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Clean Care is Safer Care: Application of Basic Infection Control Precautions by Interns at a Tertiary Care Teaching Hospital, Maharashtra

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Abstract

Background: Health care workers (HCW) can be exposed to serious types of infections if they are not following proper infection control (IC) measures. Not many studies have been performed to evaluate knowledge and awareness about IC policies and measures among interns in a tertiary care hospital. Aims & Objective: To assess knowledge and practice of interns regarding IC measures. Material and Methods: A cross-sectional, interview- based survey was conducted in D. Y. Patil Hospital, Kolhapur. 50 Interns were enrolled in this study. Participants were invited to complete the questionnaire concerning knowledge and practice about IC. Data was collected and analysed using excel sheet. Results: The overall knowledge was 88% and practice of infection control guidelines was 58%. Majority of the interns had attended an orientation course. Only 46% of the participants had received training about IC policies. Further training about IC was required by 62% of the studied trainees. About 72% of the participants had received hepatitis B virus (HBV) vaccine. Conclusion: A high number of interns were not adequately equipped with awareness and knowledge concerning IC. The majority of them require training and did not take or complete the regimen of HBV vaccine. Therefore, continued medical education and training programs should be started at the hospital level along with conferences to spread knowledge about IC.

Keywords: *Awareness; Infection Control; Interns; Knowledge; Medical Interns*

1. Introduction

Healthcare-associated infections (HCAIs), also known as nosocomial infections, are infections acquired in the hospital settings. These include new hospital acquired infections in patients as well as healthcare workers. The pooled prevalence of healthcare-associated infections was 9.0% in South-East Asian countries [1].

An estimated 1.7 million patients suffer from healthcare-associated infections at any given time [2]. Healthcare workers in general are exposed to infectious surroundings in hospitals as part of their day-to-day work. Interns, both medical and paramedical, are especially at increased risk of acquiring or spreading infections. Practice of infection control guidelines is of paramount significance, for the safety of interns, co-workers and patients [3-4].

It is natural that the medical interns, being new to the fulltime handling of patients, may be apprehensive/anxious of handling patients independently, and may not be aware of the standard infection prevention precautions, or may even lack enough seriousness to follow these guidelines. The intake of healthy neutraceutical and probiotics

may act as preventative measures against infection but following guidelines is very important for medical interns [12-18].

Past research conducted in other parts of the world about knowledge and practice of infection control guidelines by medical students reveals it to be less than satisfactory. Findings of the study will identify the current status of practice of infection control guidelines in the teaching hospital by interns and help the hospital authorities to create enabling environment towards the same if found unsatisfactory. Ultimately it should have impact on reducing the number of infections among healthcare workers and patients, and reduce the morbidity, mortality and economic burden arising out of preventable infections in the hospital settings.

2. Objectives

- To assess the knowledge of infection control guidelines among medical interns.
- To know the status of practice of infection control guidelines by medical interns.

3. Methodology

3.1. Study design: The present study was a descriptive, cross-sectional study, conducted in the month of May 2018.

3.2 Study setting: D.Y. Patil Medical College teaching hospital Kolhapur, Maharashtra.

3.3 Study participants: 50 medical interns at DY Patil Medical College Kolhapur were interviewed.

3.4 Method: Study Participants, after consenting to be part of the study, were provided with a validated questionnaire separately to be self-administered and the questionnaire was asked to be filled at one go. The questionnaire collected basic demographic information like age and gender, and also recorded their Hepatitis B vaccination status and any specific training on infection control practice at hospital prior to starting internship. The questionnaire assessed the knowledge and practice components of infection control guidelines in their current role at the hospital. The knowledge component had 10 questions and practice component 8 questions.

The questions were of multiple-choice type with 3 options to choose from; one right answer, one wrong answer and a ‘don’t know’ option. Each response was awarded one score ‘one’ for a right answer and score ‘zero’ for a wrong answer or don’t know option. Total score of participants on knowledge component was classified as Very good (score 9-10), Satisfactory (score 6-8) and not-satisfactory (score 1-5). Total score of participants on practice component was classified as Satisfactory (score 6-8), Not-satisfactory (score 1-5). Questionnaire had questions to elicit information on current sources of information on infection prevention to participants, and their interest to learn and practice infection control guidelines in future. No information on name or any other personal identifier was collected.

3.5 Data analysis: Data was be entered in excel sheet and descriptive analysis conducted. Results were presented in the form of percentages. Appropriate recommendations based on the study findings follow the results.

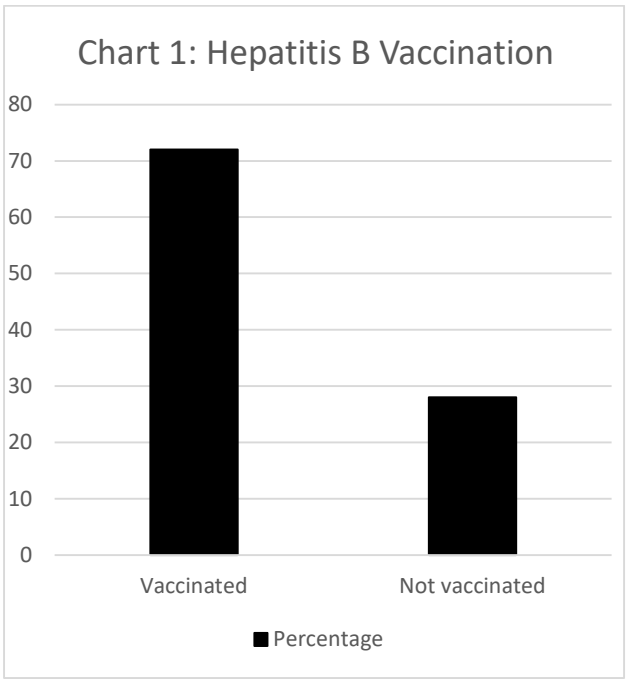
4. Results

The overall knowledge was 88%. Whereas practice of infection control guidelines was 58%. Majority of the interns had attended orientation course. Only 46% of the participants had received training about IC policies. 72% of the participants were vaccinated for Hepatitis B, 28% did not receive vaccination (Chart 1).

Most of the knowledge on infection control guidelines was obtained through MBBS curriculum. Other sources were internship orientation programme, doctors (seniors), co-interns and self-learning (Chart 2).

Knowledge parameters like use of gloves, facemasks, eye-cover and gown, facemask guidelines, risk of spilling fluids, discard needles, Standard Prevention Guidelines, etc were tested (Table 1, Chart 3).

Practice components like proper disposal of needles, use of gloves during injection, use of eye protectors, infection control precautions, reporting of injuries, sanitation practices were also assessed (Table 2, Chart 4).



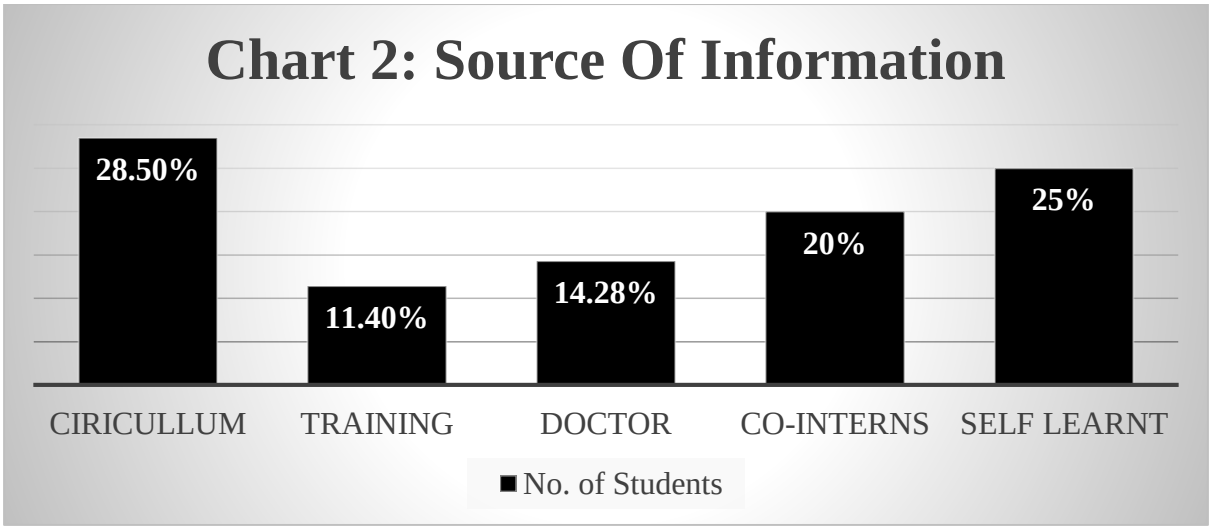
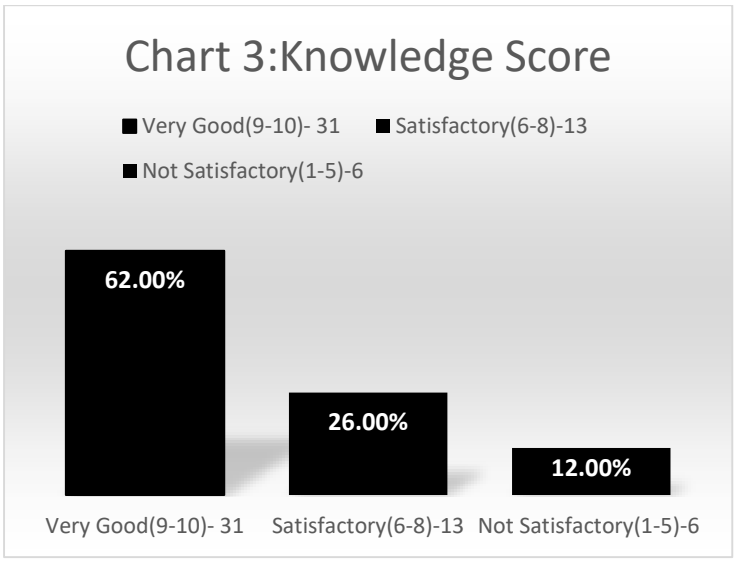


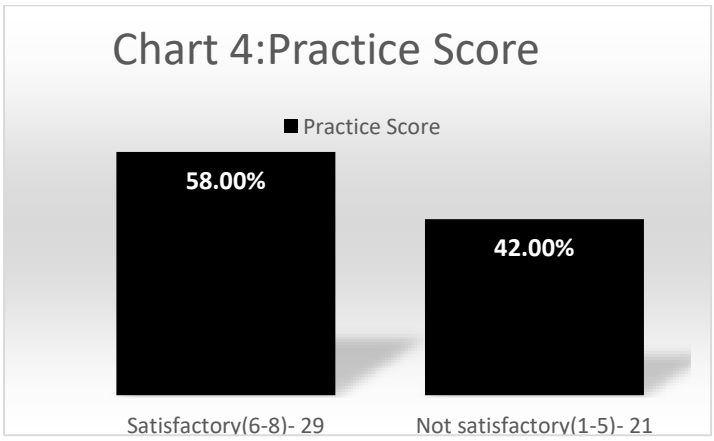
Table 1: Knowledge Parameters

Knowledge Parameter	Correct Answer	Percentage
Facemask Guidelines	41	82%
Use of Gloves, facemasks, eye cover, gown	47	94%
Risk of Spilling fluids	45	91%
Discard of Needle	41	82%
Standard Prevention Guidelines	40	80%
Sterilising Agents	33	65%

Table 2: Practice Component

Practice Component	Correct Answer	Percentage
Proper disposal of needles	35	70%
Use of gloves while giving injection	34	68%
Use of eye protectors	37	74%
Infection control precautions	41	82%
Reporting of injuries	43	86%
Sanitation Practices	41	82.%





5. Discussion

Exposure to infectious diseases is one of the most frequently identified occupational hazards faced by Health Care workers (HCW). Awareness and adequate knowledge are important requirements for all HCW.

In this study, the overall knowledge of the participants about IC measures was about 88%. Our results showed that students had more awareness about using face masks, dealing with isolated patients and IC guidelines due to it being part of the curriculum. Medical and paramedical students and trainees, being a part of the healthcare delivery system, are exposed to the same, in many teaching centres, medical and paramedical students and trainees are the first level of contact with patients.

Majority of the interns had attended orientation course in the college. They were aware about rules of safety during injection, IC guide manual and dealing with injuries. However, not all of them were aware about the use of sterilising agents although they have to face the risks associated with occupational hazards. To protect and prevent Hospital acquired injuries and/or infections, they should have adequate knowledge before their initial training period. Lack of adequate knowledge of standard precautions and isolation precautions has been reported to be

insufficient. NSIs can be seen in all the categories of HCWs. Majority of the participants had awareness regarding health hazards due to NSI. In one of the studies on needle stick injuries, except Class IV workers, rest of the HCWs were overall aware regarding hepatitis B vaccination [5]. There is a need to give emphasis as regards to awareness of postexposure prophylaxis in case of NSI.

Even though there was 88% score in the knowledge component, only 58% of the interns were able to implement this knowledge into actual practice. When it comes to the proper administration of injections, proper disposal of hospital wastes the practice was insufficient with a large number of interns not following the standard guidelines. These findings indicate the urgent need of implementing the IC policies in practice. Just having satisfactory knowledge is not useful, proper implementation is needed.

Our results showed that only 46% of the participants had received training in one or more of IC policies. Many of the participants had no previous training. Therefore, the provision of training programs that provide information about IC is a priority for medical interns. Moreover, efforts are required for bringing a reduction in the risk perception of interns through awareness campaigns and reorientation training programmes.

A large percentage (62%) of our participants had a desire for further IC training. These findings may indicate the importance of implementation of continuous training about the different IC policies for all the medical students and interns. The provision of training programs will protect both the HCW and patients from exposure to Hospital acquired infections, however, educational aims and strategies must depend on the target group. Poor IC practices will be regarded in a negative light by patients and their families.

Only 72% of the participants enrolled in our study had received HBV vaccine. This indicates the lack of protective antibody level in a large proportion of them. Therefore, the medical universities should take necessary action to inform, educate and vaccinate the HCW who may be at risk. The Ministry of Health should make HBV vaccination mandatory for all health professionals. However, there may be a false impression about prophylaxis, vaccination and treatment of HBV. Therefore, knowledge alone is not sufficient to bring about behavioural changes, but is imperative for health education personnel to remove misconceptions. This can be done by well-structured health education programs, seminars, workshop and conferences.

Serious efforts are needed to improve or review curriculum so that health sciences student's knowledge on infection prevention and control is imparted early before they are introduced to the wards. This will help in protecting both students and patients [6-7].

Similar studies conducted in Nigeria (Adegboye MB et al, 2018) [8] and South India (Jha AT et al, 2017) [9] have also established similar results. Studies to determine the impact of educational interventions have shown favourable results [10-11].

Conclusion

This study points to inadequate knowledge and practice about IC amongst interns. Only through proper education of the future HCW, the burden of HAI can be reduced. Following are our recommendations based on the study conducted:

(1) Improvement of Infection control education programs must be performed to increase safety of our HCW and patients.

(2) A medical education program should be started at the hospital level along with seminars which highlight the importance of IC policies.

(3) More emphasis should be placed on IC curriculum taught in undergraduate education.

(4) Administration of HBV vaccine to all non-immune medical and paramedical students and interns

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Isolation and characterisation of Lactobacillus species from sheep milk

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Abstract

The microbiota present in the human gut plays an vital role in the maintaining the normal health of the individual. These microbes are called as probiotics. Lactobacillus is the one of the probiotics assisting the digestion of the food and assimilation of the nutrition. The Lactobacillus are also found in the milk of milking animals. The discovery and investigation of these microbes' activities will help us to understand the pharmacological activities exhibited by these microbes. The basic techniques of isolation using the selective media and characterisation studies will help to understand the nature of these microbes ex. Gram staining, arginine hydrolysis test etc.

Keywords: Lactobacillus, Probiotics, Milk, Gram staining.

1. Introduction

The use of the microbes as functional food has explored its use in medical science. Probiotics are discovered from the different natural sources from time to time as the functional food [1–5,5]. *Lactobacillus* is found as the most preferred genera in this direction with its utility in the dairy and allied sciences [6]. The most difficult task to use these microbes as the functional food is studying the growth parameters along with the

genomic analysis. The common part observed in the case of probiotics from the same genera are its differences in physiological and biochemical characterizations [7].

Fig. 1 represents the general plating method used for the isolation of lactic acid bacteria on artificial Lactobacillus selective media by taking sheep milk samples.

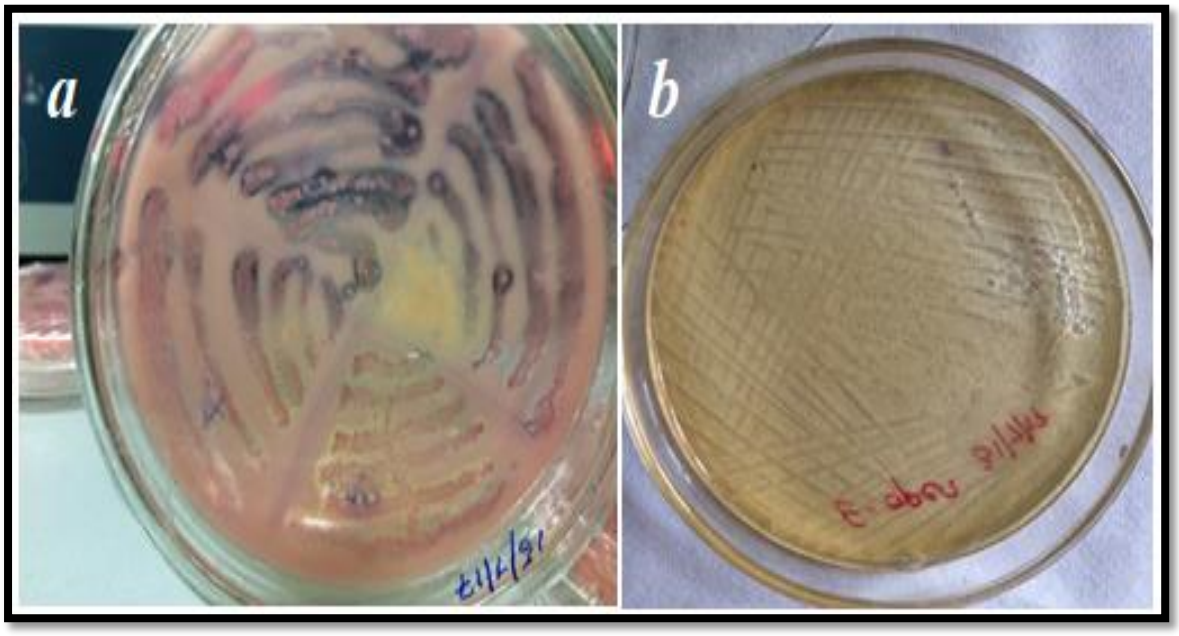

Fig.1. Isolation of the lactic acid bacteria on the a) NRCLA and b) MRS media from sheep milk samples

1. Isolation of lactic acid bacteria from sheep milk in selective media

180 milk samples were collected from the Indian sheep's breed from local places of Kolhapur, Sangli and Admapur areas of Maharashtra. The samples (50 ml) collected were stored at 5 °C until use. For bacterial enumeration, milk samples (1 ml) were kept at -78 °C in 15% glycerol before use. MRS (de Man, Rogosa & Sharpe) and NRCLA (Neutral Red Chalk Lactose Agar) broths and agar media Lactobacillus selective media were obtained as a gift sample from the Siffin Pharma, Germany [8]. MRS media was prepared by autoclaving 6.5 g MRS agar media in 100 ml distilled water, while NRCLA media was prepared by taking 5.1 g NRCLA agar media in 100 ml distilled water. The samples were inoculated on both MRS and NRCLA media by four quadrant streaking method and were incubated for a period of 48 h in a micro-aerophilic condition. After incubation, the individual colonies found on the NRCLA media were sub-cultured on MRS media and transferred into sterile MRS broth mediums. The purification of individually selected colonies were again carried out by the streak plate technique with the serial dilution method [8]. The isolated colonies were again kept at -78°C in 15% glycerol before use and were evaluated for their biochemical analysis.

Table 1. MRS media composition

Sr. no	Media ingredients	g/l
1	Proteose peptone	10.0
2	Beef extract	10.0
3	Yeast extract	5.0
4	Dextrose	20.0
5	Polysorbate 80	1.0
6	Ammonium citrate	2.0
7	Sodium acetate	5.0
8	Magnesium sulphate	0.1
9	Manganese sulphate	0.05
10	Dipotassium phosphate	2.0
Agar pH (25 ^{o}C) - 6.5 g/l (approximately)		
5.5 g/100ml distilled water at 15 lbs pressure (121 ^{o}C)		

Table 2. NRCLA media composition

Sr. No	Media ingredient	g/l
1	Peptic digest of animal tissue	3.0
2	Beef extract	3.0
3	Yeast extract	3.0
4	Lactose	10.0
5	Calcium carbonate	15.0
6	Neutral red	0.05
7	Agar	15.0
pH adjusted to 6.8 at 25°C		

Table 3. MRS broth composition

Sr. no	Media ingredients	g/l
1.	Peptone	10.0
2.	Lab-lemco powder	8.0
3.	Yeast extract	4.0
4.	Glucose	20.0
5.	Sorbitan mono-oleate	1.0 (ml)
6.	Tri-mmonium citrate	2.0
7.	Sodium acetate	5.0

8.	Magnesium sulphate	0.2
9.	Manganese sulphate	0.05
10.	Dipotassium phosphate	2.0
pH (25 °C) - 6.5 g/l (approximately)		
5.2 g/100ml distilled water at 15 lbs pressure (121 °C)		

2 Conventional lab techniques for analysis of LAB

a) Gram Staining

The Gram staining of the isolates was determined by light microscopy using Gram staining reagents. It is known that LABs are gram-positive [9]. This means that these cultures will produce blue-violet color for Gram-positive bacteria and vice-versa. The cultures were grown in MRS media at 37 °C for 24 h under micro-aerophilic conditions. Fresh cultures were used for gram staining. After incubation, the cultures were aseptically transferred into 1.5 ml of eppendorf tubes and centrifuged for 3 min at 9000 rpm. The cells were resuspended in sterile water by removing the supernatant. *L. acidophilus* from NCIM was used as positive control and *E. coli* was used as the negative control.

b) Catalase test

Catalase is an enzyme released by the microbes during the metabolic process. This enzyme act on hydrogen peroxide breaking it into water and oxygen and producing the gas bubbles. The release of the gas bubbles during the test indicates the presence of catalase enzyme.

$2H_2O_2 \rightarrow 2\ H_2O + O_2$ ---------- 1 (Equation)

The catalase test was carried out on the isolates to see their reactions to catalase. To do this, two methods can be performed. 18 h incubated cultures of isolates were grown on MRS agar at room temperature. Furthermore, for the catalase test fresh liquid cultures of LAB were used in which 3% hydrogen peroxide solution was added to 1 ml of cultures [10].

c) Gas production from glucose

This test determines the hetero-fermentative and homo-fermentative nature of the isolates by the release of CO_2 production from glucose. The overnight 1% cultures of the isolates were inoculated in MRS broths lacking citrate into the inverted Durham tubes. These cultures were further incubated for 48 h at 37 °C. The production of the CO_2 gas in Durham tubes indicates the presence of the glucose [8,11].

d) Growth at different temperatures

This test uses the bromecresol purple as an indicator in the freshly prepared MRS media. 50 µl overnight cultures of inoculum were added into 5 ml tube of modified MRS media and incubated for 7 days at 20 °C, 30 °C, 40, and 50 °C. During these incubation time, the change of the color from purple to yellow of the cells at different temperatures were observed [8,12]. *L.acidophilus* from NCIM was used as a positive control.

e) Arginine hydrolysis test

The arginine MRS modified medium and the Nessler reagent was used to view ammonia release from arginine. The freshly prepared 1% culture of the isolates was added into the MRS of 5 ml tubes containing 0.3% of L-arginine hydrochloride. The tubes were further incubated for 18 hours at 37 °C. After incubation, 50 µl of cultures were observed against the white background. 50 µl of the Nessler reagent was pipetted into the cultures and the change in the color was observed. The positive reaction was indicated by a bright orange color, while the yellow color determines the negative reaction. For the negative control, arginine free MRS was used [13].

Results and Discussion

1. Physiological and biochemical identification of LAB

All the isolates were subjected to Gram staining and they were examined under a light microscope (100X magnification). All the strains show blue-purple color staining, except *E. coli* which is used as a negative control reference. Hence all the isolated strains are found Gram-positive bacteria (Fig. 3.3. A-C), while *E. coli* shows pink color as it is Gram-negative bacteria (Fig. 3.3.D).

