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Microbiology

ANTIBACTERIAL ACTIVITY OF NEEM SEED EXTRACT AGAINST CLINICAL ISOLATES OF PSEUDOMONAS AERUGINOSA

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ABSTRACT Pseudomonas aeruginosa is ubiquitous in nature and it is found as a commensal in the skin and gastrointestinal tract of humans. It causes opportunistic infections in immune-compromised patients, not commonly seen in immune-competent patients. Various parts of the neem tree have been used as traditional Ayurvedic medicine in India. For instance, the leaves, seed and bark found to have a wide varity of antibacterial activity against both Gram negative and Gram positive bacteria. A sum of 20 clinical isolates of P. aeruginosa were subjected to antibiotic sensitivity pattern followed by detection of antibacterial activity of ethanolic extract of neem seed. we have observed increased zone of inhibition against P. aeruginosa, in particular these isolates were found to be imipenem resistant. When we compare activity of ethanolic extract of neem seed with imipenem, all isolates were better responded with neem seed. This indicates the time to look for alternative as well as promising medicine to cure such nosocomial drug resistant pathogen.

KEYWORDS : Pseudomonas aeruginosa, Neem seed extract, well diffusion method

INTRODUCTION:

Pseudomonas aeruginosa is ubiquitous in nature and it is found as a commensal in the skin and gastrointestinal tract of humans. It causes opportunistic infections in immune-compromised patients, not commonly seen in immune-competent patients. [1] It also causes serious nosocomial infections such as ventilator associated pneumonia and various sepsis syndromes because it thrives on moist surfaces of the medical equipment like catheter, causing cross infections in clinic and hospitals. [2] It typically affects the airways, urinary tract, burns, wounds, gastrointestinal system and also causes blood infections. The most prominent attributes is the resistance of this bacteria to multiple clinically important antibiotics like third generation cephalosporins (imipenem and aztreonam) and extended–spectrum cephalosporins (cefotaxime, ceftriaxone,ceftazidime). [3]

Herbal formulations are found to treat many infectious diseases since from antiquity. Plant material continues to play a vital role in the primary health care as therapeutic remedies in several developing countries. Thus, the discovery of medicinal plants as antimicrobial agents is useful in expanding the wide varieties of antibiotics available [4]. When it comes to infections, no one can go past Azadirachta indica (Neem) for its wound healing properties [5]. Various parts of the neem tree have been used as traditional Ayurvedic medicine in India. For instance, the leaves, seed and bark found to have a wide varity of antibacterial activity against both Gram negative and Gram positive bacteria. However, the leaves are in concern for its medicinal properties for wound healing. Neem leaf is effective in the healing of chronic wounds, diabetic foot and gangrene in developing conditions. [6] Thus, this study was found to evaluate the antibacterial activity of Neem seed extract against clinical isolates of Pseudomonas aeruginosa.

MATERIALS AND METHODS:

Bacterial isolates:

A total of 20 non repetitive clinical isolates of P. aeruginosa were collected from Saveetha Medical College and Hospitals, Chennai. They were processed for a battery of standard biochemical tests and confirmed. Isolates were preserved in semisolid trypticase soy broth stock and were stored at 4 ºC until further use.

Antibiotic susceptibility testing:

Antibiotic susceptibility testing was determined for this isolates to routinely used antibiotics such as to piperacillin-tazobactam, cefotaxime, ceftazidime, tetracyclin, cotrimoxazole, aztreonam, gentamicin and imipenem by Kirby Bauer disc diffusion method as per CLSI guideline. [7]

Preparation of ethanolic extract of neem seed extract:

50g of dried seed powder of Neem were taken in a separate container. To this 250 ml of ethanol was added and kept for 24 h with periodic shaking. Filtered and the filtrate was collected.

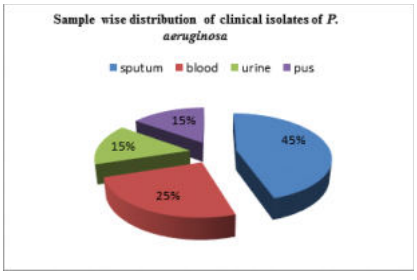
Agar diffusion method:

A sterile cotton swab were soaked into 0.5McFarland Standard P. aeruginosa isolates and were lawn cultured on Mueller Hinton agar. 50mg/ml and 100mg/ml concentrations were prepared and each dilutions were been impregnated onto the sterile discs and air dried. These discs were placed onto the lawn cultured plates and incubated at 37°C for overnight. The sensitivity results were interpretated based on zone of inhibition of bacteria. [8]

RESULTS:

Sample wise distribution of clinical isolates of *P. aeruginosa:*

Of the 20 clinical isolates of *P.aeruginosa*, 9/20 (45%) isolates were from sputum, 5/20 (25%) from blood, 3/20 (15%) from urine, 3/20 (15%) from pus.



