



IN –VITRO AN OBSERVATIONAL PROSPECTIVE STUDY OF ANTIBACTERIAL PROPERTIES OF ALLIUM SATIVUM LINN IN A TERTIARY CARE HOSPITAL OF NORTH ODISHA

Dr. Anjali Tarai*	Associate professor, Pharmacology, Pandit Raghunath Murmu Medical college, Baripada, India,757001. *Corresponding Author
Dr. Smita Patra	Department To Anatomy, Sri Ram Chandra Medical College, Cuttack India-753001.
Dr. Sabitri Beshra	Assistant Professor, Bhima Bhoi Medical College, Balangir, India-767002.
Ms. BR Abha Ayushree	Assistant Professor, PG Department of Home Science, Rama Devi Women's University, Bhubaneswar, Odisha-751022, India.
Dr. Priti Lata Panda	Professor, Microbiology, Pandit Raghunath medical college, Baripada, India-757001.

ABSTRACT **Background:** Use of traditional medicine believe that the constituents of plants are unique as they contain both active ingredients and non-active components that are useful in well-being of their patients. Plant formulations have become a better alternatives specially in developing nations as the treatment with them is relatively inexpensive and easily available without any adverse effect. Many herbal products are reported to have antibacterial properties. Antimicrobial agents currently in use have been rendered ineffective due to development of resistant strains of microbes. Hence search for development of newer antimicrobials is on, with this view the present work was undertaken to investigate the antimicrobial properties of the commonly used ingredient *Allium sativum* (garlic) in Indian kitchen and other country. **Methods:** The anti-microbial effect of *Allium sativum* (garlic) in two common organism e.g staphylococcus aureus and *Escheria.coli* were studied in the department of microbiology and pharmacology PRM Medical College, Baripada in November 2019. Garlic was collected from local market, then Aqueous garlic extract was prepared from 100 gms of freshly washed garlic cloves, Macerated in sterile ceramic mortar and pestle. The homogenate was filtered in a sterile muslin cloth and used directly for sensitivity test. The concentration used in this study is the total weight of garlic per ml. Hundred grams of raw garlic yielded 20 ml of extract (ie5g/ml). This extract was regarded as the 100% concentration. Each time the 100% garlic extract was inoculated on MH agar media and incubated at 37°C overnight for sterility. The bacterial strains were grown on Mueller –Hinton Agar at 37°C. Susceptibility test was done in Mueller Hinton (MH) agar plate uniformly seeded by means of sterile wire loop dipped in the suspension of two clinical isolates of staphylococcus aureus and *Escheria Coli* from pus and urine respectively and then it was streaked on a plate surface. The standard well diffusion method was used to screen for antibacterial activity of the herbal extract. The plates left for excess fluid to be absorbed. Wells of 5 mm diameter 4mm deep and about 2 cm apart were punched on the MH agar plate surface with sterile cork borer. Approximately 100 micro liter of the freshly prepared 100% garlic extract (v/v) were dropped into each well by micro pipette which filled them respectively. The setup were allowed to become stable for 3 hours after that it was incubated at 37°C for 24 hours. The degree of sensitivity was determined by measuring zones of inhibition in millimetre for all the individual isolates. A positive control well was equally filled with gentamycin 32 microgram per micro liter because it was available in our hospital store while sterile distilled water served as negative control. Minimum inhibitory concentration was determined and minimum bacterial count also determined. **Results:** Freshly prepared Aqueous garlic extract applied in two bacterial species viz. *Staphylococcus aureus* and *Escheria coli* which are most common prevalent. The diameter of the zone of inhibition range from 20±2 to 25±1 as compared with gentamycin 18.0±0 to 22.0±0 mm, no zone of inhibition in control group. The MIC of the garlic range from 31.25 to 62.5 mg micro liter. The MBC of garlic ranges from 20.63 to 31.25 mg micro liter. The zone of inhibition is highly significant in staphylococcus aureus. **Conclusions:** The result indicate significant antimicrobial properties possess by *Allium sativum* against *Staphylococcus aureus* and *E. Coli*.

KEYWORDS : Antimicrobial property, *Allium sativum* herb, Indigenous product.