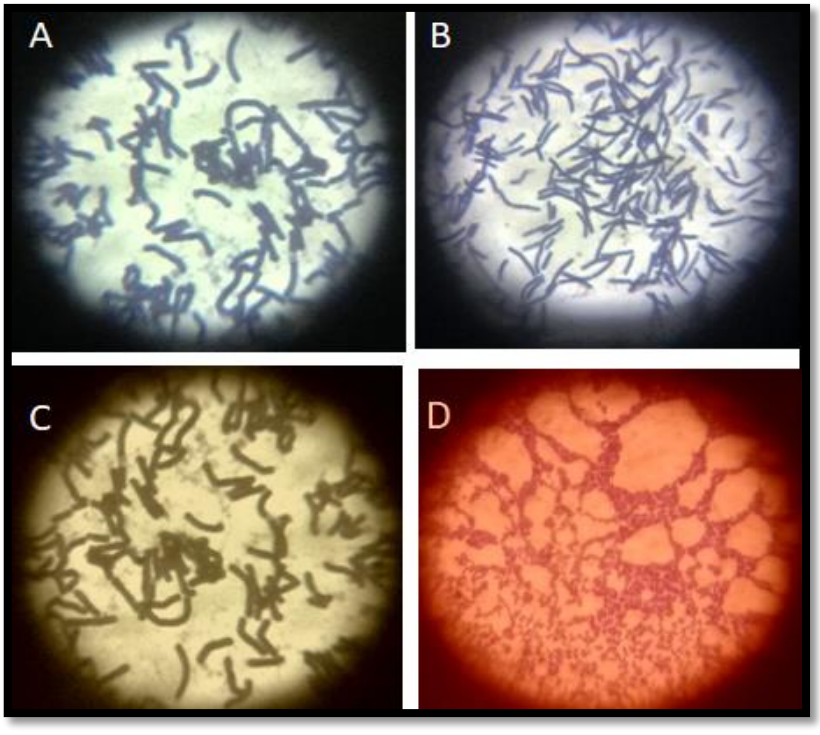

Fig. 2. Gram staining a) Sample A, b) Sample b, c) *L. acidophilus* (positive control) and d) *E. coli* (negative control) (100X)

The isolated Lactobacillus were long and rod-shaped. Isolates were tested for catalase activity. All isolates are catalase negative, as none of them given catalase activity. All strains show no gas production hence are homo-fermentative in nature (Fig. 3.4).

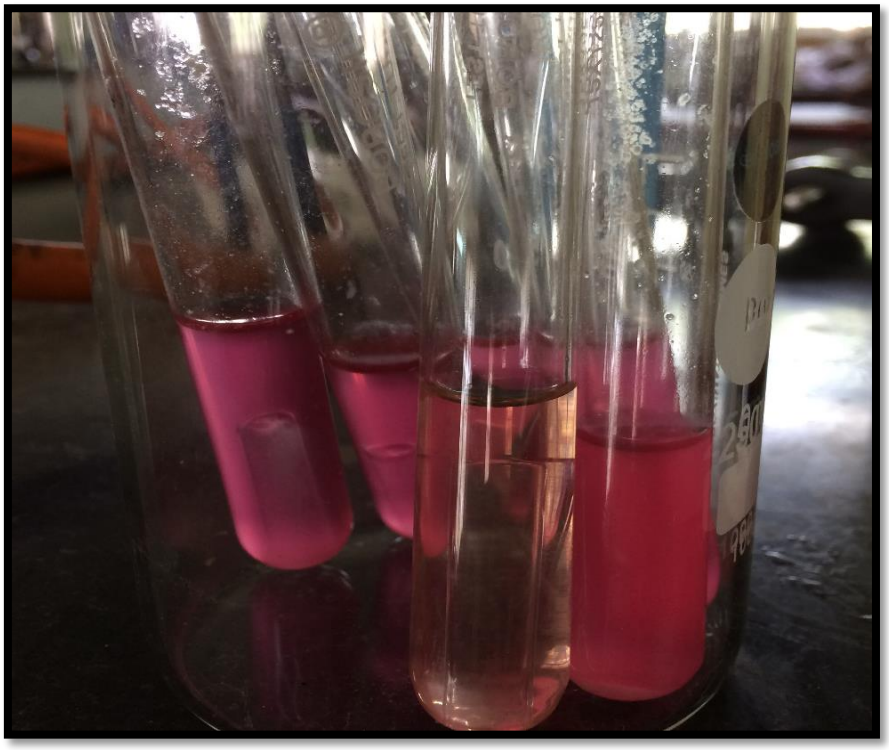

Fig. 3. Homofermentative nature observed in case of all Lactobacillus strain by Durham tube method

Another criterion for the identification of the isolates was the study of growth pattern at different temperatures. From the results of 7 days observation, all of the isolates show maximum growth between 35 °C ~ 37 °C. However, significantly very less growth is observed at 20 °C and 50 °C (Fig. 3.5).

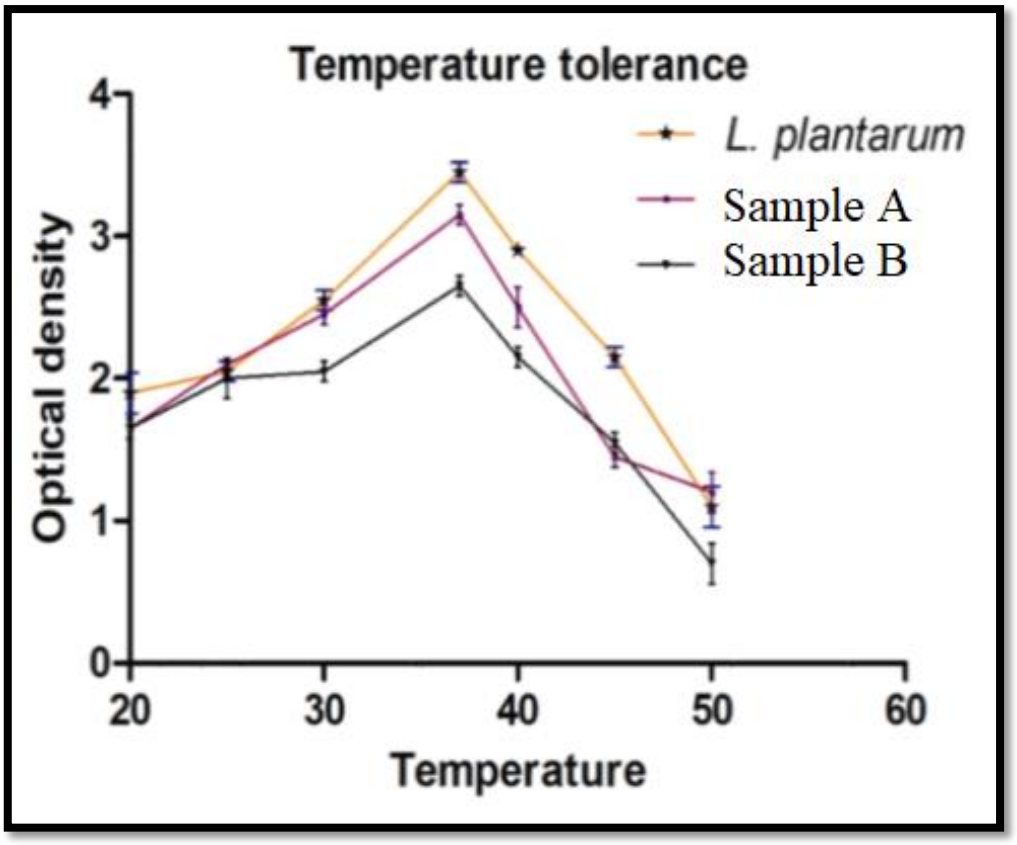

Fig. 4. Stress heat tolerance of Lactobacillus at various temperature

Arginine hydrolysis test was used as another step to follow the identification procedure. The isolates which gave the bright orange are found in producing ammonia from arginine. The yellow color indicated negative arginine hydrolysis. According to this test, both isolated strains produced ammonia from arginine.

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The origin of novel coronavirus: COVID-19

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Abstract

The new respiratory disease frequently observed are zoonoses exhibiting positive-stranded RNA viruses called Coronaviruses (CoVs). These groups of the virus are having origin from non-human species such as bats, cows and birds. The transmission of the virus to humans is reported to cause severe acute respiratory infection from cough to pneumonia. The mortality rate is increased from its origin from severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) to newly developed COVID-19. The following review states that the SARS, MERS to newly developed COVID-19 are generally types of coronavirus. The mode of infection and symptoms exhibited by all the corona types are nearly the same but with the difference in its virulence.

1. Introduction

Emerging new diseases are frequently zoonoses developed from the positive-stranded RNA viruses called Coronaviruses (CoVs) was found associated with common cold symptoms [1]. These viruses mostly infect the upper respiratory system of the mammals including humans. The source of the virus and identification of its reservoirs along with its mode of transmission between the hosts clarifies the difference. Infectious bronchitis virus (IBV) was the first coronavirus to be isolated from domestic fowls in the late 1930s [2]. But, this virus has not been reported to cause any human clinical disease. These avian coronaviruses have been found in nondomestic avian species like peafowl, pheasant, teal, turkey, penguins, pigeon, duck, and Amazon parrot [3]. Further, the coronavirus types causing minor infection in humans are 229E, NL63, OC43, and HKU1 [4]. Rarely these coronaviruses may precipitate pneumonia or bronchitis in the human host. Two species HCoV-229E and subsequently HCoV-OC43 were found to cause severe lower respiratory tract infection in immune-compromised patients and elderly patients (Jean et al., 2013, p. 43). Similarly, in 2004 Holland another novel human coronavirus (HCoV-NL63) was reported from infant (7 months) suffering from respiratory symptoms [5]. This virus also infected other children and the immune-compromised, exhibiting mild upper respiratory symptoms along with rhinorrhoea along with bronchiolitis and croup. A positive novel human coronavirus (HCoV-OC43) respiratory specimens was reported in a pediatric hospital in Montreal (2010). HCoV-OC43 is a member of the species *Betacoronavirus 1* found to infect humans and cattle showing both upper and lower respiratory tract infections along with cold-like symptoms [6]. In 2005, a novel coronavirus HCoV-HKU1, was reported in patients of Hong Kong showing pneumonitis [7]. Thus, Coronavirus is a group of related viruses that cause diseases in mammals and birds.

2. Pandemic eruption by a different novel coronavirus

Coronavirus investigators found that severe acute respiratory syndrome infected infants and old age persons. New advances in the medical sciences of coronaviruses have developed a greater understanding of the trans-species

infection, and pathogenesis of new diseases. The newly emerged zoonotic coronavirus: severe acute respiratory syndrome (SARS-CoV) was declared as a pandemic outbreak (SARS) in 2002–2003 caused by order Nidovirales, *Coronavirus* [8]. Similarly, other coronaviruses have spread from different host species from time to time causing emerging diseases.

The first human cases of SARS were reported to be caused by coronaviruses, from the source of masked palm civets and raccoon dogs which acted as an intermediate source of infection. Many survey studies reported a wide variety of sources of coronaviruses from a bat species in Asian countries. Horseshoe-nosed bats found in different locations of China and Hong Kong were found as a source of SARS-like CoVs in which these bats had found to develop their natural antibodies to tackle these coronaviruses [9]. SARS was the first reported as a pandemic in 2003, where 8,097 cases were found with 774 deaths around 30 countries globally [10].

The incubation period of SARS reported is 2 to 14 days. Few cases showed longer incubation periods for more than 14 days. Symptoms reported are the same as that of regular influenza virus, where the patients first fall ill within 2 days and become eligible to transmit the infection from symptomatic SARS after the fifth day of onset of disease with higher viral load in nasopharyngeal droplets [8].

Middle East respiratory syndrome coronavirus (MERS-CoV) was first reported in 2012. The MERS-CoV is a novel coronavirus that was earlier designated as HCoV-EMC [11]. Similarly, MERS-CoV also found to causes mild to fulminant respiratory tract infection. The clinical manifestation found in the case of MERS-CoV is similar to SARS. Bats are considered as the major suspects in terms of zoonotic infection of both MERS-CoV and SARS. MERS-CoV is a genetically the single-stranded RNA virus belonging to the family Coronaviridae same as that of other coronavirus reported. Monkeys, raccoon dogs, Himalayan palm civets, dogs, cats, and rodents are found susceptible to MERS-CoV [11]. It was stated that this virus transmitted in bats from camels. From camels, it was transmitted to humans, but the reason is unclear.

3. COVID-19 as novel coronavirus

The SARS-CoV-2 also called a COVID-19 epidemic was reported in late December 2019 in Wuhan, China. Earlier, A pneumonia of unknown cause was detected in China was first reported to the WHO Country Office in China on 31 December 2019 [12]. The outbreak was declared a Public Health Emergency of International Concern on 30 January 2020. People who become infected developed serious illnesses mostly difficulty in breathing. Older people, and those with underlying medical problems like high blood pressure, heart problems or diabetes, are reported likely to develop serious illness. Studies to date suggest that the virus that causes COVID-19 is mainly transmitted through contact with respiratory droplets rather than through the air. It was found that molecular divergence is less between SARS-CoV-2 and COVID-19. The scientist found about 4% variability in genomic nucleotides of bat SARS and a bat SARS-CoV-2.

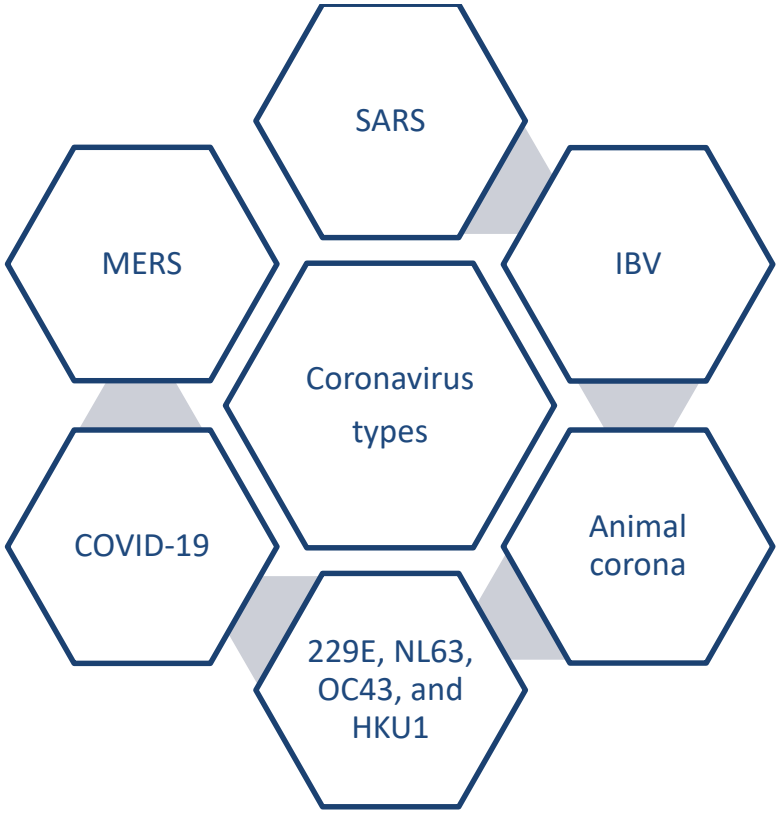

Fig.1. Types of coronavirus

4. Pathogenesis found in all above-mentioned coronavirus shows

Several distinct features such as pneumonia with epithelial cell proliferation are reported in patients infected by a coronavirus. Few reported macrophage infiltrations of the lungs along with the haemophagocytosis [13].

Symptoms	SARS	MERS	COVID-19	IBV (in the avian group, not in human)	229E, NL63, OC43, and HKU1
Fever	**	**	***	**	**
Dry cough	*	*	***	-	*
Runny nose	***	***	*	-	***
pneumonia	**	**	***	Extra-pulmonary damage	**
Shortness of breath	**	**	***	**	**

Chills and muscle aches,	**	**	***	**	**
Headache	**	**	**	-	**
Diarrhea	*	**	***	-	**
sore throat	***	**	***	-	**
rash	***	***	*	-	***
Other specific					Tachypnea (34 respirations/minute), dyspnea, and hypoxemia

*- minor symptoms, **- mostly observed symptoms, ***- majorly observed symptoms, - data not available.

Many research is going on to check that use of probiotics or neutraceuticals can prevent the infection by antioxidant mechanism along with existing drugs available in the market [14–21].

5. Mode of transmission

The interaction of the coronavirus antigenic spike protein is complementary with the host cell receptor exhibiting tissue tropism and infectivity [22]. The transmission primary mode of COVID-19 is via respiratory droplets from an infected person. This generally spreads from coughs, sneezes, or talks. The fomite borne transmission is by settling of the droplets of an infected person on the nose, eyes or mouth of a healthy individual. Coronaviruses remain for several days on surfaces of metal, glass, or plastic for several days. The transmission also spread by touching the face with hands contaminated by fomite mode. Respiratory droplets settle due to heaviness as compared to air, hence, a distance of more than six feet prevent the spread of infection from the suspected person

The basic transmission of infection is mostly by the droplet mode while it is unclear if airborne transmission occurs with COVID-19 infection. It is thus suggested the application of protective measures, including N95 masks. In general, the Coronavirus spread can be prevented by [2,4]:

- Covering the mouth during coughing and sneezing to avoid the droplets containing the virus to spread in the air
- Isolating the infected person who's already carrying the virus from healthy individuals
- Avoiding any contact with the object which contains the virus and then touching your nose or mouth

6. Conclusion

Coronavirus has affected the globe from time to time since the 20th Century by its different forms. The COVID-19 is one of the types of Coronavirus that developed pandemic infection such as SARS, MERS, etc. The word corona should be confused with the only COVID-19, but it is one type of acute respiratory infection such as SARS, MERS, etc. The symptoms observed in the case of all Corona subtypes are more or less similar. Thus Corona can briefly be called to all subtypes such as SARS, MERS and the newly evolved COVID-19.

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Study of antimicrobial sensitivity pattern in family Enterobacteriaceae in a Teaching Hospital, Kolhapur, Maharashtra.

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ABSTRACT

Infections due to gram negative bacilli (GNB) are more common and increasing antibiotic resistance amongst these infections is a worldwide problem. Enterobacteriaceae are frequently isolated from various clinical samples. Overuse or irrational use of antibiotics to treat these infections lead to develop antibiotic resistance, such multidrug resistant (MDR)-Enterobacteriaceae infections are difficult to treat. The present study was conducted to know the antibiogram of Enterobacteriaceae isolated from various clinical samples. This retrospective study was conducted in Dr D Y Patil Hospital & Research Centre, Kadamwadi, Kolhapur, Maharashtra, India over a period of one year (December 2017- December 2018). Identification and antibiotic sensitivity of Enterobacteriaceae were performed by standard microbiological procedures and VITEK II automated system. A total 235 Enterobacteriaceae GNB was isolated from various clinical samples. The most frequently isolated organism was *Escherichia coli* 45.5% followed by *Klebsiella* spp 31%, *Citrobacter* spp 11.9%, *Proteus* spp 9.3%, *Serratia marcescens* 1.2% & *Enterobacter aerogens* 0.8% in the present study. Most of the organisms were isolated from urine sample 44% followed by pus 40%. Carbapenems, Tigecycline and Amikacin were effective against MDR-Enterobacteriaceae. Most effective antibiotics were Imipenem showed 97%, Meropenem 91%, Amikacine 91%, Gentamicin 83.4%, Piperacillin-tazobactum 79.5%, Tigecycline 97% and Ciprofloxacine 41,2% sensitivity. Most resistant antibiotics were Ampicillin showed 31.4%, Ceftazidime 25.5% and Ceftrixone 24.6% sensitivity in the present study.

KEY WORDS: Antibiogram, Enterobacteriaceae, *Escherichia coli*, Imipeneme.

INTRODUCTION

Resistance to different antibiotics is a world-wide problem now days. Increasing antimicrobial resistance among Gram Negative Bacilli (GNB) is a major problem to treat infections in the community as well as in hospitalized patients (1). A Multidrug resistant infection is difficult to treat and also increases the morbidity & mortality in critically ill patients, especially ICUs.

Irrational use of antibiotics by practitioners, lack of hospital antibiotic policy leads to increase antibiotic resistance. However, the overuse and misuse of antibiotics is leading to the emergence of resistance to these life –Saving drugs. Hospital antibiograms are commonly used to help guide antimicrobial treatment and help to detect and monitor
pattern of antimicrobial resistance amongst the clinical isolates.

Gram negative bacilli are a large group of micro-organisms and amongst them Enterobacteriaceae are one of the most common bacteria isolated from various clinical samples.

Escherichia coli, Klebsiella spp, Proteus & Citrobacter spp are commonly isolated from clinical samples like urine, pus etc. other members such as *Serratia marcescens* & *Enterobacter aerogens* infections are also increasing (2).

ESBL producing GNB are clinically important because they causes multidrug resistant infections and very difficult to treat especially in patients in ICU, post-operative infections etc. [3] These enzymes are chromosomally or plasmid mediated so they show resistance to non-beta-lactam antibiotics like quinolones, aminoglycosides, chloramphenicol etc [4].

The present study was undertaken to know the antibiogram of GNB isolated from clinical samples at our teaching hospital.

MATERIALS AND METHODS

The present study was carried out to know the antibiotic sensitivity pattern among GNB in family Enterobacteriaceae isolated from various clinical samples at Dr D Y Patil Hospital and Research Centre, Kadamwadi, Kolhapur, Maharashtra over a period of one year (December 2017 to December 2018)

Clinical samples like pus, urine, sputum, body fluids etc received in clinical microbiology laboratory of Dr D Y Patil Hospital & Research Centre, Kolhapur were plated on blood agar, MacConkey agar (Hi-Media, Mumbai) for primary isolation of organisms.

Preliminary identification of GNB was performed using conventional methods including: Gram-staining, culture characteristics, lactose fermentation, and oxidase test. Further identification to species level was performed using VITEK II (ID-GN 21-341 card) automated system (BioMerieux, France) and Minimum Inhibitory Concentration (MIC) of these GNB by using AST-N280 card according to manufacturer's instructions.

Antimicrobial susceptibility test (AST): Disc diffusion method

The susceptibility of the tested isolates was carried out by Kirby-Bauer disc diffusion method on Mueller Hinton agar (Hi-Media, Mumbai) results were noted according to the Clinical and Laboratory Standard Institutes (CLSI) guidelines (CLSI, 2017)[5]. 0.5 McFarland standards used as inoculum, direct colony suspension for AST.

The commercial antibiotics discs (Hi – Media, Mumbai) used for Enterobacteriaceae were piperacillin-tazobactam (100/10 µg), ampicillin (10µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), meropenem (10µg), imipenem (10 µg), amikacin (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg) and nitrofurantoin (300 µg) for urine samples

Standard strains used were- *Escherichia coli*- 25922 as negative control and *Klebsiella pneumoniae*-700603 as positive controls as quality control for identification and antibiotic susceptibility test of test strains.

RESULTS

The total 235 GNB were isolated form different clinical samples.

