ANTI BACTERIAL ACTIVITY OF *NIGELLA SATIVA* AGAINST CLINICAL ISOLATES OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

Abdul Hannan, Sidrah Saleem, Saadia Chaudhary*, Muhammad Barkaat, Muhammad Usman Arshad

Department of Microbiology, University of Health Sciences, *Lahore Medical and Dental College, Lahore, Pakistan.

Background: Methicillin resistant *Staphylococcus aureus* (MRSA) continues to be one of the commonest pathogens encountered in clinical as well as laboratory practice. It has become a major health problem worldwide. Newer antimicrobials/agents are urgently needed to combat this problem MRSA resistance to various anti-staphylococcal agents. In the back-drop of this difficult situation *Nigella sativa* commonly known as black seed (ethanolic extract) was aimed at to evaluate if it had any anti-staphylococcal activity. **Methods:** The extract was prepared by reflux extraction method. Disc diffusion and in agar dilution methods were performed to assess the anti-bacterial activity. *Staphylococcus aureus* ATCC 25923 was used as the standard reference strain. **Results:** All tested strains of MRSA were sensitive to *N. sativa* extract at a concentration of 4 mg/disc while the extract had an MIC range of 0.2–0.5 mg/ml. **Conclusion:** The results indicated that *N. sativa* has inhibitory effect on MRSA. This finding warrants necessity of further investigation of this product of folk medicine.

KEY WORDS: MRSA, resistance, black seed, antibacterial activity, extract.

INTRODUCTION

Staphylococcus aureus is a gram positive bacterium responsible for severe morbidity and mortality worldwide. It is one of the leading cause of human infections in the skin and soft tissues, bones and joints, abscesses and normal heart valves. The organism flourishes in the hospital setting producing bloodstream and surgical wound infections.[1,2] Methicillin was introduced 1959 to treat staphylococcal infections not responding to penicillin therapy. However only within a year some strains of *S. aureus* were reported to be resistant to it. These strains were named as 'Methicillin Resistant *Staphylococcus aureus*' (MRSA). During the past four decades MRSA has spread throughout the world and has become highly endemic in many geographic areas.[3] MRSA infections are difficult to treat because of their resistance to the commonly used anti staphylococcal antibiotics viz macrolides, tetracyclines, aminoglycosides etc. Some of these MRSA strains are resistant to even the most powerful antibiotics such as vancomycin.[4] WHO has been suggesting the need to find some new antibiotics or new approaches to overcome this problem.

Nigella sativa is a herbaceous plant found in the Middle East, Europe and Western and Middle Asia. Its seeds have a great medicinal importance and have been reported to exhibit many pharmacological effects that include anti-parasitic, antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory activities.[5] The seeds have also been used to treat bacterial, fungal and parasitic infections. Its oil is used as condiment, carminative, food preservative, analgesic and to treat many ailments in different parts of world.[6]

In the light of the above pharmacological properties exhibited by *N. sativa* seeds, there is a need to investigate its efficacy against MRSA.

MATERIALS AND METHODS

At total of 99 clinical isolates of MRSA and ATCC strain 25923 were obtained from Armed Forces Institute of Pathology (AFIP), Rawalpindi and Immunology Department University of Health Sciences (UHS) Lahore.

Identification was done on the basis of morphology, cultural characteristics, biochemical reactions and susceptibility to Oxacillin discs (1 µg) using Mueller-Hinton agar supplemented with 4% NaCl.

Nigella sativa seeds were procured from Punjab Seed Cooperation Lahore. They were freed of dust and crushed in a domestic grinder and then soaked in absolute ethanol for 5 days at room temperature at the bench. The amount of ethanol was just enough to adequately cover the seeds. They were filtered under UV light using filter paper. The filtrate was put into rotary evaporator to evaporate ethanol. Prepared extract was collected and stored in refrigerator till use.

Whatman Filter paper No. 1 was used to prepare discs (6 mm). The discs were then sterilized in batches of 50 by autoclaving. The extract was diluted with propylene glycol and its different concentrations were prepared. A total volume of 250 µl was used to soak 50 discs without over or under wetting them. Discs with final concentration 0.5, 1.0, 2.0, 4.0 mg per disk were obtained. Prepared discs were stored at 4 °C in the refrigerator till use. To avoid any condensation the discs were kept at room temperature for one hour to before use.

MH agar was prepared and poured into flasks so that each flask contained 20 ml of the agar. The flasks were then autoclaved after which they were kept in water bath at 50 °C to avoid any solidification. Measured quantity of the extract was poured into each flask, mixed and poured into petri dishes. Finally plates with different concentration of the extract (0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 mg/ml) were prepared. They were allowed to solidify at room temperature and kept in refrigerator till use.