INTRODUCTION

Allium Sativum Linn (Garlic) is one of the most economically use vegetables throughout the world, either as raw vegetables for culinary purposes or, as also an ingredient in traditional and in modern medicine. *Allium* species had widely cultivated for more than 500 years. Garlic bulbs have been used as condiments, spices, seasoning, flavourings as well as for its medicinal value, while garlic leaves are consumed as green vegetables^{1,2,3}. Garlic is one of the most researched and best-selling herbal product in the market. For centuries it was used as traditional medicine for most health related disorders. Medicinal effect of Garlic is result from a combination of variety of biologically active substances which all together are responsible for its curative effect. The compounds present in garlic synergistically influences each other, so that they can have more pronounced effects.

Further, it has been also one of the richest sources of total phenolic compounds among the usually consumed vegetables, where as it is highly ranked regarding its contribution of phenolic compounds in human diet. The main responsible compounds for the flavour in garlic are sulphur containing non-volatile amino acids, among which *alliin* or *S-allyl* –cysteine sulfoxide comprises the most predominant component of garlic, makes the food more tastier. Apart from their flavour attributes, these sulphur compounds are also responsible for the renowned medicinal properties and additionally may improve biosynthesis of glutathione, which is responsible for antioxidant functions of garlic. The juice relieves earache, bronchitis, gangrene of

the lung, whooping cough, laryngeal and pulmonary tuberculosis and duodenal ulcer. In external application of the juice is used as rubefacient in skin diseases, as an eye drop and in earache.^{4,5,6}

The *Allium sativum* (liliaceae) or garlic is a hardy perennial bulbous scapigerous herb with a flat stem. The lower portion of the plant forms a bulb which consists of several smaller buds called cloves and it is surrounded by the dried thin white or pinkish basal sheaths of the foliage.^{7,8}

More than 2000 biologically active substances are present in garlic such as volatiles, aqueous and oil soluble products of sulfur along with essential oils, dietary fibres, sugars, inulin, flavonoid, antioxidants and pectin. However apart from its volatile compounds, garlic is also rich in vitamins especially vitamin B complex, B1, B2, B6 and vit C, PP, provitamin A, mineral salts, g-pattasium, magnesium, calcium and trace elements e.g iron, copper, nickel, cobalt, chromium, selenium and germanium.^{9,10} It is consumed as a rich source of other non-volatile plant nutrients with medicinal and therapeutic use, for which a specific emphasis is given to presence of flavonoids, saponins and sapogenins, phenolic compounds like nitrogen oxides and amides and proteins. L-glutamyl peptides such as *gamma*-glutamyl S-2 propenylcysteine, *gamma*-glutamyl-S-trans-1-propenyl-cysteine and *gamma*-glutamyl-S-methylcysteine are important intermediates in the metabolic pathway for ACSOs biosynthesis, being even considered as the storage pools of nitrogen and sulphur. Further, garlic has been

suggested as one of the phenolic contents present not only among the various genotypes and ecotypes, but also dependent to the farming practices and cultivation conditions. Other quality species related with the chemical composition, such as total soluble solids, PH and sugar contents, have shown a great difference among genotypes, regardless of the cultivation conditions. There is wide range of variation of chemical composition of garlic in different geographical area.

The active ingredient of garlic include enzyme alliinase, a sulfur containing compounds such as alliin, 1-2-vinylthiin, allixin and S-allyl-cysteine and sulphides such as diallyl, methylallyl and dipropyl mono-, di-, tri and tetra sulphides which are formed after the decomposition of thiosulfinate s-ajoenes and compound produced enzymatically from alliin. The main active ingredient is alliin but it is unstable and changes into different chemicals rather quickly. Garlic is a herb with complex action. It was believed as having the bacteriocidal, fungicidal, antiprotozoal, anticancer and antiviral as well as to cure everything from hemorrhoid to snake bite. The volatile nature of these bioactive compound is highly correlated with the defensive mechanisms of garlic plants against insects and several other pathogens being their release combined with cell damage and plant tissue lesions. The most characteristic volatile and odorous organo-sulfur compounds of garlic are released after the disruption of the cell membrane, causing the alpha and beta- elimination of alliin and other sulfoxides, located at the cytoplasm level, by the enzyme alliinase, located at the vacuole but not all the indications are supported by reliable or credible scientific research.^{11,12}