Table 1: isolation of GNB from different clinical samples

organism	no	%age
Escherichia coli	107	45.5
Klebsiella spp	73	31
Citrobacter spp	28	11.9
Proteus spp	22	9.3
Serratia marcescens	3	1.2
Enterobacter aerogens	2	0.8

Diagram 1: specimen wise distribution of GNB

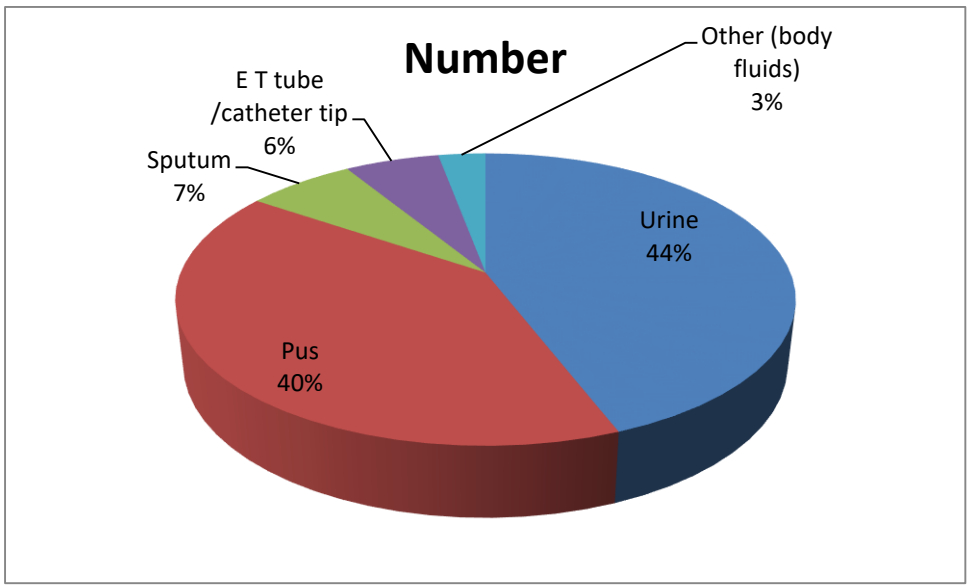


Table 2: distribution of organisms in samples

	Sample				
Organism	Urine	Pus	Sputum	Body fluids	ETtube/catheter tip
E coli (107)	57 (53.2%)	46 (42.8%)	2 (11.5%)	1 (0.9%)	1 (0.9%)
Klebsiella spp (73)	28 (38.3%)	26 (35.6%)	11 (15%)	2 (2.7%)	6 (8.2%)
Citrobacter spp (28)	9 (32.1%)	11 (39.2%)	3 (10.7%)	3 (10.7%)	2 (7.1%)
Proteus spp (22)	12 (54.5%)	9 (40.9%)	--	--	1 (4.5%)
Serracia marcescens (3)	1 (33.3%)	1 (33.3%)	--	1 (33.3%)	--
Enterobacter aerogens (2)	--	2 (100%)	--	--	--

Table 3: Antibiotic sensitivity pattern of organisms

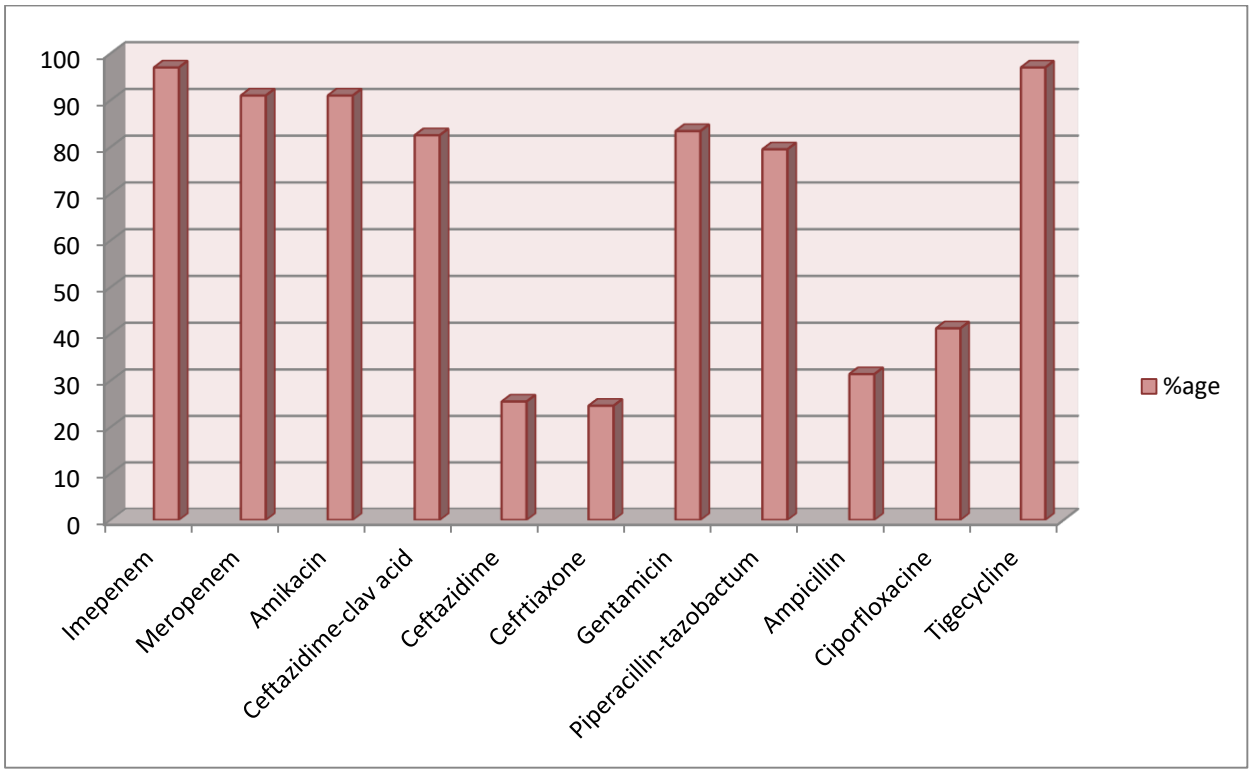
ANTIBIOTICS	**ORGANISM**					
	E coli (107)	***Kleb spp*** (73)	***Citrob Spp*** (28)	***Proteus Spp*** (22)	***Serracia Spp*** (3)	***Enterob Spp*** (2)
Amikacin	95 (88.7%)	65 (89%)	25 (89.2%)	20 (90.9%)	3 (100%)	1 (50%)
Gentamicin	90 (84.7%)	60 (82.1%)	20 (71.4%)	18 (81.8%)	3 (100%)	1 (50%)
Caftezicime-clav acid	90 (84.7%)	58 (79.4%)	22 (78.5%)	7 (77.2%)	2 (66.6%)	1 (50%)
Ceftazidime	22 (20.5%)	20 (27.3%)	8 (28.5%)	8 (28.5%)	2 (66.6%)	1 (50%)
Ceftriaxone	25 (23.3%)	15 (20.5%)	7 (25%)	7 (31.8%)	2 96.6%)	1 (50%)

Imipenem	105 (98.3%)	69 (94.5%)	26 (92.8%)	20 (90.9%)	3 (100%)	2 (100%)
Meropenem	102 (95.3%)	65 (89%)	24 (85.7%)	15 (68.1%)	3 (100%)	2 (100%)
Piperacillin-tazobactum	85 (79.4%)	60 (82.1%)	20 (71.4%)	15 (68.1%)	3 (100%)	1 (50%)
Ampicillin	37 (34.5%)	21 (28.7%)	9 (32.1%)	5 (22.7%)	--	1 (50%)
Ciprofloxacine	62 (57.%)	14 (19.1%)	8 (28.5%)	8 (36.3%)	2 (66.6%)	2 (100%)
Tigecycline	105 (98.1%)	71 (97.2%)	24 (85.7%)	20 (90.9%)	3 (100%)	2 (100%)

Table 4: Antibiogram of Enterobacteriaceae: shows sensitivity to different antibiotics

Antibiotic	sensitive	%age
Imipenem	228	97
Meropenem	214	91
Amikacin	214	91
Ceftazidime-clav acid	194	82.5
Ceftazidime	60	25.5
Cefrtiaxone	58	24.6
Gentamicin	196	83.4
Piperacillin-tazobactum	187	79.5
Ampicillin	74	31.4
Ciporfloxacine	97	41.2
Tigecycline	228	97

Diagram 2: Graph showing the %age of sensitivity to different antibiotics by Enterobacteriaceae



DISCUSSION

In the present study 235 GNB were isolated from various clinical samples.

The distribution of GNB was shown in Table 1. *Escherichia coli* was most frequently isolated from 235 clinical specimen 107 (45.5%) followed by *Klebsiella spp* 73 (31%)

Similar findings were reported from Shankarankutty et al[(1)] *E.coli* 55.3% & *Klebsiella* spp 16.6%, Zaman et al[(2)] *E coli* 38.07% & *Klebsiella* spp 15.91%, Mantravadi et al[(6)] reported 21.7% *E. coli* followed by 16.8% *Klebsiella* spp, Sahu et al[(7)] study showed *E coli* 58.5% & Klebsiella *spp* 41.5%, similar reports were noted by Vipin Kumar et al[(8)] *E coli* 58.4% & *Klebsiella* spp 22.2% in his study.

Other organisms like *Citrobacter* spp 28 (11.9%), *Proteus* spp 22 (9.3%), *Serratia marcescens* 3 (1.2%), Enterobacter *arogens* 2 (0.8%) were reported in the present study.

Specimen wise distribution of GNB was shown in diagram 1 in the present study. Most of the GNB out of 235 samples were from urine sample 104 (44.2%) followed by pus sample 95 (40.4%). Similar findings were reported by Shankarankutty et al[(1)] 75.9% from urine sample & 55.3% from pus sample, Zaman et al[(2)] reported 43.7% from urine & 27.8% from pus , Sahu et al[(7)] noted 17.1% from urine followed by 11.1% from pus .

Organism wise distribution in various clinical samples was shown in table 2. *Escherichia coli* was most frequently isolated from urine sample 57 (53.2%) followed by pus 46 (42.8%), from sputum 2 (11.5%). *Klebsiella* spp 28 (38.2%) from urine sample, 26 (35.6%) from pus, 11 (15%) from sputum, 6 (8.2%) from ET tube/catheter tip. Similar results were reported by Shankarankutty et al[(1)] 125 E.coli were isolated from urine sample followed by pus sample 21, Zaman et al[(2)] reported 73.1% E coli from urine sample & 39.2% Klebsiella spp from pus .

Citrobacter spp frequently isolated from pus 11 (39.2%), from urine 9 (32.1%). Proteus spp more frequently isolated from urine sample 12 (54.5%), pus 9 (40.9%). Serratia marcescens 3 isolates & Enterobacter aerogens 2 from pus sample.

Antibiotic sensitivity pattern of Enterobacteriaceae GNB was shown in table 3. In the present study *E coli* showed 98.30% sensitivity to carbapenem like Imipenem & 95.30% sensitivity to Meropenem, *Klebsiella* spp showed 94.5% sensitivity to Imipenem & 89% sensitivity to meropenem, similar findings were reported by Salma Nabi et al [(9)] as 98.89% sensitivity to Imipenem & 95.45% sensitivity to meropenem.

Serratia marcescens & *Enerobacter aerogens* showed 100% sensitivity to carbapenems, piperacillin-tazobactum. *Citrobacter* spp & *Proteus* spp showed 92.8% & 90.9% sensitivity to carbapenems respectively, all Enterobacteriaceae showed sensitivity to tigecycline 90% - 100%, citrobacter spp showed 85.5% sensitivity to tigecycline in the present study.

Sensitivity to ciprofloxacine in E coli 57% and 19% in Klebsiella spp in the present study, Nabi et al [(9)] showed 56.7 % to *E coli* & 32.8% to *Klebiella* spp.

Overall percentage of sensitivity to different antibiotics by Enterobacteriaceae was shown in diagram 2. In the present study imipenem showed 95.7% and 89.9% sensitivity to meropenem, Tigecycline showed 95.7% sensitivity, Amikacin showed 89.9% sensitivity, Gentamicin showed 82.3% sensitivity, piperacillin-tazobactum showed 78.5% sensitivity, 3rd generation cephalosporins like ceftazidime 25.2%, cephotaxime 24.3% sensitivity. Nitrofurantoin specifically used for isolates from urine samples showed 95% senensitivity.

High amount of resistance was noted to ampicillin and 3rd generation cephalosporins in the present study, similar results were noted by Shankarankutty et al [(1),] also by Mohamaad mehr et al [(10)],Perisamy Hariharan et al[(11)].

Present study showed high sensitivity to amikacin and gentamicin , similar results were noted by Shankarankutty et al[(1)], senisitivity to ciprofloxacin was 42.2% in the present study, similar results were noted by Perisamy Hariharan et al[(11)],Krithu Panta et al[(12)].

In the present study carbapenems, tigecycline wer most active against multidrug resistant Enterobacteriaceae similar results were reported by Zaman et al[(2)], Krithu Panta et al[(12)]

CONCLUSION

Inappropriate and overuse of different antibiotics leads to antibiotic resistance in family Enterobacteriaceae. The present study and other published studies showed carbapenems are the drug of choice to treat multidrug resistant (MDR)-Enterobacteriaceae, but some strains of this group showed resistance to carbapenems also. It is very difficult to treat the infection showing resistance to carbapenems. The present study showed high resistance to ampicillin and 3rd generation cephalosporins. To overcome this every hospital should prepare antibiotic policy.

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The role of the food and fertilizers in antimicrobial resistance in human and its preventive measures

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Abstract

Agriculture is the main occupation in India and uses plant and animal as a source of the food. In farming, many different chemicals are used to make plants and animals grow faster ex. Oxytocin which is unethical. Intensive farmers use artificial fertilizers and growth promoters such as auxin, ethylene, etc. This resulted in the bioaccumulation and health bizarre in the ecosystem. Due to this, the dreadful diseases such as diabetes and cancer have spread their roots to the lower of the society, especially in India. To cope up with the situation there is a need for organic farming which has brought the revolution in the countryside. Organic farming includes types such as vermicompost, green manure, biofertilizers, biological management, and animal husbandry. Similarly, we proposed the new form of organic farming in cities called terrace gardening. The use of household things such as clay, gunny cement bags, and kitchen waste as modern gardening can be practiced on building terrace. Different vegetables and fruits such as brinjal, ladyfinger, tomato, cucumber, watermelon, sugarcane, etc can be grown via the terrace gardening. Surplus, this will turn into a clean air ecosystem for different birds and to human being living in its vicinity.

Keywords: Agriculture, terrace gardening, organic farming,

1. Introduction

Agriculture is the main occupation in India and uses plant and animal as a source of the food. In farming, many different chemicals are used to make plants and animals grow faster ex. Oxytocin which is unethical. Human being consume plant such as fruits, vegetables, cereals, legumes, pulses, medicinal plants, and oil-seeds. Humans

also consume new nutraceutical food articles like probiotics as prophylactic agent [1–8]. While animal food consumed are chicken. egg, meat, fishes, milk etc [9]. The pollution and use of extensive fertilizers and pesticide for agriculture is creating a severe issue of the bio-accumulation generating the health hazards.

Table 1. Pollution and its hazardous effects

Pollution			
Soil	**Water**	**Air**	**Diseases**
Excessive use of fertilizers/ pesticides kills beneficial microbes and spoil soil health	Leaches as ground water in bore-well, reservoir, wells and rivers etc	Spreads in atmosphere	It weakens the plant immunity
Make soil alkaline / Saline	Increases the water toxicity	Causes many respiratory disorders	If such plants consumed by human causes gut disorders
Reduces the soil fertility and porosity	Diseases	Ex- Asthma	Ex- Cancer

2. Conventional farming and its problems

Many different chemicals are used to make plants and animals grow faster ex. Oxytocin. Intensive farmers use artificial fertilizers and growth promoters: Auxin, ethylene etc.

These chemicals do not just disappear but stay in the plants that people eat, so our food is contaminated with chemicals. Soil used to grow the plants will also be contaminated and have chemicals in it for a very long time. Animals eat the grass, which has had chemicals sprayed on to it, so the chemical get into their blood and therefore the meat that people eat. This leads to the condition called as bioacculmulation [10]. Bioaccumulation is the gradual accumulation of substances, such as pesticides, or other chemicals in an organism.

Table. 2: Food product and pesticides contaminant

Food product	**Contaminant pesticides**
Apples	Diphenylamine, Captan, Endosulfan, Phosmet, Azinphos-methyl
Bananas	Diazinon, Thiabendazone, Carbaryl
Cabbage	Methamidophos, Dimethoate, Fenvalerate, Permethrin, BHC
Carrots	DDT, Trifluralin, Parathion, Diazinon, Dieldrin
Cauliflower	Methamidophos, Endosulfan, Dimethoate, Chlorothalonil, Diazion
Cherries	Parathion, Malathion, Captan, Dicloran, Diazinon
Corn	Sulfallate, Carbaryl, Chlorpyrifos, Dieldrin, Lindane
Cucumbers	Methamidophos, Endosulfan, Dieldrin, Chlorpyrifos, Dimethoate

Grapes	Captan, Dimethoate, Dicloran, Carbaryl, Iprodione
Green Beans	Dimethoate, Methamidophos, Endosulfan, Acephate, Chlorothalonil
Lettuce	Mevinphos, Endosulfan, Permethrin, Dimethoate, Methomyl
Onions	DCPA, DDT, Ethion, Diazinon, Malathion
Oranges	Methidathion, Chlorpyrifos, Ethion, Parathin, Carbary
Peaches	Dicloran, Captan, Parathion, Carbaryl, Endosulfan
Pears	Azinphos-methyl, Cyhexatin, Phosmet, Endosulfan, Ethion
Potatoes	DDT, Chlorpropham, Dieldrin, Aldicarb, Chlordane
Spinach	Endosuslfan, DDT, Methomyl, Methamidophos, Dimethoate
Strawberries	Captan, Vinclozolin, Endosulfan, Methamidophos, Methyl Parathion
Sweet Potatoes	Dicloran, DDT, Phosmet, Dieldrin, BHC
Tomatoes	Methamidophos, Chlorpyrifos, Chlorothalonilo, Permethrin, Dimethoate
Watermelon	Methamidophos, Chlorothalonil, Dimethoate, Carbaryl, Captan

These pesticides further precipitate the chances of cancer suspect, makes the fish consumption toxic, very persistent leukemia development, toxicity to wildlife, bladder damage, male sterility etc.

3. Remedies to stay healthy [11]

1. Change lifestyle
2. Become vegetarians
3. Avoid alcohol and red meat
4. Avoid eating at Fast food restaurant
5. Daily exercise in clean air
6. Eat lots of organic food and fibers that can achieved by-
   - 6.1. No pesticides, herbicides, fungicides or chemical fertilizers are used. Organic ensures that foods are grown without the use of these harmful substances, some of which are known to cause cancer.
   - 6.2. Organic farming reduces long time exposure to pesticides and chemical fertilizers for farmers, workers, and consumers which can affect the body's ability to eliminate toxins.
   - 6.3. Diseases, pests, and weeds are managed through good soil health for natural plant resistance, selection for stronger plants, crop rotation, natural predators, and beneficial insects.
   - 6.4. Unlike conventional methods, antibiotics, pathogens, or hormones are used in raising livestock. Organic livestock have higher animal welfare standards which lower levels of pathogens present in meat.
   - 6.5. Eating organic fruits and vegetables could increase your antioxidant intake by 20-40%.
   - 6.6. 6. Organic farming ensures that food is not subject to any artificial human intervention or genetic modification.
   - 6.7. 7. Organic food cannot be irradiated. Irradiation causes changes to both macro and micronutrients in foods and creates free radicals.

Advantage of having health food are:

1 Healthy food as it contains no toxic substances

2 Natural with good taste

3 Acts as preventive measures against diseases, as unbalanced diet triggers different diseases.

4 Takes care of environmental concerns of farming

The utlity of organic farming contains [12]-

1 **Artificial fertilizers are banned in Organic farming.**

2 Organic farmers use animal manure, compost and human sewage, (which has been heated to destroy any harmful microbes) to make their crops grow.

3 'Green manure' is grown – plants are grown, then ploughed in and left to rot.

4 Many beneficial bacteria (Rhizobium etc), worms (earthworm) is natural habitat of soil and results its development

5 Crop Rotation

So this concept can be devopled in Cities by concept of terrace gardening.

4. Terrace gardening

A terrace garden is any garden on the roof of a building. Besides the ornamental benefit, roof plantings may provide food, temperature control, hydrological benefits, architectural enhancement, habitats and recreational opportunities [13]. The practice of cultivating food on the rooftop of buildings is usually mentioned as rooftop farming. Terrace gardens are commonly created at three different levels, which are:

1. On rooftop of a building,
2. Porches, window boxes, portico, balconies and such projected levels out of high-rise , above the bottom level.
3. At stage level, round the base or on roof of huge basements.

The mission and vision of terrace gardening are-

1 Design and prepare attractive gardens in home, balcony and roof tops of buildings.

2 Become the best consultant firm on roof top gardening in Kolhapur. It can also be developed in different metropolitan cities like Mumbai, Delhi etc

3 It can act as Oxygen garden

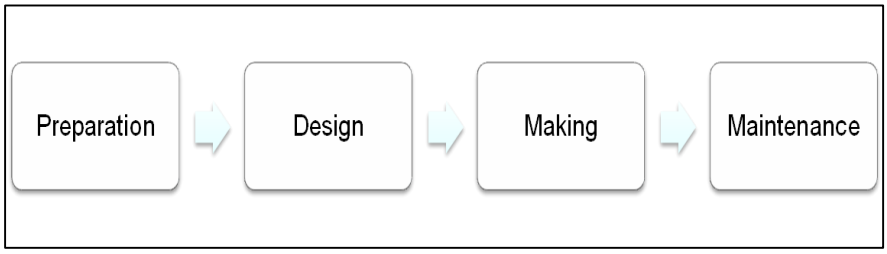

Fig. 1: Methods of terrace gardening

The steps involved in the terrace gardening are:

1 Preparation: Survey, measuring available space etc.

2 Design: Vertical, selecting materials & plants etc.

3 Making: Structure making and plantation

4 Maintenance: Post production service in short/long term

The process of the sampling includes:

1 Take a pot and place stones at the bottom. The soil mixture needs to be one part coir pith, soil and manure.

2 To this, add a few tablespoons of neem mixture and plant pseudomonas.
3 Mix well together and put it over the stones in the pot.
4 Plant a few seeds of one plant.
5 Water the soil. The plant is ready.
6 Now just remember to water it every day!
7 Once a week, dilute 30 ml of panchagavya in one litre water and spray this on the plants.
8 The right mix of soil requires regular soil, compost coir peat (or sand) and vermin-compost in equal quantities.
9 Containers of all sizes, shapes, whether of plastic, ceramic, metal or mud can be used.
10 Clay pots are more stable in windy conditions.
11 Large containers are used for planting tree. Plastic containers are used for small herbs/ plants.
12 Choose large and deep pots, avoid thin and poor quality plastic pots.
13 Use good quality potting mix for healthy growth of plants and productivity.
14 VEGETABLES FOR POTS: Tomatoes, Cucumbers, Radishes, Beans, Potatoes, Onions, Carrots, Chilies and Peppers.

Fig.2. Demonstartion of the terrace gardening

Benefits of terrace gardening

1 Conservation of soil and water.

2 Oxygen storage benefits.
3 Doesn't use pesticides and chemicals.
4 Improved air quality.
5 More pleasant and peaceful, less stressful environment.
6 Improved climate conditions- addressing heat stress.
7 It helps us to consume chemical free vegetables.
8 No need to buy vegetables from market.
9 Vegetables available in all extreme conditions ex. flood and other natural calamities etc.
10 The cost of production and maintenance is affordable.
11 Can save lot in terms for transportations, storage.
12 During the times of heavy rains or disasters, we can consume the available vegetables.
13 Even during the time of price hike in vegetables, this terrace gardening helps us by providing own vegetables.
14 Place for get together and public meetings.
15 It reduces social tensions.
16 It acts as opportunities for volunteers, employment.

5. Conclusion

The world is heading towards modernization the lifestyle is also changing affecting the human health. The busy schedule and consumption of fast food thus inviting the deadly diseases like cancer. The solution to these hurdles is utilisation of terrace gardening concept. Due to the population explosion there is need that every house of citizen in the country to be developed into the dome of oxygen bank by means of terrace gardening. The plantations at the terrace gardens will help to bring ecological imbalance and will generate organic fruits and vegetables. The terrace gardens will bring waste recycling, ecological benefits, and water conservationand health benefits. It will also attract the birds and insects making surrounding clean and happy.

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Prevalence, pattern and Impact of surfing on self-medication in silver surfers

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Abstract

Background: Silver surfers are the older persons aged 50 years & above who spend a lot of time using the internet. Self-medication is the use of one or more medications with or without physicians' diagnosis, opinion or prescription & supervision which includes the use of herbal & chemical drug. Silver surfers are more prone to self-medication because of less family responsibilities, more time, access of internet with lower cost and easy availability of medication. The objective of the study was to study the prevalence, patterns of self-medication and impact of surfing on it among silver surfers.

Methods: A observational, questionnaire based, prospective based was carried out in silver surfers in Kolhapur city. A self-structured and pre-validated questionnaire included information on socio-demographic details, general aspects of self-medication pattern, time spending on internet, source of knowledge, reason for use etc. and analyzed.

Results: Prevalence of self-medication among silver surfers was alarming high. Self-medication practices are highest in age group 51- 60 years but no significant difference among gender. Surfers are influenced for self-medication showing patterns like those who discontinued prescribed medicines without consultation are 46 % where as 34 % shifted to other medications from different pathies without consultation and 18 % added another drug by seeing advertisement or homemade remedies.

Conclusions:

It's very important to consider the media as a source of information but for health-related issues it is very risky to get influenced with advertisement and go for self-medication. Surfing and self-medication are easy but fatal or risky for health.

Keywords

Self-medication, Silver surfers, Impact of surfing, Prevalence and patterns of self-medication

Introduction

Self-medication is the use of one or more medications with or without physicians' diagnosis, opinion or prescription & supervision which includes the use of herbal & chemical drug. Self-medication is a behavior that is widely practiced worldwide. Although a universally accepted definition of self-medication does not currently exist, the World Health Organization (WHO) describes it as the ''selection and use of (herbal or chemical) medicines by individuals to treat self-recognized illnesses or symptoms'' [1] Silver surfers are the older persons aged 50 years & above who spend a lot of time using the internet, while some use holistic medicine or probiotics for the same [2, 12-18].

Self-Medication is dependent on various factors such as education, family, society, law, availability of drugs, availability of information source and exposure to advertisements. Number of reasons influence person for use of Self-medication but very common and could be enumerated are urge of self-care, feeling of sympathy toward family members in sickness, lack of time, lack of health services, financial constraint, ignorance, misbelieves, extensive advertisement and availability of drugs in other than drug shops.[3]

In India, availability of medication to the public without prescription, lower cost, cannot afford medical services, relative medical knowledge, background of medical environment, previous personal experience, personal beliefs, etc. are very promising and promoting influences for self-medication. Self-medication is one of the biggest global, health & socio-economic problems of developing countries including India. This study aimed to evaluate the impact of social media on self-medication in silver surfers to know the demographic pattern of self-medication in silver surfers and to study the influence of social media on it.

