IDENTIFICATION OF EXTRACELLULAR VIRULENCE FACTORS AND EFFECT OF PLANT EXTRACTS ON ANTIBIOTICS RESISTANT PSEUDOMONAS AEROGENOSA ISOLATED FROM CHICKEN SHOP SOIL AND CHICKEN FEATHER

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Abstract: Pseudomonas aeruginosa is a bacterium responsible for severe nosocomial, enteric infections, life-threatening infections in immune compromised persons, and chronic infections in cystic fibrosis patients. The bacterium's virulence depends on a large number of cell-associated and extracellular factors. The virulence factors play an important pathological role in the colonization, the survival of the bacteria and the invasion of tissues. During the present study samples of soil and chicken feathers were collected from different shops of Bhopal and Pseudomonas aeruginosawere isolated. Isolates were found to produce extracellular virulence factors viz protease. Isolates were found to be resistance against most of the antibiotic used to treat enteric infections. Bhopal is a city of vegetarian and non- vegetarian peoples. Presence of opportunistic pathogenic Pseudomonas aeruginosain chicken and soil sample of chicken shop is an alarming condition for non- vegetarians.

Index term: Pseudomonas aeruginosa, virulence factors, antibiotic resistance, chicken microbes, protease, hemolysin(key words)

I. Introduction

Pseudomonas aeruginosa is a highly relevant opportunistic pathogen that causes disease in both animals and human. It is typically found in soil, water, skin flora, and most man-made environments, since it requires minimal amounts of oxygen for growth, thereby allowing it to colonize a multitude of both natural and artificial environments [1]. *P. aeuroginosa* is a motile, gram negative, oxidase positive, rod shaped with single arrangement or short chains. The organism is a strict aerobe, ubiquitous and often associated with soil, water and humid environment. It affects newly hatched chickens drastically causing high mortality and mass death of embryos [2-7]. *P. aseruginosa* got a huge arsenal of virulence repertoire implicated in pathogenesis. Attributed to the numbers of extracellular virulence factors and cellular components as lipopolysaccharide, elastase, alkaline proteases, pyocyanin, pyoverdin, haemolysins, phospholipase C, rhamnolipids, biofilm, Pilli, and flagella. The complex type III secretion system recognized virulence determinant of *P. aeruginosa* capable of injecting proteins and secretion toxins into the host cell [8-15].

Despite major recent advances in the study of the virulence of the human opportunistic pathogen *Pseudomonas aeruginosa*, our understanding of the pathogenesis of *P. aeruginosa* infections remains limited. This pathogen causes a wide range of infections in humans including acute localized infections such as urinary tract infections, acute ulcerative keratitis, malignant otitis media, peritonitis, acute ventilator associated pneumonia in endotracheal incubated patients, and burn wound infections, as well as chronic localized infections such as chronic destructive lung infection s in cystic fibrosis (CF) patients. In addition, patients with severe underlying diseases reducing physical (burned patients, mechanically ventilated patients) and/or immune defense mechanisms (neutropenia, AIDS patients) are at serious risk for evolution of localized infections toward systemic disease, which is associated with dramatically elevated mortality. *Pseudomonas aeruginosa* infection can be disastrous in chronic lung diseases such as cystic fibrosis and chronic obstructive pulmonary disease. Its toxic effects are largely mediated by secreted virulence factors including pyocyanin, elastase and alkaline protease (AprA) [16]. To establish an infection, *P. aeruginosa* relies on a suite of virulence factors, including lipopolysaccharide, phospholipases, exoproteases, phenazines, outer membrane vesicles; type III secreted effectors, flagella, and pili. These factors not only damage the epithelial cell lining but also induce changes in cell physiology and function such as cell shape, membrane permeability, and protein synthesis. While such virulence factors are important in initial infection, many become dysregulated or nonfunctional during the course of chronic infection. Recent work on the virulence factors alkaline protease (AprA) and CF transmembrane conductance regulator inhibitory factor (Cif) show that *P. aeruginosa* also perturbs epithelial ion transport and osmosis, which may be important for the long-term survival of this microbe in the lung [17]. In the modern poultry industry antibiotic are used for the treatment and prevention of infectious in farm animals indented for food production and to protect public health from food borne diseases [18]. There is growing scientific evidence that use of antibiotics in food animals leads to the development of resistant pathogenic bacteria [19]. Previous study indicated that *P. aeruginosa* is resistant to various antimicrobial agents due to impermeability, multi-drug efflux, and a chromosomal AmpC β-lactamase. Prominent resistance found among α-carboxy- and Amino-penicillins, third and fourth-generation Cephalosporins, Monobactams, Carbapenems, aminoglycosides, and Fluoroquinolones [20-25].

Some bacteria have developed resistance to antibiotics that were once commonly used to treat them. The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. India is famous for its remarkable history in ayurveda and use of herbs and spices were reported previous as antibacterial agent [26]

Objective of the present study was to isolate,*Pseudomonas sp.*from chicken shop soil and chicken feather from Bhopal, *Pseudomonas* infection of chicken is of great importance because epidemics may spread rapidly through poultry flocks causing mortality in all ages. Presence of this organism will provide a base to the further study that is this producing extracellular virulence factor like enzymes, hemolysin and if yes then is it sensitive or resistant towards the common antibiotics prescribed by physicians or not, and also screening of different plant extract on this organism.