Results of antibiotic susceptibility testing:

Antibiotics	Sensitivity(20) (%)	Intermediate(20) (%)	Resistant(20) (%)
piperacillin-tazobactam	0(0)	0(0)	20(100)
Cefotaxime	0(0)	0(0)	20(100)
ceftazidime	0(0)	0(0)	20(100)
tetracycline	0(0)	0(0)	20(100)
cotrimoxazole	0(0)	0(0)	20(100)
Aztreonam	0(0)	0(0)	20(100)
Gentamicin	0(0)	0(0)	20(100)
Imipenem	2 (10)	0(0)	18 (90)

Antibacterial activity of ethanolic extract of neem seed against P. aeruginosa:

Isolates	Neem seed extract (50mg/ml) (mm)	Neem seed extract (100mg/ml) (mm)	Imipenem (mm)
1	10	24	0
2	14	20	3
3	10	25	0
4	15	25	0

5	16	23	0
6	16	26	0
7	15	26	14
8	11	23	0
9	16	25	0
10	14	23	0
11	12	21	3
12	10	25	16
13	9	28	4
14	17	24	0
15	12	28	0
16	14	22	0
17	16	22	0
18	16	26	0
19	18	21	0
20	12	25	0

DISCUSSION:

Study conducted by Dzulkarnain and coworkers in 2014, examined the antibacterial activity of ethanolic extract of neem aginst Staphylococcus aureus, Streptococcus pyogenes and Pseudomonas aeruginosa. They have found 8.7mm diameter zone of inhibition in 50mg/ml and 9.3mm diameter zone of inhibition in 100mg/ml concentration of extract against S. aureus. Simillarly, Increased zone has been observed against S. pyogenes such as 9.3mm and 10.7mm from 50mg/ml and 100mg/ml respectively. However, they did not find any known activity aginst P. aeruginosa.

In contricitory, we have observed increased zone of inhibition against P. aeruginosa, in particular these isolates were found to be imipenem resistant.

CONCLUSION:

Even though, we have found increased percentage of resistance to routinely used antibiotics, only 2 of them were shown to be sensitive to imipenem which is considered to be a last resort to treat such troublesome pathogen. When we compare activity of ethanolic extract of neem seed with imipenem, all isolates were better responded with neem seed. This indicates the time to look for alternative as well as promising medicine to cure such nosocomial drug resistant pathogen.

REFERENCES:

1. Balcht, Aldona, Raymond. 1994. Pseudomonas aeruginosa: Infections and treatment. Informal health care. 83-84.
2. Itah AY, Essien JP. 2005. Growth Profile and Hydrocarbonoclastic Potential of Microorganisms Isolated from Tarballs in the Bight of Bonny, Nigeria. World Journal of Microbiology and Biotechnology. 21(6). 1317–22.
3. Shacheraghi F, Shakibaie MR, Noveiri H. 2010. Molecular Identification of ESBL genes blaGES-1, blaVEB-1, blaCTX-M blaOXA-1, blaOXA-4, blaOXA-10 and blaPER-1 in Pseudomonas aeruginosa strains isolated from burn patients by PCR, RFLP and sequencing techniques. Int J Biol life Sci. 3(6). 138–42.
4. Zaidan MRS, Noor Rain A, Badrul AR, Adlin A, Norazah A, Zakiah I. 2005. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. Tropical Biomedicine 22(2). 165-170.
5. Sekar, S. 2008. Pharmaceutical Plant Company herbs. Retrieved February, 2009, from Metabalm: http://www.ppcherbs.com.au/metabalm.html.
6. Ncube N S, Afolayan AJ, Okoh AI. 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology. 7(12). 1797-1806.
7. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Disk Tests; Approved Standards ; Document M2-A9, 9th ed., Vol 26. Wayne, PA: CLSI; 2015.
8. Dzulkarnain SMH, Rahim IB. 2014. Antimicrobial activity of methanolic neem extract on wound infections bacteria. International Conference on Biological, Chemical and Environmental Sciences. 14. 72-76.