Materials & Methods

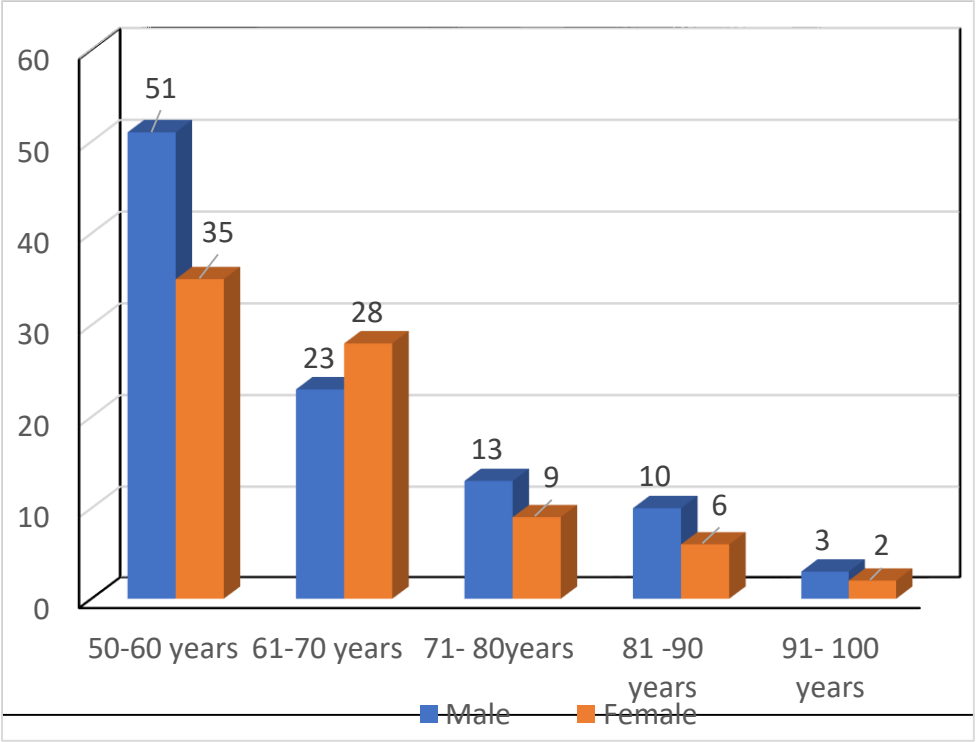
This is observational, questionnaire based, prospective, qualitative, non-interventional study. Study was conducted in various areas of Kolhapur city in July 2018. Pre-structured & pre-validated questionnaire was given to the randomly selected subjects. Consent was taken beforehand. The data was compiled & subjected to statistical analysis. Silver surfers of both genders aged 50 years & above with history of self-medication with drugs from any pathies may be on oral or topical medication were included in the study. Persons aged below 50 years of age, person taking H scheduled drugs or injectables and doctors from any pathies were excluded from the study.

Observation & Results

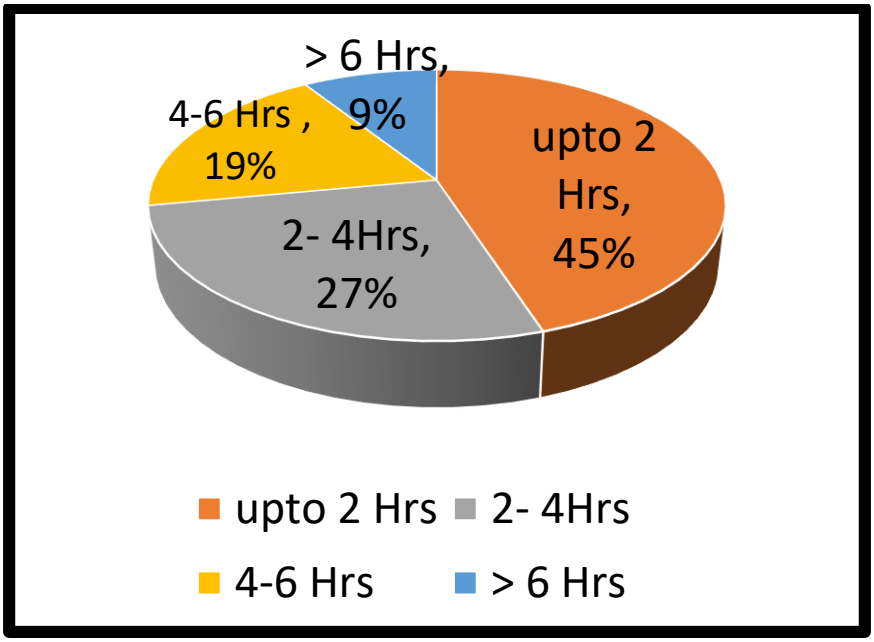
Total 192 silver surfers interviewed, 180 among them completed questionnaire, given consent to participate in the study. Silver surfers are more between 50-60 years of age group followed by 61-70 and least with 91-100 years. No significant variation found between male and female. Above data revels that in between 51-60 years of age, person may be working and can afford money and time to spend with society as well as heath wise till active to participate in social activity. Comparatively now today's females are also active among social group and in society. The survey of Harald s stated that 79% of the respondents searched for information on health topics on the internet and younger the age group, more use of internet as of seen in the study as 31% of people aged between 50 and 59 years, but only by 16% of people aged 60 years and above. [4]

Increasing age is associated with increased prevalence of chronic medical conditions [5], a higher number of medicines used, and a higher demand for all medical services, including alternative services [6,7]. High levels of self-medication practices with over-the-counter (OTC) medicines and complementary and alternative medications. Female gender and a younger age were the only variables found to be associated with CAM use in our study, in agreement with previous studies [8,9,10].

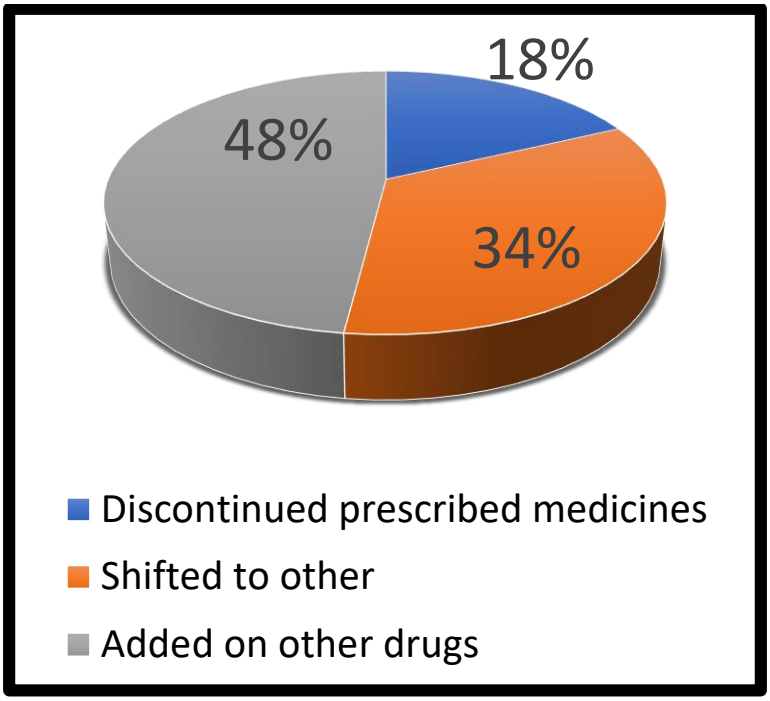
Surfing status among elderly is increasing may be due to nuclear family, lack of interaction among family members, easy availability of mobile and internet facility, improved education. Technology improved and a lot, it became a need of life as well as important tool for development of nation. We found that surfing the internet cumulatively up to 2 hours (45 %) is more common and its important for todays life pattern. This is followed by 2-4 hours (27 %) and only 9% surfers use internet for more tan 6 hours, it means the awareness of the hazards in the society is there. Pattern and impact of surfing on self-medication is highest (46 %) those who discontinued prescribed medicines without consultation. 34 % shifted to other medications from different pathies without consultation. 18 % added another drug by seeing advertisement or homemade remedies. Edward k in the study enlightened about Trends of Internet Usage in the Over 65 and possible implications for health benefit. To revel the impact of surfing among silver surfers found that with good subjective general health, 230 (21%) had internet access, versus 36 (7.1%) with poor health.[11]



Graph 1: Age & Sex Wise Distribution of Subjects (n=180)



Graph 2: Percentage of internet surfers (n=180)



Graph 3: percentage of Pattern due to impact of Social Media (n=180)

Conclusion

Self-medication is an alarming concept. This study focused on the self-medication of allopathic drugs, their use, its safety and reason for using it. It would be safe, if the people who are using it have sufficient knowledge about its dose, time of intake, side-effect on over dose, but due to lack of information it can cause serious effects such as antibiotic resistance, skin problem, hypersensitivity and allergy.

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Recent Advances in Pharmacology of *Piper nigrum* and Piperine : A Review

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Abstract

Black pepper (*Piper nigrum*) the "king of spices" is well known for its pungent aroma and flavour. "Piperine" is a major active constituent which is responsible for pungency and most of the biological activities exhibited by Black pepper. *P. nigrum* and its active principle piperine exhibit diverse pharmacological activities like antioxidant, analgesic, anti-inflammatory, anti-diarrheal, anti-thyroid, antihypertensive, antiplatelet, antidepressant, immune-modulatory, anticonvulsant, hepato-protective, etc. Nevertheless, it has got antimicrobial activities against varied bacterial species. The antibacterial activity of black pepper is due to alteration of membrane permeability, efflux pump inhibition, prevention of biofilm formation and interference of bacterial motility. Recently, antineoplastic activities of black pepper have been extensively explored through various experimentations. Piperine has been reported to enhance bioavailability of various drugs and nutrients when co-administrated. Therefore, black pepper and its active constituents have potential to replace or enhance the efficacy of conventional medical therapy which is facing resistance or toxicity issues.

Key Words : *black pepper, piperine, king of spices, antibacterial, antineoplastic, bioenhancer*

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1. Introduction

Herbs and spices are the important and inseparable part of the human diet, they are well known for their preservative and flavor-boosting property to the food and have been used in folk medicine since the ancient period [1-2]. Even today spices are used to enhance the taste of our food and also as an ingredient in drug preparations in Unani, Homeopathy and Ayurveda systems of medicine [3]. Spices includes different parts of plants like berries/fruits (black pepper, red chilli), leaves (curry leaf, coriander, mint), flower/buds (saffron, clove), bulbs (garlic, onion, leek), stem/bark (cinnamon), rhizomes (ginger, galangal, turmeric), seed (coriander) and other plant parts [4]. Black pepper (*Piper nigrum* L.) is a most commonly used spice and is acknowledged as "King of Spices" or even as "Black Gold" [5,6]. Fruits are dark green initially and turn bright orange or red after ripening. Black pepper is nothing but the cooked and dried form of unripe green berries having dark brown or grey colour [7,8]. Distribution of phenolic compounds and their kind decide the colour of pepper. Fresh green pepper is oxidised into black pepper by the enzyme o-diphenol [9]. The word "Pepper" is derived from "Pippali" in Sanskrit meaning "Berry" [8]. Major nutritional components of pepper are minerals (calcium, magnesium, manganese, copper, iron, zinc and phosphorus) vitamins (Mainly Vit A, E & K also Vit C, choline, folate, Vit. B1, B2, B3) along with dietary fibres and essential oils [6,8]. Black pepper is used not only in human dietaries but also for other purposes such as medicine, preservative, biocontrol agent and even in perfumery [10,11]. Black pepper has a worldwide distribution and cultivated in various geographical locations. Currently, it is cultivated mainly in tropical areas such as central and northern South America and Asia Pacific region. *P. nigrum* is originated in tropical evergreen forests of the Western Ghats of India[12] hence, India is known as "The Home of Black Pepper" [8,14]. The current review is an effort to compile and present updated reports of modern researches

on black pepper and its active principles, with special emphasis on their antimicrobial and other pharmacological properties.

2. Taxonomical Classification of *Piper nigrum*

Piper nigrum L. is the most popular member of family Piperaceae. The genus Piper has more than 1000 species distributed worldwide. The other well-known members of the genus include *P. longum* and *P. betle* [15].

Table 1: Taxonomical Classification of *Piper nigrum*

Rank	Scientific Name
Kingdom	Plantae
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub class	Magnoliidae
Order	Piperales
Family	Piperaceae
Genus	*Piper* L.
Species	*Piper nigrum* L.

Source: The PLANTS Database – USDA, NRCS. 2019 [16]

3. Vernacular Names

Piper nigrum has got several names in different languages, some of the common names are mentioned in Table 2.

Table 2 : Vernacular Names of *Piper nigrum*

Sr.	Language	Vernacular Name
1.	**Arabic**	Filfil ,Fulful
2.	**Assamese**	Jalook
3.	**Bengali**	Kaalaamorich.
4.	**Chinese**	Hu jiao (hu chiao)
5.	**English**	Black pepper, White pepper.
6.	**French**	Poivre commun, Poivre blanc,
7.	**Greek**	Pipéri.
8.	**Gujarati**	Kaalaamirich,
9.	**Hindi**	Kaalii mirch
10.	**Japanese**	Burakku peppaa, Koshou.
11.	**Kannada**	Menasinaballii
12.	**Marathi**	Kaaliimirii
13.	**Nepalese**	Marich.
14.	**Punjabi**	Kali mirich.
15.	**Sanskrit**	Krsna, Maricham.
16.	**Spanish**	Pimienta.
17.	**Tamil**	Milagoo, Milaagu.
18.	**Telugu**	Miryaalatiga,Savyamu.
19.	**Turkish**	Kara biber, Siah biber.
20.	**Urdu**	Kalimirch, Siyah mirch

Source: Multilingual Multiscript Plant Name Database [17]

4. Phytochemistry

During the past few decades, various researchers have investigated and isolated variety of phytoconstituents from *P. nigrum*. The survey of different literature published revealed that the Black pepper mainly contains phenolics, lignans, alkaloids, flavonoids, aromatic compounds, amides, steroids, terpenes, chalcones, starch and proteins [11,15]. It also contains essential oils up to 3.5% which constitutes pinene, sabinene, phellandrene, linalool and limonene [8]. Some of the phytochemical compounds reported are Piperine, Pipereidine, Piperamide, Piperamine, Piperettine, Pipericide, Piperolein B, Tricholein, Trichostachine, Sarmentine, Sarmentosine phellandrene, Dihydro-pipericide, Brachyamide B, (2E,4E)-N-Eicosadienoyl-pereridine, N-trans-Feruloyltryamine, N-Formylpiperidine, Guineensine, Pentadienoyl, (2E,4E)- Nisobuty-ldecadienamid, Isobutyl-eicosadienamide, Isobutyl-eicosatrienamide, Isobutyl-octadienamide, Retrofractamide Caryophyllene, Cineole, p-cymene and Carvone [11,18]. Piperine the main alkaloid from pepper was the first pharmacologically active principle to be isolated. Black pepper contains 3-8 gm% piperine [19] which has four isomers viz. Piperine, Isopiperine, Chavicine and Isochavicine. The pungent flavour of black pepper is due to piperine [8]. It is reported that Piperine, Pipene, Piperamide and Piperamine are mainly responsible for the pharmacological activities of Black pepper [8,11].

5. Pharmacological Properties of *Piper nigrum*

5.1 Antibacterial Activity

Black pepper has been investigated by various researchers and confirmed its antibacterial properties against different bacterial species. Some reports of antibacterial activities of black pepper and its active constituents against various Gram positive and Gram negative bacteria have been summarised in Table 3 & 4. Reports of Kumar *et al.* [1] and Blessy *et al.* [20] proved the antibacterial property of *P. nigrum* against *Staphylococcus* spp. Gupta *et al.* [21] reported that cold petroleum ether extract of black pepper exhibited potent antibacterial activity against *E. coli* but the extract was found ineffective against *Bacillus subtilis* and *Pseudomonas aeruginosa*. It has to be noted that antibacterial action of black pepper is due to loss of control over cell membrane permeability [22], inhibition of bacterial efflux pump, their biofilm and interference with the bacterial motilities [13]. Apart from the fruit extracts of black pepper, antibacterial activity is also exhibited by essential oils [23-25], piperine [2,3,22] piperic acid [2] and oleoresins [25]. In this regard Zarai *et al.* [2] stated that the antibacterial activity of the ethanol extracts from *P. nigrum* could be related to the presence of phenolic and flavonoid components. Black pepper oil possesses the antibacterial potency not only against human or animal pathogen but also against the aquaculture pathogens. In an experiment MIC of pepper oil nano-emulsion against *P. aeruginosa* was found to be 0.234 µl/mL. Further *in vivo* antibacterial activity tested on the shrimp (*Penaeus monodon*) revealed its effectiveness in controlling the aquaculture pathogenic bacterial infection [23].

Table 3: Antibacterial Activity of *P. nigrum* (Minimum Inhibitory Concentration)

Sr. No.	Method	MIC/MID	Extracts	Bacterial Strains							References
				S. aureus	*B. cereus*	*S. faecalis*	*K. pneumoniae*	*E. coli*	*S. typhi*	*P. aeruginosa*	
1	Tube Dilution Method	MIC (ppm)	Acetone	125	250	500	125	125	250	125	[22]
			DCM	125	62.5	125	125	125	250	125	
			Piperine	250	250	250	250	250	250	250	
2	Micro-broth Dilution Method	MIC %	Oleresins	NI	1	–	–	NI	–	–	[25]
			Oil	1	0.25	–	–	NI	–	–	
3	Well Dilution Method	MIC %	Ethanol	0.06-4	–	–	–	–	–	–	[20]
4	Tube Dilution Method	MID	Ethanol	1:4	–	–	–	1:4	–	–	[24]
			Oil	1:16	–	–	–	1:32	–	–	

MIC: Minimum Inhibitory Concentration, **MID**: Minimum Inhibitory Dilution, **ppm**: Parts per Millions, **NI**: No Inhibition, **"–"** :Not Tested

Table 4: Antibacterial Activity of *P. nigrum* (Zone of Inhibition in mm)

Sr. No.	Method	Extracts	Bacterial Strains															References
			S. aureus	*S. epidermidis*	*S. albus*	*S. xylosus*	*E. faecalis*	*S. pyogenes*	*B. subtilis*	*B. cereus*	*B. megaterium*	*K. pneumoniae*	*E. coli*	*S. enterica*	*S. typhi*	*P. mirabilis*	*P. aeruginosa*	
1	DDM	Aq (H)	NI	–	–	–	–	NI	–	–	–	–	NI	–	–	NI	NI	[27]
		Aq (C)	NI	–	–	–	–	NI	–	–	–	–	NI	–	–	NI	NI	
		EtOH (H)	22.67	–	–	–	–	23.5	–	–	–	–	10.1	–	–	19.5	NI	
		EtOH (C)	NI	–	–	–	–	9.6	–	–	–	–	11.3	–	–	9.3	NI	
		Ace (H)	10.57	–	–	–	–	NI	–	–	–	–	10.6	–	–	NI	NI	
		Ace (C)	NI	–	–	–	–	NI	–	–	–	–	11	–	–	NI	NI	
		$CHCl_3$(H)	10.20	–	–	–	–	9.4	–	–	–	–	10	–	–	10.1	NI	
		$CHCl_3$(C)	10.36	–	–	–	–	NI	–	–	–	–	10	–	–	NI	NI	
		PtEt (H)	NI	–	–	–	–	NI	–	–	–	–	11	–	–	NI	NI	
		PtEt (C)	10.70	–	–	–	–	NI	–	–	–	–	14.6	–	–	NI	NI	
		Cipro	33.41	–	–	–	–	41.4	–	–	–	–	18.3	–	–	30.7	35.3	
2	DDM (DD=4mm) EPD=40µl	CCl_4	–	–	5-9	–	–	–	5-9	–	5-9	–	5-9	–	5-9	–	–	[26]
		Benzene	–	–	5-9	–	–	–	5-9	–	5-9	–	5-9	–	5-9	–	5-9	
		$CHCl_3$	–	–	5-9	–	–	–	5-9	–	NI	–	NI	–	5-9	–	5-9	
		EtAce	–	–	5-9	–	–	–	5-9	–	NI	–	NI	–	5-9	–	5-9	
		Ace	–	–	5-9	–	–	–	5-9	–	NI	–	NI	–	5-9	–	5-9	
		EtOH	–	–	5-9	–	–	–	5-9	–	5-9	–	5-9	–	10-14	–	5-9	
		Aqueous	–	–	5-9	–	–	–	NI	–	5-9	–	5-9	–	5-9	–	NI	
		Strepto.	–	–	≥15	–	–	–	≥15	–	≥15	–	≥15	–	≥15	–	≥15	
3	DDM EPD=5µl	Ace	20	–	–	–	18	–	–	15	–	10	10	–	14	–	15	[22]
		DCM	14	–	–	–	15	–	–	12	–	12	NI	–	NI	–	14	
		Ampicillin	22	–	–	–	13	–	–	19	–	20	18	–	26	–	24	
4	DDM (DD=5mm) EPD=30µl	Aqueous	0-5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	[1]
		MeOH	4-7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
		EtOH	5-8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
5	WDM (WD=8mm)	Aqueous	25-30	–	–	–	–	–	NI	–	18	–	11-13	–	–	–	–	[4]
		EtOH	18-20	–	–	–	–	–	15	–	20	–	20-22	–	–	–	–	
		MeOH	10-12	–	–	–	–	–	19	–	20	–	20-22	–	–	–	–	
6	WDM (WD=5mm)	Pip (µl) 25	4	–	–	–	–	6	–	–	–	–	NI	–	–	–	2	[3]
		Pip (µl) 50	6	–	–	–	–	8	–	–	–	–	5	–	–	–	3	
		Pip (µl) 75	8	–	–	–	–	9	–	–	–	–	6	–	–	–	5	
		Pip (µl) 100	18	–	–	–	–	14	–	–	–	–	8	–	–	–	9	
7	WDM (WD=5mm)	MeOH	4	–		–	–	–	–	–	–	4.63	4.9	–	–	5	4.12	[19]
		Strepto	0.91	–	–	–	–	–	–	–	–	0.96	1.6	–	–	1.9	0.87	
8	WDM	EtOH	12.7	12.5	–	9.6	6	–	12	–	–	7.5	8.3	12.3	–	–	–	[2]
		MeOH	11	12	–	7.3	7.8	–	13.8	–	–	8.7	7.5	9.5	–	–	–	
		EtAce	10.7	10.5	–	13	5	–	13	–	–	NI	4.1	6.8	–	–	–	
		$CHCl_3$	8.7	10.7		5	2.3	–	11.7		–	NI	4.8	6		–	–	
		Pip	7	11.2	–	8..2	7.5	–	7.7	–	–	10.2	8	9.7	–	–	–	
		PipA	12.7	14.1	–	8.3	7.2	–	10	–	–	10.5	9	16.7	–	–	–	
9	WDM (WD=5) EPW=50µl	EtOH	14	–	–	–	–	–	–	–	–	–	13	–	–	–	–	[24]
		Oil	24	–	–	–	–	–	–	–	–	–	23	–	–	–	–	

Note : Reference antibiotics are denoted by italic and underlined font; **"–"** indicates Not Tested

NI: No Inhibition, **DDM**: Disc Diffusion Method, **WDM**: Well Diffusion Method, **WD**: Well Diameter, **DD**: Disc Diameter, **EPW**: Extract per Well, **EPD**: Extract Per Disc, , **H**: Hot, **C**: Cold, **Ace**: Acetone, **EtOH**: Ethanol, **CCl_4**: Carbon Tetrachloride, **PtEt**: Petroleum Ether, **EtAce**: Ethyl Acetated, **MeOH**: Methanol, **$CHCl_3$**: Chloroform, **Aq**: Aqueous, **Pip**: Piperine, **PipA**: Piperic Acid, **DCM**: Dichloromethane.

5.2 Antifungal Activity

Rani *et al.* [3] have determined the antibacterial (Table 4) and antifungal properties of piperine. In that experiment, poisoned food technique was used for determination of antifungal activity. They found that the maximum antifungal activity was against *Fusarium oxysporum* (14mm) and *Alternaria alternata* (17mm), minimum effect against *Aspergillus flavus* (30mm) and very least effect against *Aspergillus niger* (38mm) compared to the colony diameters of their controls 54 mm, 54 mm, 62 mm and 59 mm respectively. The fungicidal was supposed due to lysis of the fungal cell wall and cytoplasmic membrane because of the liberation of antimicrobial products. In another study [26] various Piper fruits were screened for their antibacterial and antifungal properties against some bacteria (Table 4) and *Aspergillus niger*. Different extracts (CCl_4, Benzene, Chloroform, Ethyl acetate, Acetone and Ethanol) of *P. nigrum* exhibited antifungal activity against *A. niger*, except aqueous extract. Among them, benzene extract showed the highest antifungal activity. The alkaloids and terpenoids were considered as major constituents responsible for the antimicrobial properties [26]. Recently Martinelli *et al.* [25] reported that the inhibitory activity of essential oils of black pepper against *Candida albicans* with MIC value 0.5% but *Aspergillus niger* and *Penicillium sp.* were found resistant in that study.