It has significant effect in increasing immune system. Therapeutic uses in cardiovascular diseases, cancer, liver diseases, aphrodisiac and other disorders. Therapeutically garlic has been used for a number of purposes including treatment of hypertension, hypercholesterolemia, diabetes, rheumatoid arthritis, cold, prevention of atherosclerosis and also the development of tumors. Many available publications indicates possible antibacterial, antiviral, anti hypertensive and anti thrombotic properties of garlic.^{13,14}

In Vitro studies shows that garlic have activity against many types of gram negative, gram positive bacteria including species such as *Escherichia Coli*, *Klebsiella*, *Proteus*, *Bacillus clostridium*, *Salmonella typhi* and gram positive bacteria including species such as, *Staphylococcus*, *Streptococcus* and *Mycobacteria tuberculosis*. The in vitro antibacterial activity of essential oil obtained from fresh raw bulb of garlic shows good antimicrobial and anti fungal activity against *Staphylococcus aureus*, *E. Coli* and fungus e.g *Candidiasis* respectively. (2) Res. 2013, 27(3) 380-383. In boiling and frying the antibacterial and anti-oxidant properties decreases. Crushing of garlic gloves before cooking reduce antiplatelet activity but increase anticancer activity. Even some bacteria normally resistant to antibiotic including Methicillin resistant *Staphylococcus aureus* (MRSA) or strains of bacteria resistant to several antibiotic such as *E. Coli*, *Enterococcus saprophyticus*, *Shigella* were sensitive to garlic extract.^{16,17}

Not only anti-bacterial, anti-viral, anti-fungal and anti protozoal activity also noted. The first anti bacterial properties was established in France 1721. It has activity to remove symptoms of giardiasis and *E. H* 1991. *Alliin*, *ajoene* and organosulfides from garlic are also effective anti protozoal. Louis Pasteur first described the anti bacterial effect of garlic juice in 1858. Although no reference is available, freshly prepared aqueous Garlic extract containing *alliin* have been shown to exert bacteriostatic effects on some *vancomycin* -resistant *enterococci*. An inhibitory synergistic effect was observed when used in combination with *vancomycin*. It is presumed that *alliin* regulate the sulfhydryl groups on the enzymes of the TN/546 transposon which encodes *vancomycin* resistance, enhancing susceptibility to *vancomycin*. Garlic has been used for centuries in various societies to combat infectious diseases. In India, garlic extract has been used to prevent wound infection and food spoilage. During World War I garlic was used to alleviate intestinal infections in soldiers stationed in the Balkans. It has been documented that garlic exerts a balancing inhibition between beneficial intestinal micro flora and potentially harmful enterobacteria. Inhibition observed in *E. coli* was more than 10 times greater than that seen in *Lactobacillus* case for the same garlic dose. Exactly this differential inhibition should occur is not clear, but may be due to differing composition of bacterial membranes and their permeability to *alliin*. The antibacterial properties of garlic is widely attributed to *alliin*. It was observed that at room temperature antibacterial effectiveness of garlic extract greatly reduced and this

reduction occur in lesser extent if extract stored at 0-4 O C. suggesting thermal instability. The intracellular effects of *alliin* are not well understood. It is known that *alliin* has been sulfhydryl modifying activity and as such is capable of inhibiting sulfhydryl enzymes. Cysteine and glutathione counteract the thiolation activity of *alliin* and on addition to the reaction mixture the antibacterial activity is reduced. Garlic extract were shown to decrease the oxygen uptake, reduce growth of organism, inhibit the synthesis lipids, proteins and nucleic acids and damage membranes to exert anti-fungal effect. *Ajoene*, *alliin*, diallyl tri sulphide acts by inhibiting the integrin dependant process to produce anti-viral activity.

The burden of AMR can be compared to a tip of iceberg where a major submerged portion still remains unexplored due to lack of adequate monitoring system and inadequate data.

The search for new antibacterial agents is important due to the progressively increasing resistance of clinically important pathogens to known classes of antibiotics. With the increase incidence of resistance to antibiotic, natural products from plants could be an interesting alternative. In the past years many plants have been evaluated not only anti bacterial activity but also for resistance-modifying agents. Resistance occurs due to random genetic mutations in the bacterial cell that alter the sensitivity to a single drug or to chemically similar drugs through a variety of mechanism. Many bacteria are able to develop changes in their sensitivity but *Staphylococcus aureus* and *E. Coli* have been recognised for the increasing resistance to conventional antibiotics. *Staphylococcus aureus* has persisted as one of the most important hospital and community acquired pathogens apart from causing different kinds of intoxication, it is usually involved in diverse tissue and or organ infections. *E. coli* is one of the micro organisms that has been associated with intestinal and urinary tract infections. Pathogenic and commensal strains of *E. coli* have different rate of resistance and can carry different genes.

