cup diffusion

method. Crude extract and standard antibiotic was dissolved separately at different concentrations ranging from highest concentration of 10,000 µg/ml and then dilution were performed at concentration of 5000 µg/ml, 4000µg/ml, 3500 µg/ml, 3000 µg/ml, 2500 µg/ml, 2000 µg/ml, 1500 µg/ml, 1000 µg/ml, 750 µg/ml, 500 µg/ml, 250 µg/ml and 200 µg/ml. MIC value of marine fungal crude extract was determined against clinical pathogens such as bovine and poultry. Bacterial suspensions of the test organisms were prepared and seeded on to sterilized Mueller-Hinton plates. 50µl of the extract was added to each well. Culture medium with standard antibiotic and with ethyl acetate were used in the tests as positive and negative controls. Plates were incubated at 37 °C for 20-24h and MIC was measured by zone of inhibition [16].

III. RESULTS AND DISCUSSION

Collection of pathogens from bovine:E.coli, P.multosida and klebsiella were previously collected from different districts of Andhra pradesh, based on the disease; pathogens were isolated from the infected parts of the bovine (cattle). Preliminary screening and identification of the isolated pathogens was done by Department of Epidemiology, Animal Husbandry.

Collection of pathogens from Poultry fowls:E.coli and P.multosida were previously collected from different districts of Andhra pradesh, based on the disease; pathogens were isolated from the infected parts of the poultry. Preliminary screening and identification of the isolated pathogens was done by Srinivasa hatcheries, Vijayawada.

Extraction of fungal metabolites: Different solvents such as ethyl acetate, hexane, petroleum ether and chloroform were utilized to extract the metabolites from the fermented broth. Ethyl acetate has been found to be the most suitable solvent to extract both fungal metabolites (GAPS -1 and GAPS -2).

Antimicrobial Activity: Dried fractions of GAPS -1 and GAPS -2 obtained by fractionation of the crude extract was dissolved in ethyl acetate (1000µg/ml) and tested for antimicrobial activity by agar cup plate technique against the test organisms using standard antibiotic tetracycline as a positive and ethyl acetate as negative control. It was observed extracts of GAPS -1 and GAPS -2 show prominent antibacterial activity against Escherichia coli, Pasteurella multocida and klebsiella species from bovine and Escherichia coli and Pasteurella multocida from poultry. The zone of inhibition was measured after 24hour incubation by excluding the well diameter 6mm. [Table 1 and 2].

Table 1. Anti-bacterial activity in bovine pathogens (zone of inhibition (mm)):

Pathogen	Gaps -1 (50µl)	Gaps -2 (50µl)	Ethyl Acetate (50µl)	Tetracycline (50µl)
Escherichia coli	22	18	0	32
Pasteurella multocida	28	20	0	39
Klebsiella spp	25	22	0	35

Table 2. Anti-bacterial activity in poultry pathogens (zone of inhibition (mm)):

Pathogen	Gaps -1 (50µl)	Gaps -2 (50µl)	Ethyl Acetate (50µl)	Tetracycline (50µl)
Escherichia coli	26	24	0	35
Pasteurella multocida	29	26	0	42

Minimum inhibitory concentration:

MIC value was determined for both extracts of GAPS -1 and GAPS -2 which showed better anti-bacterial activity. The MIC value of fungal extracts was ranged from 500 to 2,000 µg/ml. Lowest MIC value (500

µg/ml) was in both gaps-1 and gaps -2 found against only pathogens isolated from bovine samples, where as in case of poultry fowls lowest MIC value was observed only in gaps-2 (500 µg/ml against Pasteurella multocida) whereas MIC of gaps 1 (starts from 1000 µg/ml against E.coli). MIC values of GAPS-1 and GAPS-2 are higher than negative control and Positive control (antibiotic tetracycline (30µg/ml)) due to the impurities in fungal metabolites during extraction and complex composition of the extracted metabolites. MIC value against clinical pathogens included in this study was promising though tetracycline had much lower MIC value than the fungal isolates [Table 3 and 4].

Table 3. MIC of gaps-1and gaps-2 in Bovine pathogens expressed in zone of inhibition (mm).

Pathogen	Gaps-1 (50µl of 30µg/ml)	Gaps-2 (50µl of 30µg/ml)	Ethyl acetate (50µl of 30µg/ml)	Tetracycline (50µl of 30µg/ml)
Escherichia coli	500	1000	0	250
Pasteurella multocida	1500	500	0	250
Klebsiella spp	1000	2000	0	250

Table 4. MIC of gaps-1 and gaps-2 in poultry pathogens expressed in zone of inhibition (mm).

Pathogen	Gaps-1 (50µl of 30µg/ml)	Gaps-2 (50µl of 30µg/ml)	Ethyl acetate (50µl of 30µg/ml)	Tetracycline (50µl of 30µg/ml)
Escherichia coli	1000	1000	0	250
Pasteurella multocida	1500	500	0	250

IV. CONCLUSION

Farmers are suffering from mortality of bovine and fowls due to the chronic infections caused by different clinical pathogens though there are many antibiotics there is rate of increasing mortality. So, samples were collected from different areas where mostly dairies and poultry farms are maintaining. With the obtained results of antibacterial activity through Agar Well-diffusion method and minimum inhibitory concentrations we conclude that marine fungal isolates are very active in inhibiting the growth of clinical pathogens of bovine and poultry which could become a prominent and challenge for medicine and therapeutics in veterinary fields.

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