5.3 Antioxidant Activity

Free radicles produced in the body have potential to cause oxidative damage to the body tissues, which is associated with the equilibrium between free radicle load and adequacy of the antioxidant defence system of the body [28,11]. Various *in vivo* and *in vitro* studies have shown the antioxidant and radical scavenging properties of black pepper and its active constituents. Ethanolic extract of black pepper possesses strong radicle scavenging property [29] while aqueous and methanolic extracts have also shown *in vitro* antioxidant and radicle scavenging activity [29,30]. It is reported that black pepper oils have antioxidant [31] and DNA damage protective activities [14]. Vijayakumar *et al.* [28] demonstrated the antioxidant efficacy of black pepper and piperine in rats fed with high fat diet. They found that the simultaneous supplementation with black pepper or piperine lowered thiobarbituric acid reactive substances (TBARS) and conjugated dienes (CD) levels and maintained superoxidase dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and reduction in glutathione (GSH) levels near to the those of control rats when compared to rats fed with high fat diet. They concluded that black pepper, as well as its active principle piperine, given at small doses have significant protective action against high-fat induced oxidative stress to cells. *Piper nigrum* is rich in enzymes glutathione peroxidase and glucose-6-phosphate dehydrogenase which are known to protect against oxidative damage [29]. Zarai *et al.* [2] also noticed antioxidant property of black pepper extracts and its components, among them ethanol extract, piperine and piperinic acid exhibited the highest antioxidant activity which was comparable to α-tocopherol.

5.4 Antineoplastic Activity

Although chemotherapy is one of the principal modes of treatment for cancer patients their narrow margin of safety and progressive developing resistance are major problems [32,33]. The use of herbal medicines or natural products alone or in combination with conventional anti-cancer agents has been shown to produce beneficial effects [34]. In this context Black pepper is reported to possess anti-neoplastic, anti-mutagenic and anti-metastatic properties [11,15]. Selvendiran *et al.* [35] presented evidence that the piperine effectively inhibit B(a)P-induced lung carcinogenesis in albino mice by offering protection from protein damage and also by suppressing cell proliferation. In a study piperidine exhibited antiproliferative activity against HEp2 cells (Human epithelioma cells of larynx) dose dependently. Following treatment with extract for 24 hrs the cells changed their shape to round, lost surface morphology and died at a concentration of 50% [36]. Cancer preventive and anti-cancer activity was exhibited by piperine free *Piper nigrum* extract (PFPE) against N-nitrosomethylurea (NMU) induced mammary tumors in rats by exhibiting cytotoxic effects against MCF-7 breast cancer cells that were mediated through the induction of the apoptotic pathway [37]. Recently Sriwiriyajan *et al.* [38] isolated two bioactive compounds, (-)-kusunokinin and piperlonguminine from *P. nigrum* and studied anticancer activity against breast cancer cell lines. It

was found that both (-)-kusunokinin and piperlonguminine inhibited the growth of breast cancer cells by inducing cell cycle blockage, and cell apoptosis on luminal MCF-7 and basal MDA-MB-468 breast cancer cell lines through reduction of topoisomerase II expression and the induction of p21 on the cell cycle. They also mentioned that mechanism of cell apoptosis of MCF-7 and MDA-MB-468, treated with both compounds, was through the suppression of bcl2 and the induction of p53, bax, cytochrome c, caspase-3, caspase-7 and caspase-8 but not caspase-9. Hence it has been postulated that *P. nigrum* is a novel therapeutic spice for the treatment of colorectal carcinoma. Ethanolic extracts of *P. nigrum* (EEPN) possess the high amount of total phenol content (TPC) which is having significant positive correlations in dose dependent cellular inhibition and cytotoxic properties against colorectal carcinoma cell lines [39].

5.5 Analgesic Activity

Analgesic activity of piperine was determined in mice using acetic acid-induced writhing and tail flick assay. It revealed the intraperitoneal (i.p.) administration of piperine (30, 50 and 70 mg/kg) significantly inhibited ($P<0.01$) the acetic acid-induced writhing in mice, similar to the effect of the standard analgesic indomethacin (20 mg/kg i.p.). In the tail flick assay, piperine (30 and 50 mg/kg, i.p.) and morphine (5 mg/kg, i.p.) caused a significant increase ($P<0.01$) in the reaction time of mice. Pre-treatment of animals with naloxone (5 mg/kg i.p.), reversed the analgesic effects of both piperine and morphine in the tail flick assay. Hence it was concluded that the piperine exhibits significant analgesic properties which were supposed to be mediated via opioid receptor activation [40]. Similarly, Sabina *et al.* [41] noticed analgesic activity of piperine by acetic acid-induced writhing and hot plate methods. Piperine administrated in doses 20 and 30 mg/kg intraperitoneally in mice exhibited analgesic activity comparable to that of standard analgesic indomethacin (10 mg/kg i.p.). Further they suggested that the analgesic property is due to reduction in local prostaglandin levels. Tasleem *et al.* [42] used analgesy-meter, hot plate and acetic acid–induced writhing test and tail immersion method to determine the analgesic activity of *P. nigrum* extracts and piperine. They found that piperine (5 mg/kg) and ethanol extract (15 mg/kg) after 120 min and hexane extract (10 mg/kg) after 60 min exhibited significant analgesic activity by tail immersion method, in comparison to ethanol extract (10 mg/kg) using analgesy-meter in rats. However, with hot plate method piperine (5 and 10 mg/kg) and hexane extract (5 mg/kg) produces significant analgesia after 120 min. While in the writhing test, ethanol extract (15 mg/kg) significantly stopped and piperine (10 mg/kg) completely terminated the writhes in mice.

5.6 Anti–Inflammatory Activity

Piperine in doses of 5 and 10 mg/kg, P.O. significantly inhibited carrageenan-induced paw edema in rats, which was accompanied by a concurrent reduction in PGE_2 levels in affected paws. It revealed that the piperine has an anti-inflammatory property which is attributed to inhibition of prostaglandin release [43]. In a similar experiment of carrageenan-induced paw edema, piperine administrated in doses 10 and 15 mg/kg, P.O. produced anti-inflammatory activity after 30 min. lasted till 60 min while hexane and ethanol extracts of pepper showed the duration of action equal to 120 min at lower doses i.e. 10 mg/kg [42]. Recently Wang *et al.* [44] reported that dichloromethane fraction of black pepper (piperine as a major component) exhibited anti-inflammatory activity by suppressing expression or production of IL-1β, IL-6, and TNF-α and by reducing oxygen-free radicals through increased superoxide dismutase activity and decreased malonaldehyde levels on permanent focal cerebral ischemia injury in rats.

5.7 Antipyretic Activity

In vivo antipyretic study was carried out by Sabina *et al.* [41]. In the study baker's yeast was administrated subcutaneously in the mice to produce pyrexia. Control group showed a significant increase in the rectal temperature. Group administrated with piperine (20 and 30 mg/kg i.p.) showed dose dependant decrease the rectal temperature compared to control as well as reference antipyretic indomethacin (10 mg/kg p.o.).

5.8 Bioavailability Enhancing Activity

Concept of bioenhancer is based on a traditional system of Indian medicines. The first bioavailability enhancer discovered was the piperine, in 1979. The bio-enhancing dose of piperine is approximately 15 -20 mg/person/day in divided doses which is very less than the LD50 dose of piperine. Piperine enhances bioavailability by inhibition of hepatic and non-hepatic drug metabolizing enzymes, increases blood supply to the gastrointestinal tract and enhancing drug transport across the cell membrane [45]. Piperine has been screened for its bioenhancer potential and reported to enhance the bioavailability of various drugs viz. herbal ingredients (curcumin), antibiotics (ampicillin, cefotaxime, ciprofloxacin, norfloxacin, gatifloxacin, metronidazole), NSAIDs (diclofenac, nimesulide, ibuprofen), antiepileptics (phenytoin, pentobarbitone, carbamazepine), β-blockers (propranolol, atenolol), other drugs (fexofenadine, pentazocine, beta-carotene, omeprazole, theophylline) and the antineoplastic agent docetaxel [45-47]. Piperine has been found to be bioequivalent with commercially available rifampicin preparation Risorine (rifampicin 200 mg, isoniazid 300 mg and piperine 10 mg). Co-administration of piperine enhances drug uptake and delays its elimination, so the effective plasma concentration of a drug is maintained for long duration and the also the dose rifampicin have minimized from 450 to 200 mg [45].

5.9 Antidiarrhoel Activity

Shamkuwar and Shahi [48] noticed that aqueous extract of Black pepper potentiated the antidiarrhoeal, antimotility, and antisecretory activity of Kutajarishta (a herbal antidiarrhoeal formulation) significantly in mice with castor oil and magnesium sulphate induced diarrhoea. In this context Malik and Gilani [67] explained the mechanism by which pepper acts on the gastro-enteric system. They mentioned that the antisecretory, antidiarrheal and antispasmodic activities are through activation of opioid receptors and by blocking Ca^{++} Channels, however spasmodic activity is through activation of the cholinergic system.

5.10 Anticonvulsant Activity

Bukhari *et al.* [40], screened anticonvulsant activity of piperine in Pentylenetetrazole and Picrotoxin induced seizer models. Piperine when administered in doses 30, 50 and 70 mg/kg, i.p. showed significant anticonvulsant property by delaying the onset of seizers. Pentylenetetrazole (PTZ) produces seizures by inhibiting gamma aminobutyric acid (GABA). Hence inhibition of PTZ induced seizers indicates the GABA-ergic activity of piperine.

5.11 Larvicidal Activity

The ethanol extract of black pepper and its fractionated constituents exhibited potent larvicidal activity against the pyrethroid-resistant strain of *Aedes aegypti*. The main larvicidal constituent of the extract identified was the piperolein-A and piperine. Crude ethanol extract of black pepper found most toxic with LC_{50} value 0.9 ppm followed by piperolein-A (LC_{50}=1.46 ppm) and piperine (LC_{50}=1.53 ppm). There was a gradual loss in the larvicidal activity during the process of fractionation of *P. nigrum* ethanol extract [49].

5.12 Other Pharmacological Activities

Apart from potential activities reviewed above, some of the other pharmacological properties have been mentioned in Table 5.

Table 6: **Various Pharmacological Activities of *Piper nigrum***

Sr.	Activity	References
1.	**Antihypertensive**	[50]
2.	**Anti-asthematic**	[6], [51]
3.	**Anti-platelet**	[52]
4.	**Anti-apoptopic**	[53], [54]
5.	**Antideppressent**	[55], [56]

6.	**Anti-thyroid**	[14], [57]
7.	**Immunomodulary**	[8]
8.	**Hepatoprotective**	[14], [58]
9.	**Carminative**	[8]
10.	**Digestive-stimulant**	[11]
11.	**Fertility Enhancer**	[56]
12.	**Melanocyte-proliferative**	[47], [59]
13.	**Neuroprotective**	[47]
14.	**Production Enhancer**	[60], [61]
15.	**Hypoglycemic and hypolipidemic**	[68]

6. Toxicology and Safety Profile

Although Black pepper is being used since ancient period and is listed safe for its intended use as a spice by FDA [45], there are some controversial reports related to its safety [10]. It is reported that topical use of pepper oil may over stimulate the kidneys while pepper powder can cause an erythematous lesion on the skin. Sometimes burning sensation in the foetus can be noticed hence its use should be avoided in pregnancy [8]. Previously Piyachaturawat *et al.* [62] had conducted acute toxicity tests and found LD_{50} values 400, 330,200, 43, and 15.1 for single dose through i.m., i.g., s.c., i.p. and i.v. respectively. Most of those animals died of respiratory failure within 3-17 min. Some authors stated its carcinogenicity, structural resemblance with known natural carcinogens [10] and DNA damage promoting ability [8]. On the other hand toxicological investigations have revealed that black pepper and piperine are non-genotoxic [63], non-mutagenic [64] and having no immunotoxic effects [65]. It is also reported that black pepper or piperine when administrated in doses 250 times that of normal human intake had expressed no adverse effects [66]. Sabina *et al.* [41] while studying ulcerogenic effects of piperine on gastric mucosa noticed that piperine produced significantly less gastric ulcers compared to indomethacin and exhibited dose dependant decrease in ulcer index.

7. Conclusion

It has been observed that most of the currently available antibacterial, antifungal even antineoplastic chemotherapeutic agents and some anthelminthic agents are becoming ineffective and resistant against their respective targets. Furthermore, these agents and many other frequently used drugs (NSAIDs, etc.) have proved to possess a variety of adverse effects. In this regard, *P. nigrum*, piperine and other active principles have been reported to possess wide spectrum pharmacological properties with much higher safety profile, hence can be considered as an alternate to the conventional medicine. Although, further detailed studies and controlled clinical trials need to be performed for more precise investigations. Furthermore, there is a good scope to standardize and formulate different therapeutic preparation containing black pepper or piperine along with other drugs to enhance their bioavailability and potency in the treatment of different ailments and diseases.

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OXYDENTS, FREE RADICALS, REACTIVE OXYDEN SPECIES (ROS) AND ANTIOXIDANTS

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Abstract

The presence of free radicals in biological materials was discovered less than six (6) decades ago. Soon then, Denham Harman hypothesized that oxygen radicals may be formed as by-products of enzymic reactions in vivo. Oxidants broadly defined as endogenous or exogenous substances, which have the capacity to bring about the oxidation of target molecules, either directly by abstraction of electrons, or indirectly through the production of highly reactive intermediate chemical entities. The Most common ROS includes, superoxide anion (O_2),Hydrogen peroxide(H_2O_2), hydroxyl radical(OH^-),hypochlorous acid(HOCl),peroxyl radicals, nitric oxide.peroxynitrite ($ONOO^-$), Superoxide anion (O_2)-

Historical Background of Free Radicals

The presence of free radicals in biological materials was discovered less than six (6) decades ago. Soon then, Denham Harman hypothesized that oxygen radicals may be formed as by-products of enzymic reactions in vivo. In 1956, he described free radicals as a Pandora's box of evils that may account for gross cellular damage, mutagenesis, cancer, and, last but not least, the degenerative process of biological aging. [1]

In second era, McCord and Fridovich (1969), discovered the enzyme superoxide dismutase (SOD) and, found that free radicals are important in biology. Many researchers then were inspired to investigate oxidative damage inflicted by radicals upon DNA, proteins, lipids, and other components of the cell.

A third era began with the first reports by Mittal and Murad, 1977, describing advantageous biological effects of free radicals and provided suggestive evidence that the superoxide anion through its derivative, the hydroxyl radical, stimulates the activation of guanylate cyclase and formation of the "second messenger" cGMP.[2]

Now there are many evidence showing that living organisms have not only adapted to an unfriendly coexistence with free radicals but also have the advantageous use of free radicals. Important physiological functions that involve free radicals or their derivatives include the following regulation of vascular tone, sensing of oxygen tension and regulation of functions that are controlled by oxygen concentration, enhancement of signal transduction from various membrane receptors including the antigen receptor of lymphocytes, and oxidative stress responses that ensure the maintenance of redox homeostasis.[3, 34-40] The term “oxidative stress” was coined

only 30 years ago. Oxidative stress is a situation when steady-state ROS concentration is transiently or chronically enhanced [4]

Free radical production and Reactive Oxygen Species(ROS)

Oxidants broadly defined as endogenous or exogenous substances, which have the capacity to bring about the oxidation of target molecules, either directly by abstraction of electrons, or indirectly through the production of highly reactive intermediate chemical entities. Free radicals are chemical species which contain one or more unpaired electron in their atomic structure which is responsible for its reactivity,[5] and are capable of independent existence for very brief intervals of time. ROS is a collective term that includes all reactive forms of oxygen, including both the radical and non radical species that participate in the initiation and/or propagation of radical chain reactions. Accumulation of ROS beyond the immediate needs of the cell brings oxidative degradation of critical molecules, such as DNA, proteins, and lipids. [6]

Endogenous sources of oxidant radicals

1.Along Electron transport chain(ECT) during oxidative phosphorylation in mitochondria during ATP synthesis- As a consequence of normal aerobic respiration, mitochondria consumes O_2 ,reducing it by sequential steps to produce H_2O (requires 4 electrons).Inevitable byproducts of this process are O_2^-, H_2O_2 and –OH radicals.[7]

2. In Peroxisomes-These are the organelles responsible for degrading fatty acids and other molecules, produces H_2O_2 as a byproduct further degraded by catalase. Under certain conditions ,some of the peroxide escapes degradation, may enter other compartment of cell and may cause oxidative DNA damage.[8]

3.During infection and inflammation- Phagocytic cells like neutrophils, macrophages destroy bacteria or virus infected cells with oxidative burst of nitric oxide(NO),O_2^-,H_2O_2, and OCl^-.[8]

4.During hypoxia- Hypoxic conditions are created when oxygen is limited to the cells and mitochondria pumps ROS.[9]

Exogenous sources of oxygen radicals

1.Ionizing radiations- UV rays and ionizing radiation,converts H_2O into OH^-(hydroxyl) radicals which are very toxic. Ionizing radiation induces mitochondrial reactive oxygen species production accompanied by upregulation of mitochondrial electron transport chain function.[10]

2.Cigarette smoke and pollution –The oxides of nitrogen(NO) in cigarette smoke(about 1000ppm) cause oxidation of macromolecules and deplete antioxidant levels.[11]

3. Excess Iron and copper salts –Excess Iron and copper salts promote generation of oxidizing radicals from peroxides(H_2O_2 converts to OH^- radicals) [12]

4.Drugs/Xenobiotic-Drugs like Acetaminophen in toxic doses produces excess oxidant radicals,[13] through cytochrome P450 in liver.

5.Natural toxic chemicals from plants-Cytochrome P450 enzymes in animals constitute one of the defense system against natural toxin chemicals from plants. The induction of these enzymes prevents acute toxic effect but produces oxidants as by-products.[8]

Types of ROS

The Most common ROS includes, superoxide anion(O_2),Hydrogen peroxide(H_2O_2), hydroxyl radical(OH^-),hypochlorous acid(HOCl),peroxyl radicals, nitric oxide.peroxynitrite ($ONOO^-$),

Superoxide anion (O_2)-The superoxide(much less active) is an anionic radical formed by the reduction of molecular oxygen through the acceptance of a single electron. They are generated in mitochondria during electron transport through ECT during oxidative phosphorylation is generally accompanied by escape of up to 1-2% of electrons that are trapped by molecular oxygen.[14] The enzyme required to convert oxygen to superoxide is NADPH oxidases(NOX/DUOX). NADPH enzyme comprised of seven isomers NOX1-NOX5 and DUOX1-DUOX2.Activation of NOX family protein will produce ROS.[15] Superoxide anions are also generated by cytochromes P450 which catabolize various endogenous compounds and xenobiotics. Activated phagocytes also possess metabolic pathway for the production of superoxide radicals in response to bacterial infection. Superoxide anion can also initiate the cascade of arachidonic acid metabolism, resulting in the formation of more superoxide and in the liberation of Fe2+ from ferritin stores. [16] Superoxide anion is less active due to negative charge of the species however its protonation generates the per hydroxyl radical (HO_2`) with a higher oxidizing potential

Hydrogenperoxide (H_2O_2)

superoxide dismutase (SOD) converts superoxide to hydrogen peroxide and molecular oxygen; however, superoxide can also spontaneously dismutate to form hydrogen peroxide and singlet oxygen, a strong oxidizing agent. [17] The reaction potential of Hydrogen peroxide is very low,however it is converted into the hydroxyl radical with higher oxidizing capacity.H_2O_2 in presence of myeloperoxidase(neutrophil-derived enzyme), can be converted to hypochlorous acid having reactive potential . H_2O_2possesses a unique(forROS) capacity to cross biological membranes with turn it into a classical signaling molecule.[18]

Hydroxyl radical(OH^-)

Hydrogen peroxide themost reactive free radical in vivo, is formed by the reaction of $O_2^{\bullet-}$ with H_2O_2 in the presence of Fe^{2+} or Cu^+ (catalyst).(Fenton reaction.)[19] or result of Haber-Weiss cycle that Involves a reduction of ferric ions(Fe^{3+}) by superoxide anions into ferrous ions followed by the Fenton reaction. Hydroxyl radical is the most reactive ROS due to its low half life and can oxidize almost any molecule in its proximity including DNA, Phospholipids and proteins.[20]Oxidation of this molecules results in the accumulation of 8-oxoguanine (8-oxoG) and other oxidized nucleic bases, malondialdehyde (MDA), and 4-hydroxynonenal (HNE) as typical lipid peroxidation products and in protein damage manifested in the increase of the protein carbonyl content.[21]

Peroxyl radicals

Peroxyl radicals are produced primarily during lipid peroxidation. Although lipid peroxidation found to play a useful role in some biological processes, peroxidation of membrane i.e. hydroxyl radical can react with a poly-unsaturated fatty acid(removes one hydrogen) not only altering its structural and functional integrity but also generating multiple fatty acid peroxyl radical which spontaneously reacts with other lipids, proteins, or nucleic acids thereby propagating a cascade of electron transfer and bring oxidation of these substances. The cell injury produced by lipid peroxidation of cell membranes may affect membrane fluidity, increase in permeability, alter electrical potential, and may bring lysis of cell.[22] Proteins, both structural and enzymatic are also denatured by these free radicals. Toxic oxygen metabolites can also directly attack nucleic acids causing base hydroxylation, crosslinking or scission of DNA strands that can result in cell death and mutation. [23]

lipid peroxidation steps-The oxidation of lipids by ROS consists of three steps

1) Initiation step

The hydroxyl radical extracts a hydrogen atom from a methylene carbon of a polyunsaturated fatty acid forming a carbon-centered lipid radical. The lipid radical can interact with molecular oxygen to give rise to a peroxyl radical.

2) Propagation step

The peroxyl radical is converted to a lipid hydro peroxide by extracting a hydrogen atom from a methylene carbon to form a new lipid radical, thus, initiating a propagating chain of lipid peroxidation. The hydro peroxides can be further degraded to hydrocarbons, alcohols, ether, epoxides and aldehydes. Of these products, malondialdehyde and 4-hydroxynonenal have additional ability to inactivate phospholipids, proteins, and DNA by bringing about cross-linking between theses molecules.[24]

3) Termination step

The chain reaction is stopped by interaction between the radicals themselves, or between the radical and antioxidants forming nonradical product or unreactive radical. The chain is generally ended when the lipid radicals interact with vitamin E forming a lipid alcohol and a tocopherol radical.[24]

Singlet oxygen($^1O_2$)

Singlet oxygen is very reactive high energy species.It does not contain unpaired electron and so it is not a free radical .It is largely involved in photochemical reactions, such as energy transfer due to type II photosensitization, thermal decomposition of endoperoxides and dioxetanes and during reaction when hypochlorous acid combines with hydrogen peroxide to form water gives singlet oxygen. Singlet oxygen is a very reactive ROS and induces various carcinogenic, genotoxic and mutagenic effects through its action on polyunsaturated fatty acids and DNA. [25]

Nitric Oxide (NO.), Peroxynitrite (ONOO) (Reactive nitrogen species)

Nitric oxide radical ($NO^{\bullet}$) is formed in biological tissues from the oxidation of L-arginine to citrulline by nitric oxide synthase(NOS)s.[26] One of the most important reactions under physiological conditions is that of superoxide and nitric oxide radicals resulting in peroxynitrite

$$NO + O_2^- \longrightarrow OONO^- \text{(peroxynitrite)}$$

This reaction helps to maintain the balance of superoxide radicals and other ROS and is also important in redox regulation.. The protonated form of peroxynitrite (ONOOH) is a powerful oxidizing agent may cause depletion of sulfhydryl (-SH) groups and oxidation of many molecules causing damage similar to that observed with OH.

Antioxidants

Halliwell & Gutteridge (1989) have defined antioxidants as substances that are able, at relatively low concentrations, to compete with other oxidizable substrates and, thus, to significantly delay or inhibit the oxidation of these substrates. There exists a balance between their formation and removal (redox state). To maintain an oxido/redox balance, cells protect themselves from the toxicity of excess ROS/RNS in different ways, enzymatic and nonenzymatic antioxidants.

a)Enzymatic antioxidants (Endogenous)

Superoxide dismutase (SOD),Catalase(CAT), Glutathione peroxidase(GP_X),Glutathione reductase(GR_X)

b) Non-enzymatic antioxidants

i) Metabolic antioxidants-Lipoid acid, Glutathione, L-arginine, uric acid, bilirubin.

ii)Nutrient antioxidants (exogenous)-Vitamin E, Vitamin C(ascorbic acid), Carotenoids, Trace elements (Se,Cu,Zn,Mn)

Mode of action of antioxidants

1.Preventive antioxidants-They act by binding to and sequestering oxidation promoters and transition metal ions, such as iron and copper, which contain unpaired electrons and strongly accelerate free radical formation. Example includes transferrin and lactoferrin (which bind ferric ions), ceruloplasmin (which binds Cu, catalyzes the oxidation of ferrous ions to ferric due to its ferroxidase activity, and increases the binding of iron to transferrin), haptoglobins (which bind hemoglobin), hemopexin (which binds heme), and albumin (which binds copper and heme). [27]

2.Enzyme antioxidants The endogenous enzymes act on specific ROS after they are formed and degrade them to less harmful products. Examples include superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH)). SODs convert the superoxide radical to hydrogen peroxide, which is not a free radical by itself, but is a precursor of the highly reactive hydroxyl radical. The SODs, catalase, and GHS constitute the major intracellular enzymatic antioxidants.[27]

3.Scavenging or chain breaking antioxidants-Chain-breaking antioxidants act by presenting themselves for oxidation at an early stage in the free radical chain reaction and giving rise to low energy products that are unable to propagate the chain further. Lipid-soluble scavengers(vitamin E (α-tocopherol), β-carotene, and coenzyme Q (CoQ). [28] and water-soluble scavengers ascorbic acid, various thiols, uric acid, and bilirubin, act in cellular environments act as chain breaking antioxidants.