AIMS AND OBJECTIVES

The present In-vitro observational prospective study was conducted to evaluate possible antibacterial property of freshly prepared aqueous garlic extract on gram +ve bacteria (*Staphylococcus aureus*) and gram -ve bacteria (*Escheria. coli*).

METHODS

In-vitro a prospective observational study was undertaken to evaluate the antibacterial property of freshly prepared aqueous garlic extract (*Allium Sativum*) on the ATCC (American type culture collection), *Staphylococcus aureus* strain 25923 and *Escheria coli* strain 25922. The freshly culture isolates *Staphylococcus aureus* (gram +ve bacteria) and *Escheria. Coli* (gram -ve) bacteria were tested with the standardised aqueous garlic extract, compared with the CLSI (Clinical laboratory standard international) recommended antibiotic for pus and urine sample. MIC of CLSI recommended antibiotic compared with the MIC of the freshly prepared garlic extract. The LAB isolated organism (gram +ve and gram -ve) bacteria were used from the stock samples of pus and urine maintained in the department of Microbiology, PRM medical college and hospital Baripada from November, 2019 to April, 2020 in collaboration with department of Pharmacology, PRM MCH Baripada^{4,5}. This is the part of the study of resistance/sensitivity pattern of anti microbial agents in PRM medical college and hospital, Baripada, Mayurbhanja and it was approved by institutional ethical committee. IEC registration no EC/ New/INSTI/2020/975.

Inclusion Criteria-

- Gram +ve bacteria *Staphylococcus aureus*
- Gram -ve bacteria *Escheria coli*
- Freshly prepared aqueous garlic extract

Exclusion Criteria-

- Other gram +ve and gram -ve organism.
- Aged garlic extract.

Allium sativum (garlic) were collected from local market and its aqueous garlic extract was prepared from hundred grams of freshly washed garlic cloves macerated in sterile ceramic mortar and pestle. The homogenate was prepared and filtered in a sterile muslin cloth. The filter was used directly for sensitivity test. The concentration used in this study is the total weight of garlic per ml. Hundred grams of raw garlic yielded 20 ml of extract eg (5g/ml). This extract was regarded as

the 100% Concentration. Each time the 100% garlic extract was inoculated on nutrient agar media and incubated at 37°C overnight for sterility.

Susceptibility Test

The organism chosen were grown in sterile peptone water overnight. Then the turbidity of the test organisms was matched with McFarland standards. Mueller Hinton (MH) agar plate was uniformly seeded by means of sterile wire loop dipped in the suspension of the two multiresistance organism such as *Escheria. Coli* isolated from clinical material urine and *Staphylococcus aureus* isolated from pus was prepared and streaked on the plate surface. The agar well diffusion method are described by Uhlman et al was used for the screening the antagonistic activity of the extracts against pathogenic microbes. The plate left for excess fluid to be absorbed at 37°C. Wells of 5 mm length and 1 mm diameter, 4 mm deep and about 2 cm apart were punched on the MH agar plate with a sterile cork and borer. Then approximately hundred micro liter of the freshly prepared aqueous garlic extract 100% concentration were dropped into the each well which filled them used for screening of the antimicrobial property of the *Allium sativum* (garlic) extracts against the *Staphylococcus aureus* and *Escheria. coli*. The set up were allowed to become stable for 3 hours and plates were incubated at 37°C for 24 hours. The Bacterial growth was inhibited by extract leads to the formation of inhibition zones. The anti microbial activity was evaluated by measuring the diameter of inhibition of visible zones of the microbial growth produced by the diffusion of the extract with help of thread from no bacterial growth (or zero growth) in mm. The minimum inhibitory concentration i.e., MIC was defined as the lowest concentration of extract where no vitality or able to inhibit the bacterial growth was observed after 24 hours on the basis of zones of inhibition of bacterial growth. A positive control well was equally filled with gentamycin 32 micro gram per micro litre as available in our hospital store while sterile distilled water served as negative control. Minimum inhibitory concentration and minimum bacterial count were also determined.