Nine strains were picked up at random for screening purpose. Broth cultures equal to 0.5 McFarland standards were made from each strain. A sterile cotton swab was dipped into the broth and a lawn was made on to the MH agar plate. The discs were placed and the plates were incubated at 35 °C overnight. Vancomycin 30 µg and propylene glycol discs were used as positive and negative controls respectively. The diameter of the clear zones around the discs was measured in mm with digital calipers (Sylvac Fowler ultra-cal II).

MIC of *N. sativa* extract was performed against all test strains. Five hundred µl of the broth culture was put into the wells of the multi inoculator (Mast Diagnostic, UK). Agar plates with extract were taken, dried in hot air oven at 55 °C for 4–5 minutes and inoculated with multipoint inoculator and incubated overnight. Three control plates were also set, one with MH agar inoculated with all strains to confirm the viability of the cultures, second control plate contained medium only and the third plate had extract incorporated medium to check the sterility of medium and the extract. MIC was recorded as the lowest concentration of the extract at which visible bacterial growth was completely inhibited. The experiment was performed in triplicate.

RESULTS

The average zone of inhibition of the extract at its different concentrations is shown in Table-1. Disc containing propylene glycol (dilutent of the extract) produced no zone of inhibition whereas vancomycin disc used as positive control produced significant zone of inhibition (>15 mm according to CLSI standards). The results of disc diffusion assay are further explained by Figure-1.

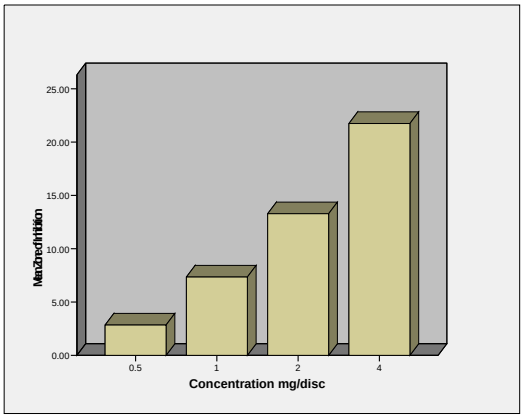

Figure-1: Bar chart showing correlation between zone size and dose (mg/disc)

MIC range of black seed extract on 99 strains of MRSA strains is shown in Table-2. MIC_{90} and MIC_{100} were found to be the same viz 0.5 mg/ml. ATCC 25923 was inhibited at 0.5 mg/ml. Figure-2 explains the cumulative percentage of MRSA inhibited at different concentration of the extract.

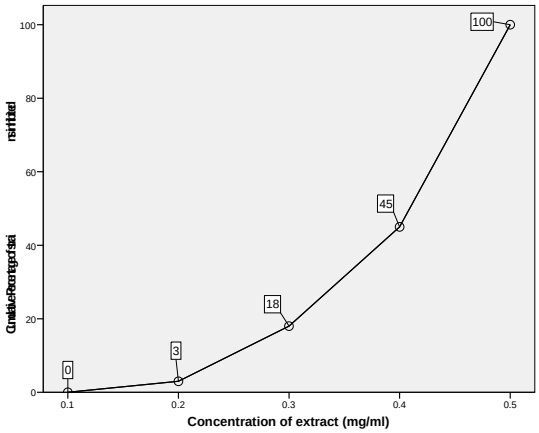

Figure-2: Cumulative percentage of MRSA inhibited at different concentration of black seed extract.

Table 1: Inhibitory effects of black seed extract and vancomycin on MRSA and one ATCC strain

Strain ID	Black seed extract (zones in mm)				Vancomycin disc
	0.5 mg	1.0 mg	2.0 mg	4.0 mg	30 µg
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
UHS.Imm 12	0±0*	0±0	10.8±0.32	22.22±1.01	19.91±0.38
UHS.Imm 25	0±0	0±0	10.13±1.22	21.22±1.35	20.86±0.62
AFIP 23845	9.92±1.87	12.67±1.53	14.55±1.71	25.79±3.34	20.66±0.99
AFIP 2079	0±0	8.9±0.7	13.51±1.83	21.07±0.89	20.66±0.77
AFIP 26763	0±0	12.56±0.9	16.5±0.5	24±2.09	21.85±0.17
AFIP 23807	0±0	0±0	11±2.09	16.28±0.62	19.88±0.21
AFIP 23197	0±0	8.6±1.52	14±2	20.07±0.47	20.53±0.44
AFIP 21089	10.20±0.72	18.2±0.71	23.8±12.63	32.6±2.58	21.9±0.48
AFIP 23804	0±0	0±0	10.02±1	18.52±1.52	20.36±0.74
ATCC 25923	8.3±0.58	8.5±1.42	12.5±1.31	15.68±0.72	21.31±0.79

Table-2: Minimum inhibitory concentration of *Nigella sativa* against 99 strains of MRSA.