Important antioxidants

a) Enzyme antioxidants

1.Superoxide Dismutase(SOD)- Superoxide dismutase destroys the free radical superoxide by converting it to peroxide which is further destroyed by catalase or glutathione peroxidase (GHPX) reaction. SOD converts the superoxide radical to the less-reactive H_2O_2. There are three forms of SOD are cytosolic,Copper-zinc superoxide dismutase (Cu, Zn-SOD)(SOD-I),active site is constituted by a copper and a zinc atom bridged by a common ligand. Mitochondria Mn-SOD(SOD-II) and extracellular SOD (ECSOD). Generally, SOD catalyses the dismutation by successive oxidation and reduction of the transition metal ion at the active site in a ping-pong-type mechanism with high reaction rates. [29]

2.Catalase(CAT)-Is a tetrameric haem-enzyme consisting of 4 identical tetrahedrally arranged subunits. Catalase react with H_2O_2 to form water and oxygen molecule, a nontoxic product. **3.Glutathionsystem-** Glutathione system (glutathione, glutathione peroxidase, glutathione transferase and glutathione reductase) is a key defence against H_2O_2 and other peroxides. Glutathione is a tripeptide with L-gamma-glutamyl-L-cysteinyl glycine in both its reduced and dimeric forms.The selenoprotein Glutathione peroxidase(GPx) enzyme removes H_2O_2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power. Besides hydrogen peroxide, GPx also reduces lipid or nonlipid hydro peroxides while oxidizing glutathione (GSH).[30]

Nutrient antioxidants

Vitamin E. Vitamin E is a fat-soluble vitamin ,widely known for its biological function as a chain breaking lipid soluble antioxidant. Vitamin E is a chiral compound with eight stereoisomers: α, β, γ, δ tocopherol and α, β, γ, δ tocotrienol. Only α-tocopherol is the most bioactive form in humans. Because it is fat-soluble, α-

tocopherol safeguards cell membranes by protecting it against lipid peroxidation by free radicals. It acts through the antioxidant protection of LDL-Cholesterol. Vitamin C increases the resistance of LDL to oxidation by recycling vitamin E and other phenolic antioxidants in lipoprotein particles[31] .Low intake of vitamin E,C,A and beta carotene is associated with low immune responses and increase risk of cancer. The dietary sources of vitamin E are vegetable oils, wheat germ oil, whole grains, nuts, cereals, fruits, eggs, poultry, meat. Cooking and storage may destroy natural d-α-tocopherol in foods. The recommended dietary allowance for vitamin E is 10mg for men and 8mg for women. Although controversial, the use of long-term vitamin E supplementation in high dose should be approached cautiously.

Vitamin C. Vitamin C also known as ascorbic acid($AscH^{-}$), is a water-soluble vitamin. Vitamin C has been shown to efficiently scavenge superoxide, hydrogen peroxide, hypochlorite, the hydroxyl radical, proxyradicals and O_2. It donates a hydrogen atom to a free radical and neutralizing it, becoming an ascorbate radical but this radical is very stable. Ascorbic acid can also act to protect membranes against peroxidation by enhancing the activity of tocopherol, the chief lipid soluble, chain breaking antioxidant. Natural sources of vitamin C are acid fruits, green vegetables, tomatoes. Ascorbic acid is a labile molecule, therefore it may be lost from during cooking.

β-carotene and vitamin A- Beta-carotene is a fat soluble member of the carotenoids which are considered provitamins because they get converted to active vitamin A(retinol).β-carotene is a strong antioxidant and is the best quencher of singlet oxygen.Vitamine acts as antioxidant and as an immunostimulant by modulating the growth and function of T-cells, B-lymphocytes and natural killer cells. Beta-carotene is present in many fruits, grains, oil and vegetables (carrots, green plants, squash, spinach) .Lycopene(in tomato) a carotenoid, possesses antioxidant and anti proliferative properties in animal.

Flavonoids. Flavonoids are polyphenolic compounds which are present in most plants. Over 4000 flavonoids have been identified and classified into flavanols, flavanones, flavones, isoflavones, catechins, anthocyanins, proanthocyanidins. Flavones and catechins (in green tea) has potent antioxidant activity.[32] They have been reported to prevent or delay a number of chronic and degenerative ailments such as cancer, cardiovascular diseases, arthritis, aging, cataract, memory loss, stroke, Alzheimer's disease, inflammation, infection. The main natural sources of flavonoids include green tea, grapes (red wine), apple, cocoa (chocolate), ginkgo biloba, soybean, curcuma, berries, onion, broccoli, etc.

Metals – Zinc a metallic divalent cation bound to protines in cell and dell membranes. Zinc present in many zinc metalloenzymes in biological system. Zinc maintains integrity of biological membranes by stabilizing thiol groups and phospholipids and protects against oxidative injury Zinc finger as transcription factors for interacting with DNA and regulating the activity of gene.[33] Selenium forms the active site of several antioxidant enzymes including glutathione peroxidase. At low dose, health benefits of Se are antioxidant, anti-carcinogenic and immunomodulator.Se is a trace mineral found in soil, water, vegetables (garlic, onion, grains, nuts, soybean), sea food, meat, liver, yeast

Transitional metal binding proteins–metal binding protein ferritin for iron and caeruloplasmine for copper act as crucial component of antioxidant defense system by sequestering iron and copper so that they are not available to drive formation of hydroxyl radical.

Fig-1

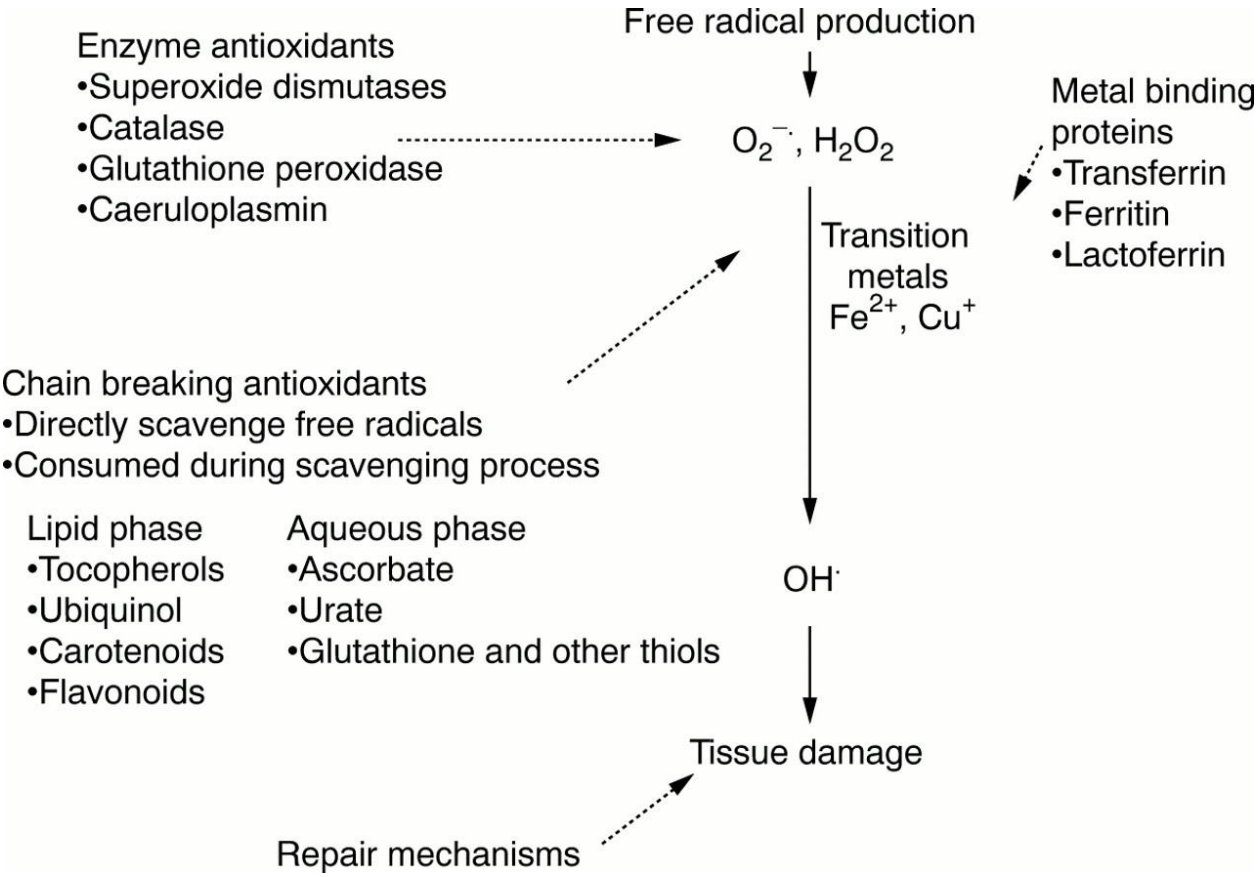

Fig -1 Journal of clinical pathology. Antioxidants in health and diseases vol 54 issue 3.

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Role of Regulatory Agencies in Pharmaceutical Industry

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Abstract

Regulatory agencies aim for protection of human health in pharmaceutical sector. Since the purpose of a drug is to diagnose, mitigate, cure, prevent or treat diseases or ailments in living organisms. They are products intimately linked with the advances in research and regulation. People as well as Government of any country spent loads of money on drugs because of the role they play important role in saving loves, restoring health, preventing diseases and spread of epidemics. So, a drug ought to be safe, efficient and possess good quality. The pharmaceutical industry, while chasing for an international market, is constrained to comply with national regulations. Regulatory agencies are important for meeting the requirements to meet the regulatory requirements for legal procedures with respect to drug development process in a country. Each country has a regulatory agency, which enforces the rules and regulations. They also issue guidelines for regulation of drug development, licensing and registration.

Keywords: Regulatory agencies;Pharmaceutical industry; Drug

1. Introduction

The field of Drug regulatory science, both pharmaceutical and biopharmaceutical is emerging rapidly. Regulatory agencies as well as evaluation agencies which can be either government sector or private sector are increasing their vision emphasizing more and more delivering the public with drug products of high quality, efficacy and safety. Various regulatory agencies and industries, including better quality dossiers has contributed to improving the licensing dossier review procedure for pharmaceuticals and also for biopharmaceuticals.[1]. Regulations and derived procedures have increased globally and this can be partly explained by the great progress of science in the last 10 years [2]. Before a drug product is marketed, drug reviewers in these regulatory agencies need to apply review science to thoroughly evaluate whether the research results support the safety, effectiveness and quality control of the new drug product. Apart from this, as science is progressing, the existing regulations are also being constantly adapted as well as updated. They aim to contribute an environment where decisions about the benefits and risks of medicines are made in a scientifically robust and transparent way to serve patients. Prior to the approval of the drug and their monitoring, safety of prescribed drugs becomes higher priority in any regulatory agency.

The level of regulations changes significantly across countries. The developed countries have set up stringent laws and regulations governing the flow of pharmaceuticals within their territories while developing countries offer a level of relaxation to back their domestic market and encourage pharmaceutical growth. In developing countries regulatory capture is adequate and government has little capability [3]. However, these countries also keep their vision higher for moving parallel by updating themselves to their existing system of approvals and evaluation. Same thing is true with reference to type of medicinal products too. The Over the counter (nonprescription) products do not need the same extent of regulation just like the prescribed drugs at the time of registration (marketing authorization) or in ongoing usage. The regulatory philosophy for biopharmaceuticals has differed from that for synthetic organic drugs, because of special concerns over biological contaminants, process variability, biological assays and preclinical testing limitations. But with the advancement in analytical and purification technology have now started to challenge this difference [4]**.**

In case of cancer vaccine (CaVs) no guideline or regulation exist that specifically mentions CaVs[5]. WHO and CHMP guidelines putforths cancer vaccines which are intended in treating diseases and immunogens such as monoclonal antibodies (mAbs) are not mentionedin the vaccine guidance. The recommendations in these documents may nevertheless be considered as relevant for the development of CaVs[6]**.** Reporting of ADRs and responding to the same for the safety of patients is one of the most concerned duties of regulatory bodies. Such drugs usually need to be thoroughly reinvestigation or withdrawn from market. Internet is one of the best players in drug safety issues. Websites are most used media for drug label changes as well as warnings and they conduct nationwide surveys on drug safety surveys. The Ryan Haight Online Pharmacy Consumer Protection Act passed by Congress in2008, amends the Controlled Substances Act to prohibit the delivery, distribution, or dispensing of a prescription drug over the Internet without a valid prescription. The regulations to control the purchases by internet are also an area of consideration. The harmonization of regulation is encouraging for industries and is an emerging area nowadays. International harmonization such as ICH as well as regional harmonization such as ASEAN regulations demonstrates the co-operation and willingness of new world for providing the public best medical care. Many countries regulate the price that consumers pay for pharmaceuticals. The regulated price is

normally is not par the market price [8]. Even it has been observed that countries with strict price regulation (France, Italy, and Japan) have lower prices than the less regulated markets of the United States and the United Kingdom [9].

International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH) is a collaborative initiative between the EU, Japan and the United States with observers from WHO, EFTA (European free trade area) and Canada. ICH harmonization focuses primarily on technical requirements for new, innovative medicines. However, developing countries have limited resources and thus are mostly generic markets and might be having difficulties in implementing numerous sophisticated and stringent ICH standards. Pharmaceutical regulatory harmonization helps in safety and efficacy of good quality pharmaceuticals. World Health Organization (WHO) supports harmonization on regional,inter-regional, national and international levels [10]. WHO's role in drug regulation is four-fold. First, issuing the necessary regulations and standards through its Expert Committees. Second, supporting regulatory capacity building leading to implementation of drug regulation on national level and its harmonization on regional and Global level. Third, in some areas for essential products by ensuring safety, efficacy and quality of essential medicines and all vaccines through ―prequalification. Fourth, WHO has a vital role in facilitating exchange of regulatory information [11].

2. Need of regulatory agencies

Regulatory Agency is an organization which regulates industries, such as pharmaceuticals, medical devices, banking and energy. Regulatory Agency also has a specific importance within the healthcare system (pharmaceuticals, medical devices,foods and biologics) and most companies, whether multinational pharmaceutical companies or small, innovative entrepreneurs, have special departments for Regulatory Affairs profession.Drug development and their commercialization has a highly regulated path including drug registration i.e.marketing approval.In today's environment, competition is severe and reduces the time required to reach the market is critical to product and hence company achieves success. The proper conduct of Regulatory Affairs activities hassubsequent economic importance to the company. Inadequate data reporting will prevent positive evaluation for a marketing evaluation. A new drug costs many millions of currencies to develop and even a short delay in launching a product in market has subsequent market considerations. Worse conditions are failures which fully report all available data or the release of product bearing any error which might need a product recall [5]. This will lead to loss of millions of units, not to mention the reduction of health professionals, investors and patients. Regulatory agencies become the bridge between the Government and the company.

3. Scope of regulatory agencies in pharmaceuticals

India is rising in pharmaceutical sector day by day and there is a need of regulatory agency to meet the current needs of industries for matching competition globally. Regulatory affairs professionals bridge the pharmaceutical industries and worldwide regulatory agencies. They are required to be well versed in the laws, regulations, guidelines and guidance of the regulatory agencies. The pharmacy students need preparation with latest development for serving the industries and hence there is a growing need for incorporation of current requirements of pharma industries in their regular curriculum. The regulatory affairs work synonymously with marketing and R&D to develop and manufacture innovative products which will take advantage of new technological and regulatory developments to accelerate in to market. New products adding to the significant revenues to company's revenue thus decreasing time to equate large material gains in market. Employing adaptive clinical trial strategies, obtaining quick approval from regulatory authorities and avoiding pitfalls in processes can accelerate development of new products and help to reduce costly errors and time lags.[11].

Table 1. Regulatory agency of different countries

Sr. No.	Country	Name of Regulatory Authority
1	Afghanistan	Medicines Regulatory Authority
2	Australia	Therapeutic Goods Administration (TGA)
3	Bangladesh	Directorate General of Drug Administration (DGDA)
4	Brazil	Agencia Nacional de Vigiloncia Sanitaria (ANVISA)
5	Canada	Health Canada
6	China	China food and Drug Administration (CFAD)
7	Europe	European Medicines Agency (EMEA)
8	Germany	Federal Institutes for Drugs and Medicinal Devices
9	India	Central Drugs Standard Control Organization (CDSCO)

10	Japan	Pharmaceuticals and Medical Devices Agency (PMDA)
11	New Zealand	Medicines and Medical Devices Safety Authority (MEDSAFE)
12	Nigeria	National Agency of Food and Drug Administration and Control (NAFDAC)
13	Pakistan	Drug Regulatory Authority of Pakistan (DRAP)
14	Saudi Arabia	Saudi Food and Drug Authority
15	South Africa	Medicinal Control Council
16	South Korea	Ministry of Food and Drug Safety (MFDS)
17	Sri Lanka	Cosmetics Devices and Drug Regulatory Authority (CDDA)
18	Switzerland	Swiss Medic, Swiss Agency for Therapeutic Products
19	UK	Medicines and Healthcare Product Regulatory Agency (MHRA)
20	USA	Food and Drug Administration (FDA)

Table 2. International Organizations

Sr. No.	Name of Organisation
1.	World Health Organization (WHO)
2.	International Conference on Harmonization (ICH)
3.	International Organization for Standardization (ISO)
4.	American Society for Testing and Materials (ASTM)

4. Central Drugs Standard Control Organisation (CDSCO)

The Central Drugs Standard Control Organisation (CDSCO) working under Directorate General of Health Services, Ministry of Health & Family Welfare, Government of India is the National Regulatory Authority (NRA) of India. Itsheadquarters is located at FDA Bhawan, Kotla Road, New Delhi andaccommodates6Zonal offices, 4Sub-Zonal offices, 13 Port offices and 7Laboratories wide-spread across the country.

The Drugs & Cosmetics Act,1940 and rules 1945 have entrusted various responsibilities to central & state regulators for regulation of drugs &cosmetics. Itforesees uniform implementation of the provisions of the Act & Rules made there under for ensuring the safety, wellbeing and rights of the patients by regulating the drugs and cosmetics. CDSCO is constantly developingfor upbringing of transparency, uniformity and accountability in its services ensuring safety, efficacy and quality of the medicines and medicinal products manufactured, imported and distributed across country.

Under the Drugs and Cosmetics Act 1940 and rules 1945, CDSCO is responsible for all the drugs approval, Conducting Clinical Trials, deciding the standards for Drugs and controlling the quality of imported Drugs in the country and coordinatingwith the activities of State Drug Control Organizations by advising with a view of bringing about the uniformity in the administration of the Drugs and Cosmetics Act, 1940.In addition to this,Both the CDSCO and state regulators are responsible for granting the licenses of various specialized categories of critical Drugs such as blood and blood products, I. V. Fluids, Vaccines and Sera [6].

4.1 Role of CDSCO

- Approval of new drugs and clinical trial.
- Import registration and licensing.
- License approving of blood banks, Large Volume Parenterals (LVP), Vaccines, DNA products and some medical devices.
- Amendment to Drugs& Cosmetic act and rules.
- Banning of drugs and cosmetics.
- Granting the test license, personal license,NOCs for export.

- Testing of new drugs.
- Oversight and market surveillance through inspectorate of centerover and above the state authority.

5. U.S. Food & Drug Administration (USFDA)

The Food and Drugs Act of 1906 was the first of more than 200 laws that constitute one of the world's most comprehensive and effective networks of public health and consumer protections. Today, the FDA regulates $1 trillion worth of products every year. It ensures the safety of all food products except for poultry, some egg and meat products; ensuring safety and effectiveness of all drugs consisting of biological products such as blood, vaccines, tissues for transplantation, medical devices, animal drugs and feed(Table 3). They make sure that cosmetics, medical and consumer products that emit radiation do no harm to humans and environment.

5.1 Role of FDA

The role of FDA's regulatory authority has very extensive approach. FDA's responsibilities are very closely related with several other government agencies. It's always confusing for consumers for contacting the appropriate regulatory agency.

Table 3- Categories approved by FDA

Categories	**Examples**
Foods	Dietary supplements, Bottled water, Food additives, Infant formulas, Other food products (excluding meat, poultry and some egg products)
Drugs	Prescription drugs (both brand-name and generic), Non-prescription (over-the-counter) drugs
Biologics	Vaccines, Blood and blood products, Cellular and gene therapy products, Tissue and tissue products, Allergenics
Medical devices	Simple items like tongue depressors, Complex technologies like heart pacemakers, Dental devices Surgical implants and prosthetics
Electronic products emitting radiations	Microwave ovens, X-ray equipment, Laser products, Ultrasonic therapy equipment, Mercury vapor lamps, Sunlamps
Cosmetics	Color additives found in makeups, Skin moisturizers and cleansers, Nail polish and perfume
Veterinary Products	Livestock feeds Pet foods Veterinary drugs and devices
Tobacco Products	Cigarettes Cigarette tobacco Smokeless tobacco

5.2 Product Approval

FDA is responsible for protecting public health by regulating human drugs and biologics, animal drugs, medical devices, tobacco products, food (including animal food), cosmetics, and electronic products that emit radiation. But not all those products undergo premarket approval — that is, a review of safety and effectiveness by FDA experts and agency approval before a product can be marketed. In some cases, FDA's enforcement efforts focus on products after they are already for sale. That is determined by Congress in establishing FDA's authorities.

- Companies and manufacturers, but they have authority to inspect them as per cGMP guidelines.
- Compounded drugs.
- Tobacco and tobacco products.
- Cosmetics
- Medical foods such as special nutrients, but they should comply good manufacturing practices.
- Infant formula- but they are subjected to regulatory oversight.
- Dietary supplements- prior notification of 75 days is required before marketing.
- Food label, including nutrition facts panel.[7]

6. Medicines & Healthcare productsRegulatory Agency (MHRA)

The Medicines and Healthcare products Regulatory Agency regulates medicines, medical devices and blood components for transfusion in the UK. It is recognized globally as an authority in its field; the agency plays a leading role in protecting and improving public health and supports innovation through scientific research and development.

The agency has 3 centres:

- The Clinical Practice Research Datalink (CPRD), a data research service that aims to improve public health by using anonymized NHS clinical data
- The National Institute for Biological Standards and Control (NIBSC), a global leader in the standardization and control of biological medicines
- The Medicines and Healthcare products Regulatory Agency (MHRA), the UK's regulator of medicines, medical devices and blood components for transfusion, responsible for ensuring their safety, quality and effectiveness

MHRA is an executive agency of the Department of Health. It employs more than 1,200 people and has facilities in London, York and South Mimms, Hertfordshire.

6.1 Advisory bodies

MHRA has created several independent advisory committees to provide impartial advice to ministers about the regulation of medicines and medical devices. These committees may also establish working groups to address specific problems. Members of these committees may receive a fee and claim some expenses.

- Advisory Board on the Registration of Homeopathic Products
- Herbal Medicines Advisory Committee
- The Review Panel
- Independent Scientific Advisory Committee for MHRA database research
- Medicines Industry Liaison Group
- Innovation Office

7. Therapeutic Goods Administration (TGA)

The Therapeutic Goods Administration (TGA) is part of the Australian Government Department of Health, and is responsible for regulating therapeutic goods including prescription medicines, vaccines, sunscreens, vitamins and minerals, medical devices, blood and blood products.Almost any product for which therapeutic claims are made must be entered in the Australian Register of Therapeutic Goods (ARTG) before it can be supplied in Australia.

As part of the Department of Health, the TGA safeguards and enhances the health of the Australian community through effective and timely regulation of therapeutic goods.

7.1 Role of TGA

The TGA is responsible for ensuring that therapeutic goods available for supply in Australia are safe and fit for their intended purpose. These include goods Australians rely on every day, such as vitamin tablets and sunscreens, through to goods used to treat serious conditions, such as prescription medicines, vaccines, blood products and surgical implants.

7.2 The TGA regulates the supply of:

- Regulating medicines

The regulation of medicines includes features like:

Medicines tested having higher level of risk are evaluated for safety, quality and efficacy; Based on different levels of risk, classification of medicine is done; assessment for quality and safety of ingredients with lower risk in medicines.

- Regulating medical devices

The regulation of medical devices includes:
Implementation of regulatory controls for manufacturing process of medical devices based on different levels of risk to the user, medical devices are classified; Inclusion of medical device in Australian Register of Therapeutic Goods.