II. Material and Methods:

Isolation of *Pseudomonas*species: strains were isolated from soil and chicken feather of four different chicken shops. For this soil samples and feathers were collected in clean sterile poly bags. Sample were brought to the laboratory and processed for further use. Soil samples were serially diluted; feathers were dipped in the sterile distilled water. Both the samples were then inoculated in nutrient agar medium and incubated at 37^{o}C. Isolates were identified by cultural characteristics, Gram staining and biochemical characterization [27].

Extracellular enzyme production: screening and characterization of Extracellular protease were done by agar plate method [28].

Antimicrobial susceptibility testing:

Antimicrobial susceptibility of Amoxicillin AMX 25 mcg, Ciprofloxacin CIP 5 mcg, Ceftriaxone CRO 30 mcg, Cefuroxime CXM 30 mcg, Cotrimoxazole CMX (Trimethoprim/ Sulphamethoxazole 25 mcg), Gentamycin CN10 mcg and Ofloxacin OFX 5 mcg were tested by disc diffusion method [28].

Hemolysis test: Isolates were screened for beta hemolysis by agar plate method [28].

Extraction of plant material:

The fresh ginger rhizomes and garlic cloves were collected, cleaned, peeled, sliced and dried at room temperature. After drying, pieces of Allium sativum (Garlic),Zingiberofficinale (Ginger) and leaves of basil were grinded to fine particles in isolated manner utilizing a suitable grinder. Ten grams of the powder Allium sativumand Ten grams of Zingiberofficinale was weighed and macerated in 50 milliliters of D.W, 96% ethyl alcohol, each alone. The containers were left at 250 C for 3 days (72 hrs). After that, the suspensions were filtered and the filtrates (extracts) were delivered into sterile, clean containers with suitable labeling and kept at 4^{0}C until used for additional assay [29].

Antibacterial activity of plants extract

The disk diffusion method is used to evaluate antimicrobial activity of the each plant extract. The plant extract loaded over sterile filter paper discs (8 mm in diameter). Ten ml of Mueller-Hilton agar medium was poured into sterile Petri dishes (as a basal layer) followed with 15 ml of seeded medium previously inoculated with bacterial suspension (100 ml of medium/1 ml of 10^{7} CFU) to attain 10^{5} CFU/ml of medium. Sterile filter paper discs loaded with plant extract concentration of (10 mg/ml) were placed on the top of Mueller-Hilton agar plates. The plates were kept in the fridge at 5 °C for 2 h. to permit plant extracts diffusion then incubated at 37 °C for 24 h. The presence of inhibition zones were measured by Vernier caliper, recorded and considered as indication for antibacterial activity [30]

III. Result and Discussion:

Tables:

Plant species	**Common name**	**Plant part used**	**Extract pH**
Zingiberofficinales	**Ginger**	**root**	**5.8**
Allium sativum	**Garlic**	**root**	**5.7**
Ocimumtenuiflorum	**Holy Basil**	**leaves**	**6.1**

Plant extract	**Diameter of Zone of inhibition (in cm)**			
	Isolate 1	**Isolate 2**	**Isolate 3**	**Isolate 4**
Zingiberofficinales	**0.9**	**1.2**	**0.9**	**0.8**
Allium sativum	**1.4**	**0.9**	**1.1**	**1.2**
Ocimumtenuiflorum	**0.3**	**0.4**	**0.3**	**0.5**

Pseudomonas aeruginosa is a serious opportunistic pathogen infection among individual with compromised immune system. Therefore, this study investigated occurrence, detection of virulence factor like extracellular enzymes, hemolysin and antimicrobial sensitivity/ resistance and. During the present study *P. aeruginosa* was isolated from soil and chicken feather obtained from four different chicken shops. Isolates were found to produce extracellular enzymes like protease. *P. aeruginosa* protease are considered as important virulence factor which damage host tissues and interfere with host antibacterial defense mechanisms. It was reported that *P. aeruginosa*biofilm cells are not completely killed by antibacterial [31]. The remarkable ability of *P. aeruginosa* is to adapt and thrive in a wide variety of environments is due in part to its extensive versatility, which contributes significantly to its potential as a pathogen. Hemolysins may be destroying red blood cells by lysis. During present study *P.aeruginosa*were found to beresistant against most of the antibiotics commonly used by physicians in Bhopal except gentamycin but it is found to be sensitive against ginger and garlic root extract and slight sensitive against holy basil leave extract/ It can be interpreted from the present investigation is that extracellular protease, hemolysin production and antimicrobial resistance may lead to existence of serious types of*P.aeruginosa*infectionif contaminated chicken will be consumed by immunocompromised patient. Further investigations are required to check weather ginger, garlic and basil extract help to eradicate *Pseudomonas aeruginosa* from chicken before consumption.

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