MIC (mg/ml)					
MRSA	MIC Range*	(MIC_{45})	MIC_{50}	MIC_{90}	MIC_{100}
N=99	0.2–0.5	(0.4)	>0.4	0.5	0.5

*MIC_{45} is mentioned as this was the actual reading on the plates where 45% of the strains were inhibited at 0.4 mg/ml. The finding is written in brackets as it is not conventional to mention MIC_{45}

DISCUSSION

Black seed extract has been extensively studied for its antimicrobial activity against a wide range of bacterial, fungal and parasitic organisms. However only a limited data is available so far regarding its efficacy against MRSA. The present study was therefore designed to evaluate this aspect of *N. sativa*.

The results of disc diffusion assay demonstrated that MRSA strains were completely inhibited at 4 mg/disc (zone size >12 mm was considered to be significant).[7] However a concentration of 0.5 mg per disk failed to inhibit any of these strains.

The quantity of the extract required to inhibit bacteria was low in agar dilution assay as compared to the disc diffusion technique. This is likely because of the difference in methodology of the two assays. In agar dilution method the extract is directly incorporated into the medium therefore the bacteria are brought into direct contact with all components of the extract rather than relying on diffusion of constituents through the disc. The concentration of the ingredients is always higher next to the disk and decreases gradually. Since bacteria give rise to a new generation every 18–20 minutes therefore inoculated plates containing discs at 37 °C help bacteria to grow while components of the extract need some time to diffuse through the disc and exert their effects.[8] These factors point out lack of sensitivity of agar diffusion assay therefore it is recommended that interpretation of results of diffusion tests must be based on the comparison between dilution and diffusion methods. Such comparisons are useful in establishing reference standards.

A group of researchers studied total of 8 MRSA and 4 MSSA (methicillin sensitive *Staphylococcus aureus*) strains and evaluated them against aqueous and ethanolic extracts of *N. sativa*. MIC value was found to be 0.04 mg/ml for both MRSA and MSSA which differs from our value of MIC.[7] The reason may be the difference in the method of extract preparation and method adopted to perform MIC.

In another study the researchers tested methanolic black seed extract against *S. aureus*. MIC_{56} for methanolic extract was 0.125 g/100ml whereas our study proves MIC_{90} to be 0.5 mg/ml. Zone of inhibition was more than 20 mm at 15 mg per disc and 8 mg per disk for chloroform and methanolic extract respectively whereas our study proves that a concentration of 4 mg/disc is enough to give a significant zone.[9]

It is very important to develop guidelines for all procedures adopted in evaluating antibacterial activity of black seed and analyze extracts of Black seed of different regions for the actual ingredient which is responsible for their antibacterial activity. There is also an urgent need that a standard method may be devised for extract preparation.

CONCLUSION

It may be concluded from this study that *N. sativa* seed extract has antimicrobial activity against MRSA. It is expected that using natural products as therapeutic agents will probably not elicit resistance in micro-organisms. It is essential that research should continue to isolate and purify the active components of this natural herb and use in experimental animals.

ACKNOWLEDGMENT

We acknowledge our gratitude to University of Health Sciences, Lahore for fully supporting this research activity.

REFERENCES

1. Haddadin AS, Fappiano SA, Lipsett PA. Methicillin resistant *Staphylococcus aureus* (MRSA) in the intensive care unit. Postgrad Med J. 2002;78:385–92.
2. Jones ME, Karlowsky JA, Draghi DC, Thornsberry C, Sahm DF, Nathwani D. Epidemiology and antibiotic susceptibility of bacteria causing skin and soft tissue infections in the USA and Europe: a guide to appropriate antimicrobial therapy. Int J Antimicrob Agents. 2003;22:406–19.
3. Mark CE, Ashley RD, Gaynor R, Edward J, Brian G. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). PNAS. 2002;99:7687–92.
4. Tenover FC, Biddle JW, Lancaster MV. Increasing Resistance to Vancomycin and Other Glycopeptides in *Staphylococcus aureus*. Emerg Infect Dis. 2001;7:327–32.
5. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. Phytother Res. 2003;17:299–305.
6. Salem ML. Immunomodulatory and therapeutic properties of *Nigella sativa* L. Seed. Int Immunopharmacol. 2005;5:1749–70.
7. Dadgar T, Asmar M, Saifi A, Mazandarani M, Bayat H, Moradi A, Bazueri M. Antibacterial Activity of Certain Iranian Medicinal Plants Against Methicillin-Resistant and Sensitive *Staphylococcus aureus*. Asian J Plant Sci. 2006;5:861–6.
8. Lusby PE, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. Arch Med Res. 2005;36:464–7.
9. Mashhadian NV, Rakhshandeh H. Antibacterial and antifungal effects of *Nigella sativa* extracts against *S. aureus*, *P. aeroginosa* and *C. albicans*. Pak J Med Sci. 2005;21:47–52.

Address for Correspondence:

Dr. Sidrah Saleem, Research and Teaching fellow (Microbiology), University of Health Sciences Lahore- Pakistan
Email: sidu_78@yahoo.com