- Other therapeutic goods regulated by the TGA

Risk management approach is applied by TGA for the regulation of blood components, tissue and cellular products, sterilant and disinfectants.
TGA does not regulate Veterinary medicines, Health insurance, Cosmetics, Healthcare professionals, Chemicals, Food.

7.3 TGA regulation

The Australian community expects therapeutic goods in the marketplace to be safe, of high quality and of a standard at least equal to that of comparable countries and regulates therapeutic goods through:

- Pre-market assessment;
- Post-market monitoring and enforcement of standards; and
- Licensing of Australian manufacturers and verifying overseas manufacturers' compliance with the same standards as their Australian counterparts.

8. European Medicines Agency (EMA)

EMA is governed by an independent Management Board. Its day-to-day operations are carried out by the EMA staff, based in London, overseen by EMA's Executive Director.EMA is a networking organisation whose activities involve thousands of experts from across Europe. These experts carry out the work of EMA's scientific committees.

Management Board-The Management Board consists of 36 members, appointed to act in the public interest, who do not represent any government, organisation or sector.The Board sets the Agency's budget, approves the annual work programme and is responsible for ensuring that the Agency works effectively and co-operates successfully with partner organisations across the EU and beyond.

Executive Director-The Agency's Executive Director is the legal representative of the Agency. He is responsible for all operational matters, staffing issues and drawing up the annual work programme.

Agency staff-The Agency's staff support the Executive Director in carrying out his responsibilities, including administrative and procedural aspects of EU law related to the evaluation and safety-monitoring of medicines in the EU.

Scientific Committees-EMA has seven scientific committees that evaluate medicines along their lifecycle from early stages of development, through marketing authorisation to safety monitoring once they are on the market. In addition, the Agency has a number of working parties and related groups, which the committees can consult on scientific issues relating to their particular field of expertise.These bodies are composed of European experts made available by national competent authorities of the EU Member States, which work closely with EMA in the European medicines regulatory network.

8.1 Role of EMA

To fulfil its mission, the European Medicines Agency (EMA) works closely with national competent authorities in a regulatory network. The Agency also implements policies and procedures to ensure it works independently, openly and transparently and upholds the highest standards in its scientific recommendations. EMA brings together scientific experts from across Europe by working closely with the national regulatory authorities in European Union (EU) Member States, in a partnership known as the European medicines regulatory network.

The network pools resources and expertise in the EU and gives EMA access to thousands of European scientific experts who take part in the regulation of medicines.

Ensuring the independence of its scientific assessments is a high priority for EMA. The Agency takes care to ensure that its scientific experts, staff and Management Board do not have any financial or other interests that could affect their impartiality.EMA strives towards being as open and transparent as possible about how it reaches its scientific conclusions. EMA's European public assessment reports describe the scientific basis for EMA's recommendations on all centrally authorised medicines.EMA also publishes a large amount of information in lay language about its work and about medicines. For more information, see Transparency. The Agency also seeks to publish clear and up-to-date information on how it operates, including planning and reporting documents and information on funding, financial management and budgetary reporting.

9. Canada's Health Care System

Canada's publicly funded health care system is dynamicreforms have been made over the past four decades and will continue in response to changes within medicine and throughout society. The basics, however, remain the same-universal coverage for medically necessary health care services provided based on need, rather than the ability to pay.

The organization of Canada's health care system is largely determined by the Canadian Constitution, in which roles and responsibilities are divided between the federal, and provincial and territorial governments. The provincial and territorial governments have most of the responsibility for delivering health and other social services. The federal government is also responsible for some delivery of services for certain groups of people. Publicly funded health care is financed with general revenue raised through federal, provincial and territorial taxation, such as personal and corporate taxes, sales taxes, payroll levies and other revenue. Provinces may also charge a health premium on their residents to help pay for publicly funded health care services, but non-payment of a premium must not limit access to medically necessary health services.

There is more to health than the health care system. The responsibility for public health, which includes sanitation, infectious diseases and related education, is shared between the three orders of government: federal, provincial/territorial and local or municipal. However, these services are generally delivered at the provincial/territorial and local levels.

9.1 The Federal Government

The federal government's roles in health care include setting and administering national principles for the system under the Canada Health Act; financial support to the provinces and territories; and several other functions, including funding and/or delivery of primary and supplementary services to certain groups of people. The federal government provides tax and cash transfers to the territories and provinces in support of health through the Canada Health Transfer. The federal government is also responsible for health protection and regulation (e.g., regulation of pharmaceuticals, food and medical devices), consumer safety, and disease surveillance and prevention. It also provides support for health promotion and health research.

10. World Health Organization (WHO)

WHO began when Constitution came into force on 7 April 1948-a date we now celebrate every year as World Health Day. WHO is now more than 7000 people from more than 150 countries working in 150 country offices, in 6 regional offices and at our headquarters in Geneva.

10.1 WHO remains firmly committed to the principles set out in the preamble to the Constitution.

- Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.
- The enjoyment of the highest attainable standard of health is one of the fundamental rights of every human being without distinction of race, religion, political belief, economic or social condition.
- The health of all peoples is fundamental to the attainment of peace and security and is dependent on the fullest co-operation of individuals and States.
- The achievement of any State in the promotion and protection of health is of value to all.
- Unequal development in different countries in the promotion of health and control of diseases, especially communicable disease, is a common danger.
- Healthy development of the child is of basic importance; the ability to live harmoniously in a changing total environment is essential to such development.
- The extension to all peoples of the benefits of medical, psychological and related knowledge is essential to the fullest attainment of health.
- Informed opinion and active co-operation on the part of the public are of the utmost importance in the improvement of the health of the people.
- Governments have a responsibility for the health of their peoples which can be fulfilled only by the provision of adequate health and social measures.

10.2 Role of WHO

WHO works worldwide to maintain safety and health, and serve the vulnerable. Its goal is to ensure that a billion more people have universal health coverage, to protect a billion more people from health emergencies, and provide a further billion people with better health and wellbeing. For universal health coverage, WHO:

- improves access to essential medicines and health products
- focus on primary health care to improve access to quality essential services
- improve monitoring, data and information.
- train the health workforce and advise on labor policies.
- work towards sustainable financing and financial protection

- support people's participation in national health policies

For health emergencies, WHO:

- prepare for emergencies by identifying, mitigating and managing risks
- detect and respond to acute health emergencies
- support delivery of essential health services in fragile settings.
- prevent emergencies and support development of tools necessary during outbreaks

For health and well-being, WHO:

- address social determinants
- promote intersectoral approaches for health
- prioritize health in all policies and healthy settings.

Through its work, WHO address:

- human capital, noncommunicable diseases prevention
- mental health promotion
- climate change in small island developing states
- antimicrobial resistance
- elimination and eradication of high-impact communicable diseases.[12]

11. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) is unique in bringing together the regulatory authorities and pharmaceutical industry to discuss scientific and technical aspects of drug registration. Since its inception in 1990, ICH has gradually evolved, to respond to the increasingly global face of drug development. ICH's mission is to achieve greater harmonisation worldwide to ensure that safe, effective, and high-quality medicines are developed and registered in the most resource-efficient manner.[13]

12. International Organization for Standardization (ISO)

ISO is an independent, non-governmental international organization with a membership of 164 bodies. Through its members, it brings together experts to share knowledge and develop voluntary, consensus-based, market relevant International Standards that support innovation and provide solutions to global challenges. International Standards make things work**.** They give world-class specifications for products, services and systems, to ensure quality, safety and efficiency. They are instrumental in facilitating international trade**.**ISO has published 22593 International Standards and related documents, covering almost every industry, from technology, to food safety, to agriculture and healthcare. ISO International Standards impact everyone, everywhere.

12.1 Role of ISO

ISO International Standards ensure that products and services are safe, reliable and of good quality. For business, they are strategic tools that reduce costs by minimizing waste and errors and increasing productivity. They help companies to access new markets, level the playing field for developing countries and facilitate free and fair global trade.[14]

13. The American Society for Testing and Materials (ASTM)

ASTM International is a globally recognized leader in the development and delivery of voluntary consensus standards. Today, over 12,000 ASTM standards are used around the world to improve product quality, enhance health and safety, strengthen market access and trade, and build consumer confidence. Leadership in international standards development is driven by the contributions of our members: more than 30,000 of the world’s top technical experts and business professionals representing 140 countries. Working in an open and transparent process and using ASTM’s advanced IT infrastructure, our members create the test methods, specifications, classifications, guides and practices that support industries and governments worldwide.

13.1 Role of ASTM

ASTM International standards are the tools of customer satisfaction and competiveness for companies across a wide range of markets. Through more than 140 technical standards-writing committees, it serves a broad range of industries: metals, construction, petroleum, consumer products and many more.

When new industries-like nanotechnology, additive manufacturing and industrial biotechnology-look to advance the growth of cutting-edge technologies through standardization, many of them come to ASTM International.ASTM standards are passports to a successful global trading strategy. Its high quality, market-relevant standards, developed in

accordance with the guiding principles of the World Trade Organization, fuel trade by opening new markets and creating new trading partners for enterprises everywhere. For businesses ranging from Fortune 500 leaders to emerging start-ups, our standards help level the playing field to foster competition in the global economy. Beyond standards development, ASTM offers certification and declaration through our subsidiary, the Safety Equipment Institute, as well as technical training programs and proficiency testing. All our programs complement our standards development activities and provide enterprise solutions for companies, government agencies, researchers and laboratories worldwide.The American Society for Testing and Materials was formed in 1898, founded by Charles B. Dudley, Ph.D., a chemist with the Pennsylvania Railroad. In 2001, it changed its name to ASTM International. ASTM's world headquarters are in West Conshohocken, Pennsylvania, with offices in Belgium, Canada, China, Peru and Washington, D.C. [15]

14. Conclusion-

Drug Regulatory agencies are boon and helping hands in regulation and maintaining the safety, wellbeing and rights of a patient and people consuming the medicines and medicinal products. Every country has their own regulatory agency whereas, there as some which are regulating worldwide. Although various agencies play differently in regulating, their prime objective is to achieve and deliver safe, high quality product to the market, thus minimizing the errors.

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Formulation and Characterization of Bicalutamide Loaded Polymeric Microcapsules

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ABSTRACT:

Microcapsular system offers great potential of drug delivery for such candidate drug with low water solubility. Bicalutamide is an anticancer drug used in prostate cancer treatment. Bicalutamide has low aqueous solubility. In present research work an attempt was made to prepare Microcapsules by using ionic gelation method. Ionotropic gelation involves merely the interaction of an ionic polymer with oppositely charge ion to initiate cross-linking. The prepared Microcapsules were characterized using different techniques UV spectroscopy, DSC and FTIR. SEM analysis was done shows excellent results smoothed shaped chitosan Microcapsules enclosed with drug. The Microcapsules showed a good capacity for the encapsulation and loading of BCa. Various kinetic models analyzed in-vitro drug release profile of BCa-Chitosan Microcapsules. The prepared polymeric Microcapsules shows a higher solubility and increased drug release when in contact with biological fluids and their polymeric nature allows obtaining the desired controlled and sustained drug release.

KEYWORDS: Bicalutamide, Ionic Gelation, Polymeric Microcapsules, Release Kinetic Study.

1. INTRODUCTION:

Microcapsule is a colloidal drug delivery system composed of an oily or an aqueous core enclosed by a thin polymeric membrane. Microcapsules have recently generated lot of attention in the area of controlled release with availability of biocompatible and biodegradable polymers. Nanotechnological devices can be crucial approaches for the constancy of the carried drug. Anticancer drugs that suffer rapid plasmatic metabolization will be protected by using such strategies. [1] Besides protecting the carried chemotherapeutic, nanoparticles can also be used to avoid cellular mechanisms such as multidrug resistance, because they allow entry into the cancer cells and act as an intracellular anticancer drug reservoir. [2] Biodegradable polymers have been used frequently as drug carriers due to its impressive bioavailability, superior encapsulation, control release and fewer toxic properties. [3] Polymer based nanocarriers are more advantageous as drug carriers compared with conservative formulations due to the risk of drug targeting and intravenous administrations without any possibility of embolization. [4] Chitosan is a biodegradable natural linear biopolyaminosaccharide obtained by alkaline deacetylation of chitin, polymer with great potential for pharmaceutical applications due to its biocompatibility, high charge density, non-toxicity and mucoadhesion. It has been shown that Microcapsules not only improves the

dissolution of poorly soluble drugs but also exerts a significant effect on fat metabolism in the body. Gel formation can be obtained by interactions of chitosan with low molecular counter ions such as polyphosphates, sulphates and crosslinking with glutaraldehyde. [5] Chitosan Microcapsules were rapidly absorbed by human tumor cells. In an work it is demonstrated that the encapsulation of docetaxel within LNCs dramatically increased the drug biological half-life, providing substantial accumulation at the tumoral site in mice bearing subcutaneously implanted C-26 colon adenocarcinoma. [6] Bioadhesive properties can be obtained by coating the Microcapsules surface with polymers presenting positive charge, such as the polysaccharide chitosan and its derivatives. The adsorption process of chitosan onto Microcapsules occurs via ionic interactions between chitosan. [4] The capability to engineer biocompatible polymers with controllable properties is greatly desirable. Many controversies surround the management of prostate cancer to include screening practices, diagnosis, and treatment options.[7, 8] Although various medical organizations do not recommend routine screening for this malignancy, [9] other organizations, such as the American Urological Association and the American Cancer Society, have published screening guidelines for the early detection of prostate cancer.[10, 11] Results from the PLCO (Prostate, Lung, Colorectal and Ovarian Cancer Screening) trial in the United States, sponsored by the National Cancer Institute, and the European Randomized Study on Screening, should provide definitive answers to the question of whether or not prostate-specific antigen (PSA) screening can ultimately reduce mortality from prostate cancer.[12]

Ionic gelation (IG) was former known as 'ion-induced gelation'. Ionotropic gelation involves merely the interaction of an ionic polymer with oppositely charge ion to initiate cross-linking. Unlike simple monomeric ions, the interaction of polyanion with cations (or polyanion with polycation) cannot be completely explained by the electro-neutrality principle. Chitosan-based nanoparticles prepared by ionotropic gelation with the polycation of acidic pH, and TPP (tripolyphosphate) as the anionic oligomeric partner leads to the formation of colloidal systems.[13] An attempted was made for developing sustained release mucoadhesive loaded chitosan nanoparticles, which can serve as intranasal drug delivery system which shows the controlled release of drug up to several hours. [14] Unlike the microemulsion method, another advantage of the IG method is the ease with which the degree of cross-linking and therefore the 'buffering' activity can be manipulated. Systematic stability studies for particles generated by both techniques are essential before it can be directly extrapolated for industrial scale up.[15] Compared with other colloidal carriers, polymeric Microcapsules shows a higher stability when in contact with biological fluids and their polymeric nature allows obtaining the desired controlled and sustained drug release.[16] Various attempts have been made for enhancing the solubility of BCa, β-cyclodextrin Inclusion complexation,[17] Nanostructured lipid carriers,[18] Nanodispersion,[19, 20] instead of this Microcapsular system is more advantageous because of its sustained release, incremental drug selectivity and effectiveness, improvement of drug bioavailability and alleviation of drug toxicity. Microcapsules, with submicron size, can be administered intravenously, reach to the target and release the encapsulated drug. [21, 22]

1. EXPERIMENTAL SECTION:

1.1. Material and Methods: Chitosan (95% deacetylation), Sodium tripolyphosphate was purchased from Sigma Aldrich. Bicalutamide were purchased from 3S Corporation, Mumbai, India. Tween – 80 and Acetic acid purchased from Merck Specialties Pvt. Ltd. Mumbai. All other materials were of pharmaceutical and analytical grades and were used as received.

1.2. Methods:

2.2.1 Differential scanning calorimetry (DSC): Preparation of Polymeric Microcapsules:

Bicalutamide loaded polymeric Microcapsules were prepared by using previously mentioned method [13, 24] with slight modifications. BCa loaded Chitosan nanoparticles were prepared by ionic gelation of chitosan solution with sodium tripolyphosphate (0.30%). Tween 80 (0.3%) solution use as a re-suspending agent to prevent aggregation at ambient temperature while stirring. 50 mg of BCa and various concentrations of chitosan (F1-F5) dissolved in acetic acid in aqueous solution (1%) under magnetic stirring at room temperature for 45 min. the formulation batches with the different composition of the materials given in the table no.1.

Table 1: Formulation Batches of BCa-Chitosan Microcapsules

Sr. No.	Materials	Batches					
Drug : Polymer Ratio		F1	F2	F3	F4	F5	F6
		1:1	1:2	1:3	1:4	1:5	1:6
1.	Bicalutamide (mg)	50	50	50	50	50	50
2.	Chitosan (mg)	50	100	150	200	250	300
3.	Acetic acid (%)	1	1	1	1	1	1
4.	Sodium Tripolyphosphate (%)	0.84	0.84	0.84	0.84	0.84	0.84
5.	Tween 80 (%)	0.30	0.30	0.30	0.30	0.30	0.30

2. RESULT AND DISCUSSION:

2.1. UV-Visible Spectroscopy: A primary stock solution of Bicalutamide (50 mg) was prepared in phosphate buffer solution (7.4 pH). All the measurements were performed at room temperature. The standard solutions were prepared by the proper dilution of the primary stock solution with buffer and for linearity study; serial dilutions were made for Bicalutamide in the range of ppm concentrations were prepared by diluting the stock solution with buffer.

2.2. Entrapment efficiency, Loading efficiency and Loading: Percent Entrapment Efficiency was determined by analyzing the methanolic solution containing BCa after centrifugation under UV-Visible spectrophotometer (Shimadzu, UV- 1800, Japan) and subsequent UV Analysis. The percent entrapment was calculated using the formula mentioned below.

$$\text{Entrapment Efficiency (\%EE)} = \frac{\text{BCa. T} - \text{BCa. S}}{\text{BCa. T}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, BCa.T = total amount of BCa taken; BCa.S = amount BCa present in supernatant

To calculate the percent loaded drug, 10 mg of BCa loaded PNCs was dispersed into 10 mL methanol and ultrasonicated until the drug was extracted into methanol and centrifuged 15000 rpm for 20 min. The absorbance maxima are recorded at 321 nm and compared with the calibration curve of BCa in methanol. The percent Drug Loading ability was calculated using the equation 2. [23].

$$\text{Drug Loading Ability (\%DL)} = \frac{\text{BCa. PNCs}}{\text{W. PNCs}} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where, BCa.PNCs = amount of Bicalutamide on loaded PNCs; W.PNCs = total amount of PNCs taken.

Percent Entrapment Efficiency was determined by analyzing the methanolic solution containing BCa after centrifugation using UV-Visible spectrophotometer respective values of all parameters depicted in table no.2 from all six batches F2 shows best results.

Table 2: Showing Drug Loading, Entrapment and Loading Efficiency

Batches	Entrapment efficiency (%)	Loading efficiency (%)	Loading Ability (%)
F1	63.66±0.61	1.74±0.52	64.90 ±3.0
F2	88.36±1.20	2.64±0.55	85.30 ±2.1
F3	66.40±1.23	2.10±0.62	80.39 ±2.54
F4	73.42±1.28	1.45±0.38	80.45 ±2.6
F5	73.74±0.87	1.89±0.24	81.90 ±1.9
F6	85.28±1.18	2.20±0.89	3.40 2.8

2.3. *in vitro* Dissolution Study: In-vitro release profile of BCa from the microspheres was examined in Phosphate Buffer (pH 7.4) using USP II, six stage dissolution rate test apparatus (Electrolab). Microcapsules equivalent to 100 mg of drug packed in filter paper and was suspended in dissolution medium at 50 rpm and 37 ± 0.5°C. [24] An aliquot of 2 mL was withdrawn simultaneously at intervals of one hour and same volume of fresh medium was replaced. The samples were filtered through Whatman filter paper and analyzed spectrophotometrically at 272 nm for amount of drug released.

2.4. Particle Size and Zeta Potential:

Dynamic light scattering is used to determine the particle size of dispersed sample. PNCs dispersed in water and diluted several times. Nanoplus Particulate System (Micromeritics, USA), which determines the average particle size by intensity distribution, did the measurement. Similarly, surface charge on particle was determined using Zeta potential measurements on Nano Plus 3, using flow cell containing a micro volume of dispersion interacted at the platinum electrode interface. All measurements were done in triplicate and STD calculated. The Zeta potential is the overall charge acquired by particles in a particular medium, and its value gives an indication of the potential physical stability of NCs dispersion. If all the particles have a large positive or negative ZP, they will repel each other, and the system is considered stable. The higher the value, the more stable the system. [25] Microcapsules withparticles size were observed at optimum range in F2formulation with PDI 0.105. which is depicted in tabel no. 3.

Table 3: Particle Size and Zeta Potential of Formulated Batches

Batch	Particle size (nm)	Polydispersity index	Zeta potential (mV)
F1	121.53	0.091	20.3
F2	130.5	0.105	25.6
F3	135.4	0.074	21.0
F4	140.4	0.22	24.0
F5	152.03	0.04	24.7
F6	162.33	0.063	25.2

1.1. Fourier Transform Infra-red (FTIR) Spectroscopy: Confirmation of chitosan, Bicalutamide and prepared Microcapsules and was analyzed using FTIR spectrophotometer (IR Affinity-1, Shimadzu Japan). 1:100 ratio of the sample was mixed with dried KBr, examined under spectrophotometer. Using diffuse reflectance spectrum the transmission was calculated after scanning sample from 4000 – 500 cm^{-1}. Figure 1 shows FTIR spectra of neat BCa, which had characterized by sulphate at about (1528 cm-1), hydroxyl group (3,582 cm-1), carbonyl (1,690 cm-1), amide (3,338 cm-1) and nitrile (2,230 cm-1). Hydrogen bonding and steric interference are often used, as a tool to describe the interaction of drug and carrier and carbonyl group remains a dominant hydrogen bond acceptor that can form a hydrogen bond with the hydrogen of Chitosan, evident from the decreased peak intensity of carbonyl group and C–H peak broadening in BCa-Chitosan system. Compared to Bicalutamide peaks, physical mixture peaks were smoothened signifying a strong physical interaction was exists in between Bicalutamide and Chitosan.

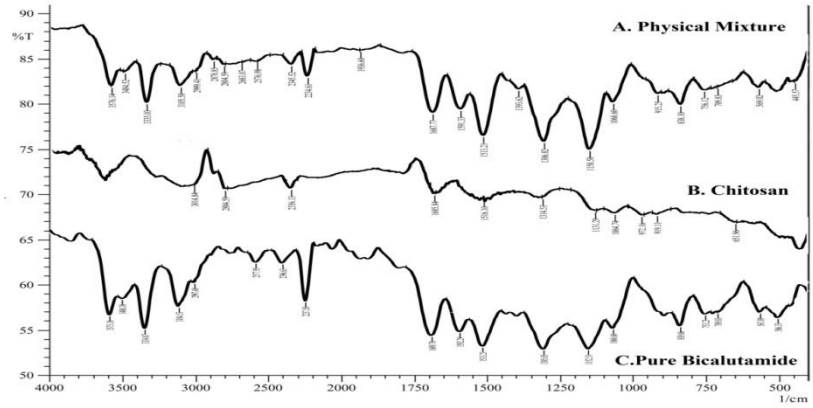
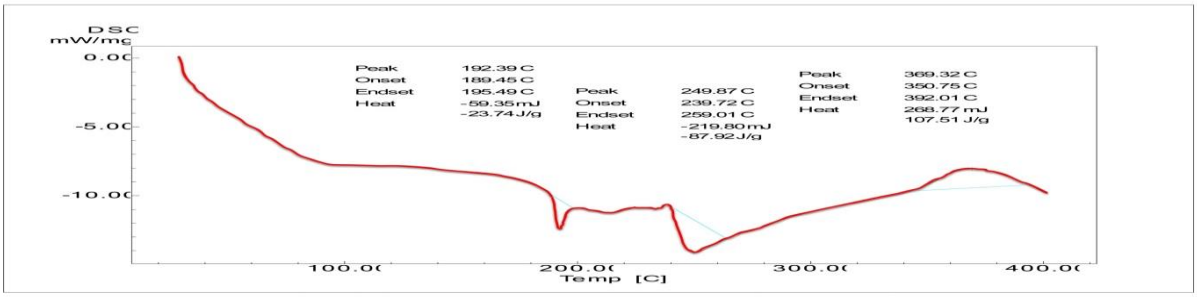
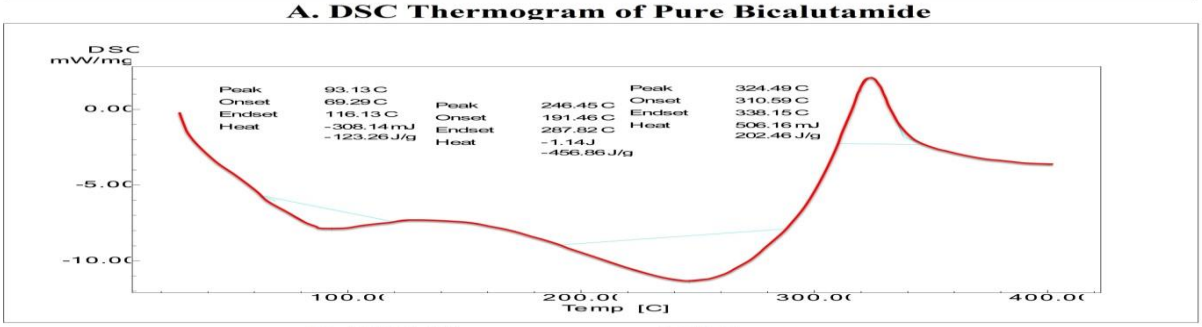


Figure 1 Fourier Transform Infra-red Spectrum of Physical mixture (A), Chitosan (B), Pure Bicalutamide (C).