Statistical Analysis

The antimicrobial activity of aqueous extract of garlic was evaluated by measuring the diameter of zone of inhibition produced by micro-organism such as *staphylococcus aureus* and *Escherichia coli* by help of thread from no growth or zero growth and as mentioned in the observation table.

Minimum inhibitory concentration of garlic (MIC) ranges from 31.25 to 62.5 mg µl. The MBC (minimum bacterial count) ranges from 20.63 to 31.25 mg µl. It was correlated with the previous study done by Papidopoulou (2005 Food Bioscience 43, 41-6).⁶ The activity of extract is greater than that of the gentamycin.⁸

As it is a single study independent t test is applied.

RESULTS

The freshly prepared aqueous garlic extract implicated in two most prevalent and common bacterial species viz. *Staphylococcus aureus* and *Escheria coli*. The diameter of the zone of inhibition range from 20±2 to 25±1 as compared with gentamicin 18.0±0 to 22.0±0 mm of *E. coli* as per (Tab-1) and (Tab-3). The MIC of the garlic range from 31.25 to 62.5 mg micro liter. The MBC of garlic ranges from 20.63 to 31.25 mg micro liter. The main inhibitory effect of fresh aqueous extract of garlic is due to essential oil and alliin. The freshly prepared garlic extract have highly significant anti bacterial activity against *staphylococcus aureus* than *Escheria coli* as per (Tab-2) and (Tab-4).

Table-1: Average Diameters Of Zone Of Inhibition Against E-coli & Staph. Aureus Among Allium Sativum Extract, Gentamycin And In Distilled Water.

Sl. No.	Drug	Dose	Diameter of Zone of Inhibition in mm
1	Distilled Water(-ve control)	100µl	No zone of inhibition
2	Garlic extract(E.COLI)	100µl	20± 2 to 25±1 mm
3	Garlic (test drug)(Staph. Aureus)	100µl	21±2 to 26± 1mm
4	Gentamycin 32 micro g.	100µl	18±0 to 22±0 mm

Table-2: Comparison Of Antibacterial Activity Of Extracts Of Allium Sativum Vs Gentamycin Against Staphylococcus Aureus & E. Coli.

Variables	Garlic extract	Gentamycin	t-value [95% CI]	P-value
Min zone of inhibition in mm	20 ± 2	18 ± 0	1.41 [-4.08 to 8.08]	0.29
Max. Zone of inhibition in mm	25 ± 1	22 ± 0	4.25 [-0.04 to 6.04]	0.05
Min zone of inhibition in mm	21 ± 2	18 ± 0	2.12 [-3.08 to 9.08]	0.17
Max. Zone of inhibition in mm	26 ± 1	22 ± 0	5.66 [0.96 to 7.04]	0.02*

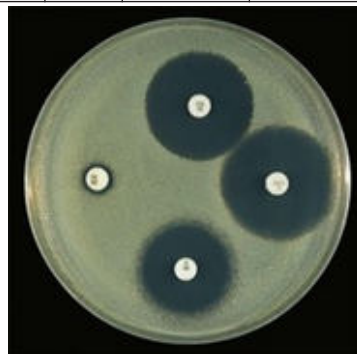


Figure 1. Zone Of Inhibition Of Staphylococcus Aureus By 100% Concentration Of Raw Garlic Extract.



Figure 2. Zone Of Inhibition Of E. Coli By 100% Concentration Of Raw Garlic Extract.

DISCUSSION

The most common prevalent organism were selected for this study is *staphylococcus aureus* and *E. coli*. The freshly prepared aqueous garlic extract was used for its antibacterial property. Garlic is rich in several sulphur containing compound such as alliin, allicin, ajoenes, flavonoid and pectin. The main ingredient in aqueous garlic extract is allicin but it is unstable. The antimicrobial activity of garlic is attributed to allicin activity that was reported towards a wide variety of micro-organism including antibiotic resistance, gram-positive and Gram negative bacteria. Aqueous garlic extract reported to inhibit the growth of several pathogenic bacteria with varying degree of susceptibility. Meriga et al reported that aqueous garlic extract showed anti bacterial activity towards gram negative *KL Pheumonea* and *Escheria .coli*. as well as gram positive e.g. *Bacillus subtilis* and *staphylococcus aureus* strains.

More over garlic extract prevented the growth of entero pathogenic *Escheria Coli* strain and other pathogenic intestinal bacteria which are the main cause of diarrhea in humans and animals. Besides antibacterial activity of garlic, it was reported to prevent toxins produce by bacterial infection. Allicin showed effectiveness towards methicillin resistant *staphylococcus aureus* (MRSA). Allicin anti microbial activity is due to its chemical interaction with enzymes containing thiole. thioredoxin reductase, RNA polymerase and alcohol dehydrogenase by oxidising protein cysteine or glutathione residues under physiological conditions.^{18,19} Allicine is a dose related biocide that can influence essential metabolism of cysteine proteinase and thus kill all eukaryotic cells due to presence of thiol group in all living

cells. Under certain conditions alliin, a major component of garlic shown to be anti-bacterial, degrades to diallyltrisulfide. This chemical is more stable than the extremely volatile alliin and is easily synthesised. It has been suggested that microbial cells are more affected than human cells because they do not have an intracellular thiol content adequate to counterbalance the thiol oxidation by alliin and alliin derived products. Ajoene has been shown to inhibit phosphatidyl choline synthesis in trypanosomes.^{20,21,22}

Aminoglycosides are potent bacteriocidal antibiotics targeting the bacterial ribosome. Several mechanisms have been evolved in bacteria which confer them with antibiotic resistance. These mechanisms can chemically modify the antibiotic to render it inactive through physical removal from the cell or modify the target site so that it is not recognised by the antibiotic. In *Escheria coli* the main mechanism of resistance to aminoglycosides are active drug efflux and enzymatic inactivation.

Several reports indicate different anti biotic combinations assayed in vitro and used in the clinics. However the combinations of natural products and clinically used anti biotics are less reported. The data obtained in this study indicate the potential anti-bacterial activity of aqueous extract of garlic.

CONCLUSIONS

During the global problem of resistance of antimicrobial agents which lead to more use of indigenous products, that shown by the in vitro data obtained in this study, inhibit the growth of bacteria by *Allium sativum* 100% concentration as per (tab-1&2) due to bacteriocidal or bacteriostatic effects, easily available and less costly. Herbal garlic extract preparation can effectively prevent the infections of micro-organisms depending on the organism the susceptibility to garlic extract was comparable to the conventional antibiotic gentamycin. In the 1940s the antimicrobial activities exhibited by garlic were first reported to be due to alliin, a volatile compound extracted from raw garlic sience with other antimicrobials compared to when use alone. When used in combination with antibiotic or anti fungals, alliin enhanced the antimicrobial activities of these substance and improved the antimicrobial efficacy. Hence it is likely that combination therapy of alliin with additional anti-microbial drugs could serve as viable alternative for combating raising the anti-microbial resistance. This review focuses on the antimicrobial activities exhibited by alliin alone as well as in combination with other substances. The mechanism of action of alliin elucidated by some of the studies are also highlighted in present review in order to provide a compressive overview of this versatile bioactive compound and mechanistic evidence supporting its potential use in antimicrobial therapy. Then alliin has been widely investigated for its putative inhibitory activities against a wide range of micro organisms. Alliin has demonstrated a preference for targeting the thiol containing proteins and/or enzymes in micro-organism. It has also demonstrated the ability to regulate several genes essential for virulence of micro-organism. Recently it was reported that alliin may function better in combination. It particularly recommended for the elderly helping to reduce body fat and slow down the ageing processes, through strengthening the immune system to prevent cold and flu like symptoms. However the risk of drug interaction with garlic especially in elderly and those with chronic diseases is attracting more and more interest. The poly phenols from aqueous garlic extract significantly inhibit the growth of *Escheria coli* and *Staphylococcus aureus*. So further study are needed to understand the real health benefit and its effect in human body in alone or with other antibiotics for its synergistic activities to other organisms. Therefore it is our duty to join hands together to say no to antibiotic unless it is indicated and use them within their permissible levels as from indigenous groups. Because if it is not done today, our future generation would be in a world without Antibiotic where for treating even a common conditions would become difficult. The Ethano pharmacological research can utilize the common plants of our country for novel drug development and can improve the traditional health care systems.

Acknowledgement---

Dr Manoj Kumar Dash (statistician)
ASST. professor, Dept of Community medicine
PRM MCH, Rangamatia, Baripada, Mayurbhanja, Odisha.
Mail id- drmkdash@icloud.coms

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