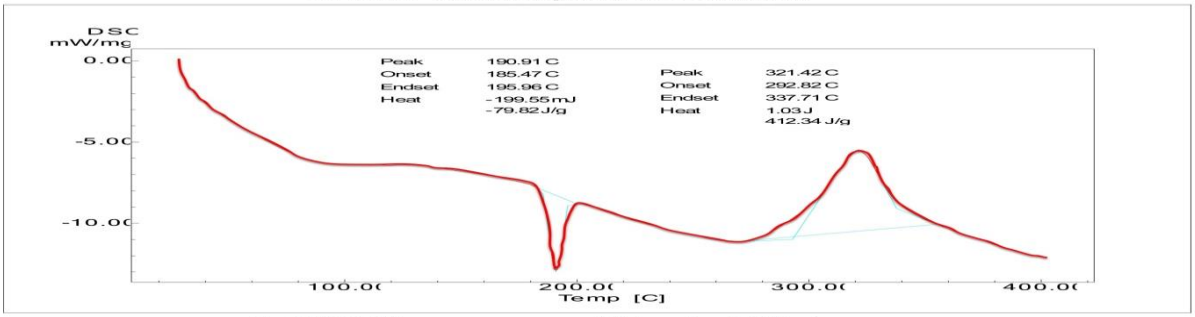
2.5. Differential Scanning Calorimetry (DSC) Analysis: Thermal analysis was carried out on DSC Instrument (Perkin Elmer 4000, USA) from 20 ^{0}C to 400 ^{0}C at a heating rate of 20^{0}C/min in a nitrogen atmosphere. Thermal behavior of drug (BCa), Chitosan and prepared polymeric Microcapsules (PNCs) was determined using 3 mg of sample capped into aluminum pan sealed using the high-pressure press. The DSC graph was recorded with the empty aluminum pan as a reference. Neat BCa (Fig. 2 A) melting can be observed from the sharp endothermic peak at 192 °C. The physical mixture showing melting of amorphous BCA present within the Microcapsules at around 190°C. A slight melting point depression of BCa is observed in physical mixture when compared with the neat BCa signifying that there is some interaction between AP and BCA. This inclination was also observed and remains almost unaffected to temperature, whereas BCa-



A. DSC Thermogram of Pure Bicalutamide



B. DSC Thermogram of Chitosan



C. DSC Thermogram of Physical Mixture

Chitosan shows a very elusive peak around 278 °C in physical mixture, which has masked the melting endothermic peak observed in neat BCa denoting presence of amorphous phase of BCA in BCa-Chitosan system. The DSC Thermogram of Pure Bicalutamide, Chitosan and Physical Mixture is depicted in figure no. 2.

Figure 2: DSC Thermogram of Pure Bicalutamide (A), Chitosan (B) and Physical Mixture (C).

2.6. Scanning Electron Microscopy (SEM): The surface morphology analysis of prepared polymeric Microcapsules (PNCs) was done using Scanning Electron Microscope (SEM, S-4800, Hitachi, Japan). The samples were sputtered with gold prior to analysis thereby samples get conductive. The accelerating voltage of 15.0 kV was set during the measurement and images were captured at different magnification. Morphological characteristics of BCa-chitosan Microcapsules and pure BCa were examined using SEM and are presented in Figure 3. Microcapsules appear uniform and spherical carrying porous structures within. The SEM images revealed that the capsules were spherical in shape, nearly monodisperse in capsule diameter, and had a smooth non-porous shell wall. The capsule shell walls were free of the thick layer.[26]

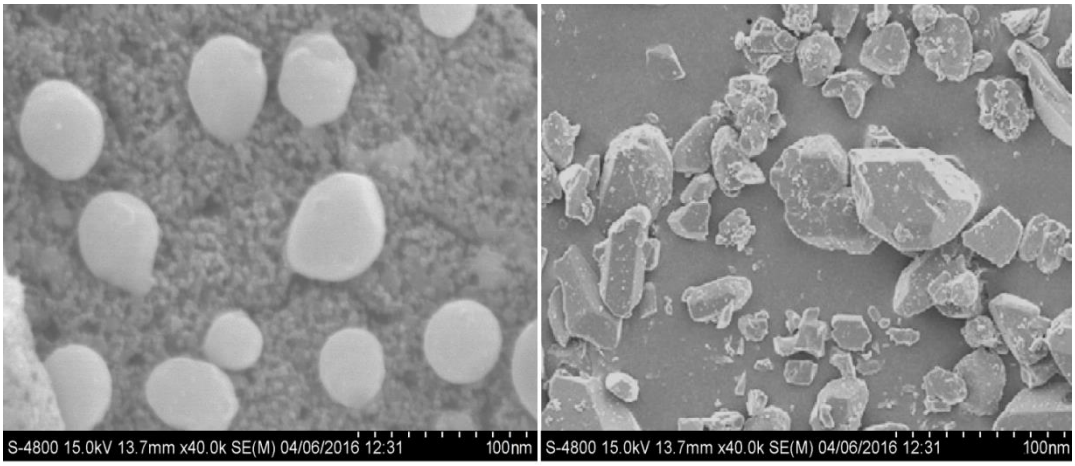


Figure 3: Scanning Electron Microscopy (SEM) of BCa-chitosan Microcapsules and pure BCa

2.7. Dissolution Kinetics Studies: The composition of Microcapsules influences the mechanism of drug encapsulation through the adsorption mechanism on the polymer or entrapment within the core. The localization of drug in the Microcapsules structure affects its release kinetics. [4] BCa-Chitosan Microcapsules confirmed improved dissolution profile when compared with neat pure BCa. This may be due to an increase of BCa wettability because of chitosan possesses hydrophilicity. Significantly improved release profile from BCa-Chitosan was found as compared to neat BCa. The *in-vitro* release profile of BCa-Chitosan Microcapsules was analyzed by various kinetic models which are depicted in figure no. 4. The kinetic models used were zero order, first order, Higuchi and Korsemeyer-Pappas equation (Table no. 4). The releases constant were calculated from the slope of the respective plots. Higher correlation was observed in the Higuchi equation. For planer geometry, the value of n=0.5 indicates a Fickian diffusion mechanism, for $0.5<n<1.0$, indicates irregular (non Fickian) and n=1 implies class II transport. Both dissolution and diffusion profile of the drug from the nanoparticles showed fitting to Higuchi plot with zero order release kinetics and indicated Non Fickian diffusion mechanism for the release of the drug from the nanoparticles. The diffusion profile of the drug from the nanoparticles confirmed to Higuchi plot with zero order release kinetics and indicated Non-Fickian diffusion mechanism for the release of the drug from the nanoparticles.

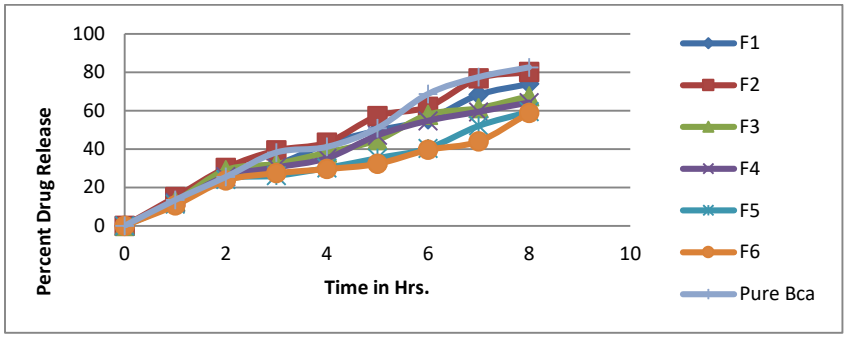


Figure 4: *in vitro* Dissolution profile BCa-Chitosan Microcapsules and Pure BCa

Table 4: Various Parameters of the Model Equation on the *In-vitro* Release Kinetics of BCa-Chitosan Microcapsules

Batches	First order		Higuchi		Korsemeyer-peppas	
	K	R^2	K	R^2	R^2	n value
F1	0.057	0.698	13.55	0.969	0.727	0.757
F2	0.057	0.723	13.57	0.981	0.924	0.631
F3	0.058	0.683	12.96	0.958	0.654	0.615
F4	0.054	0.654	15.04	0.959	0.670	0.706
F5	0.051	0.631	12.62	0.974	0.649	0.656
F6	0.040	0.545	14.25	0.966	0.691	0.654

3. CONCLUSION:

In the present study, a process for Bicalutamide loaded microcapsules has been successfully demonstrated. The release significantly affected by optimizing the sonication rate. Prepared Microcapsules shows greater wetting properties and drug release. The microcapsules were characterized using UV spectroscopy, DSC and FTIR spectroscopy studies. The surface morphology was studied by using SEM analysis; it seems rounded shaped chitosan Microcapsules enclosed with drug. The Microcapsules showed a good capacity for the encapsulation and loading of BCa. I*n-vitro drug* release profile of BCa-Chitosan Microcapsules was analyzed by various kinetic models. The results of fitting the release kinetic model to the experimental data of BCa release suggested an anomalous behavior. This strategy can successfully be inferred to number of other drug candidates in a cost effective way for preparation of sustained release formulations with improved.

Conflict of Interest: author declares no conflict of interest.

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Short communication: Whole genomic analysis and phylogenetic comparison of *Lactobacillus rhamnosus* and *Lactobacillus plantarum*

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1. Introduction

The Lactobacillus strain was isolated from sheep milk by general plate culture method on MRS broth [1–8]. The use of microbes as functional food maintain health and prevent many disease and disorder. The isolation and research of new strains of probiotic strains especially lactobacilli proven to be useful to satisfy the increasing demand of the population. In the current study, the probiotic potential of Lactobacillus strains isolated from sheep milk was investigated. The Lactobacillus strains were identified and evaluated for tolerance against gastric acidity and bile toxicity, along with the adhesion to HT-29 cells. The study proved the antimicrobial activities and antibiotic susceptibility. Survival of the strains through the host intestine was examined by the 12 week Wister rat feeding and faecal analysis trial. The in-vivo trials not only proved the adhesion but also the survival of the Lactobacillus plantarum MCC 3595 inside the intestinal lumen of the host. Thus, the isolated strain can act as the functional food by the further clinical investigation. The isolation of genomic DNA with amplification of 16 S rDNA region helps to identify the exact nature of the one strain with the others [9]. The common part observed in the case of probiotics from the same genera are its differences in physiological and biochemical characterizations [1]. Thus, to identify the exact difference between the closely related Lactobacillus

from the same genera the whole genomic sequencing is carried out; this help to discover the special pharmacological importance of the discovered microbes belonging from the same family or the genera.

The present research deals with the whole genomic analysis of the Lactobacillus. In the current study, the two Lactobacillus strains are first time isolated from sheep milk; as earlier, it was reported to be found only in the human gut. Thus, these isolated cultures are comparatively studied with the existing culture of *L. acidophilus* obtained from NCIM (National Collection of Industrial Microorganism), Pune.

2. Whole genomic sequencing of Lactobacillus strains

The DNA libraries were generated as per manufacturers instruction using 'NextSeq-500/550 High-Output Kit version 02.5' (for 300/cycles) followed by sequencing carried out by NextSeq-500 sequencing_system-Illumina (The Netherlands). Trimmomatic v0.36 software was used for trimming of the reads applying sliding-window of 04:15 settings and minimum Phred score of 33. The reads were generated by removing the adaptor sequence. The read qualities were demonstrated by FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc). The assembling of data was carried out by de novo using SPAdes_v3.12.0. The further analysis was covered in the contigs >500 bp length and > 5x coverage.

3. Phylogeny identification and relationship of LAB

The phylogeny studies were carried out by the common ancestor's background determinations of the isolates. The Neighbor-Joining method is used in finding the evolutionary gaps and similarities. The phylogeny tree with branch length sum is given as = 02.19005408 [10]. The tree is constructed with a scale, the same branch lengths are having the same units and determine the same evolutionary distances. The Maximum Composite Likelihood method is used in determining the evolutionary distances and are calculated as in the units with the proper substitutions per locations. The analysis involves 24 nucleotide sequences (Fig. 3.10). The gap and missing links with its database are eliminated from the tree. 1011 positions data sets are finalized for the study. The study is conducted by using the evolutionary MEGA7 software.

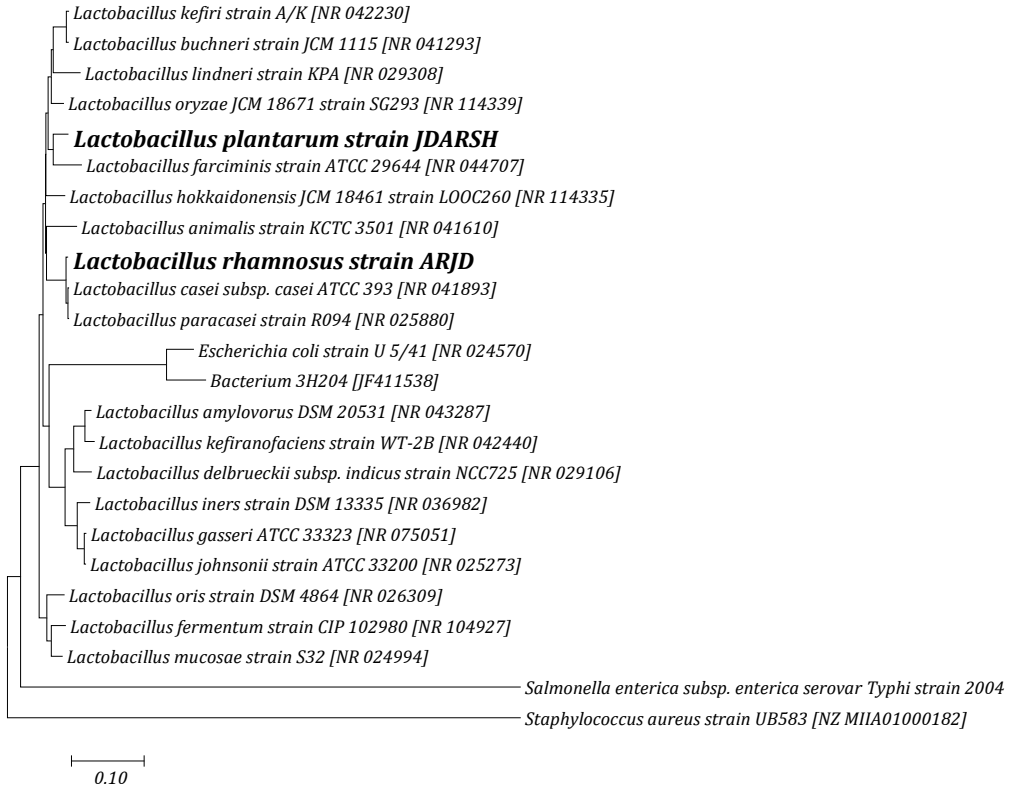

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Fig 1. Evolutionary relationships of taxa by Neighbor-Joining method

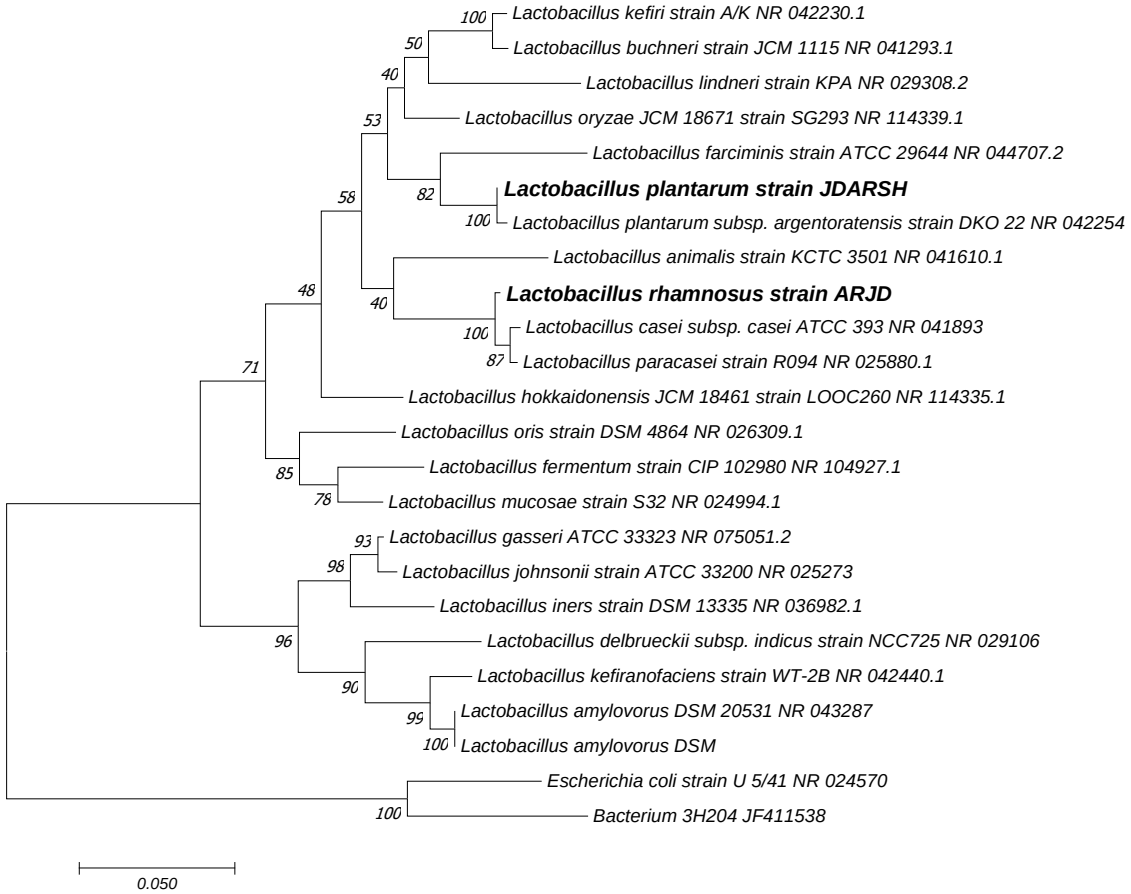

Fig 2. Evolutionary relationships of taxa by Maximum Likelihood method

Tamura-Nei model was used to study the evolutionary history by Maximum Likelihood method [11]. The chart prepared is a data sheet of the tree with the highest log likelihood (-07826.77) [12]. The similarity index in the form of the percentage i.e. associated similar taxas are clustered together and showed in next branches. The study was conducted using the Neighbor-Join and BioNJ algorithms studies plotting the initial tree(s) for the heuristic search using the Maximum Composite Likelihood (MCL) approach. The evaluation of the data is carried out by the topology superior log-likelihood value. The data sheet is presented as the tree with branch lengths with the substitutions at each branch. The analysis used 24 nucleotide sequences (Fig. 3.11). The gap and missing links with its database are eliminated from the tree. 1289 positions data sets are finalized for the study. The study is conducted by using the evolutionary MEGA7 software.

3.3.4 Whole genomic analysis of Lactobacillus strains (Lp, Lr)

a) Lactobacillus plantarum (Lp)

LOCUS PYBS01000000: 31 rc DNA linear BCT 27-MAR-2018
DEFINITION *Lactobacillus plantarum* strain JDARSH, whole genome shotgun sequencing project.
ACCESSION PYBS00000000, VERSION, PYBS00000000.1
DBLINK Bio-Project: PRJNA439183; Bio-Sample: SAMN08741665
KEYWORDS-WGS.
SOURCE: *Lactobacillus plantarum*
ORGANISM: *Lactobacillus plantarum*
Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus.

REFERENCE-1: (bases 1 to 31)
AUTHORS: Patil, A., Suryavanshi, M., Disouza, J. and Pawar, S.
TITLE: Draft genome sequence of *Lactobacillus plantarum* strain JDARSH MCC3595
JOURNAL: Unpublished
REFERENCE: 2 (bases 1 to 31)
AUTHORS Patil, A., Suryavanshi, M., Disouza, J. and Pawar, S.
TITLE: Direct Submission
JOURNAL submitted (20-MAR-2018): Microbial Culture Collection, National
Centre for Cell Science Pune, Pashan Sutarwadi, Pune, Maharashtra 411021, India
COMMENT: The *Lactobacillus plantarum* whole genome shotgun (WGS) project has the project accession PYBS00000000. This version of the project (01) has the accession number PYBS01000000, and consists of sequences PYBS01000001-PYBS01000031.
Bacteria and source DNA available from MCC 3595.
Annotation was added by the NCBI Prokaryotic Genome Annotation
Pipeline (released 2013). Information about the Pipeline can be found here: https://www.ncbi.nlm.nih.gov/genome/annotation_prok/
##Genome-Assembly-Data-START##
Assembly Date: MAR-2018
Assembly Method: SPAdes v. 3.9
Genome Representation: Full
Expected Final Version: Yes
Genome Coverage: 102.0x
Sequencing Technology: Illumina HiSeq
##Genome-Assembly-Data-END##
##Genome-Annotation-Data-START##
Annotation Provider: NCBI
Annotation Date: 03/20/2018 12:37:21
Annotation Pipeline: NCBI Prokaryotic Genome
Annotation Pipeline
Annotation Method: Best-placed reference protein set; Gene MarkS+
Annotation Software revision: 4.4
Features Annotated: Gene; CDS; rRNA; tRNA; ncRNA; repeat_region
Genes (total): 3,128
CDS (total): 3,060
Genes (coding): 2,980
CDS (coding): 2,980
Genes (RNA): 68
rRNAs: 3, 1, 1 (5S, 16S, 23S)
Completer RNAs: 1, 1 (16S, 23S)
Partial rRNAs: 3 (5S)
tRNAs: 59
ncRNAs: 4
Pseudo Genes (total): 80
Pseudo Genes (ambiguous residues): 0 of 80
Pseudo Genes (frame shifted): 39 of 80
Pseudo Genes (incomplete): 27 of 80
Pseudo Genes (internal stop): 24 of 80
Pseudo Genes (multiple problems): 10 of 80
CRISPR Arrays: 1
##Genome-Annotation-Data-END##
FEATURES- Location/Qualifiers source: 1….31
/organism= "Lactobacillus plantarum"
/mol_type= "genomic DNA"
/strain= "JDARSH"
/isolation_source= "milk sample"
/culture_collection= "MCC: 3595"
/db_xref="taxon: 1590" /country="India: Kolhapur" /collection_date= "2016"
/collected_by= "Mr. Abhinandan Patil"
WGS: PYBS01000001-PYBS01000031
Thus, *Lactobacillus plantarum*is designated as sample A (Lp) for the further experimental processes.

***b) Lactobacillus rhamnosus* (Lr)**

LOCUS PYRN01000000, 38 rc DNA; linear- BCT 18-SEP-2018

DEFINITION: *Lactobacillus rhamnosus* strain ARJD, whole genome shotgun sequencing project.

ACCESSION: PYRN00000000

VERSION: PYRN00000000.1

DBLINK: Bio-Project: PRJNA445595; Bio-Sample: SAMN08793894

KEYWORDS- WGS

SOURCE: *Lactobacillus rhamnosus*

ORGANISM: *Lactobacillus rhamnosus*

Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus.

REFERENCE: 1 (bases 1 to 38)

AUTHORS: Patil, A., Suryavanshi, M., Disouza, J. and Pawar, S.

TITLE: Draft genome sequence of *Lactobacillus rhamnosus* strain ARJD MCC 3594

JOURNAL- Unpublished

REFERENCE- 2 (bases 1 to 38)

AUTHORS-Patil, A., Suryavanshi, M., Disouza, J. and Pawar, S.

TITLE-Direct Submission

Journal Submitted (25-MAR-2018) Microbial Culture Collection, National Centre for Cell Science Pune, Pashan Sutarwadi, Pune, Maharashtra 411021, India

COMMENT: The *Lactobacillus rhamnosus* whole genome shotgun (WGS) project has the project accession PYRN00000000. This version of the project (01) has the accession number PYRN01000000, and consists of sequences PYRN01000001-PYRN01000038.

Bacteria and source DNA available from MCC 3594.

Annotation was added by the NCBI Prokaryotic Genome Annotation

Pipeline (released 2013). Information about the Pipeline can be found here: https://www.ncbi.nlm.nih.gov/genome/annotation_prok/

##Genome-Assembly-Data-START##

Assembly Date: MAR-2018

Assembly Method: SPAdes v. 3.9

Genome Representation: Full

Expected Final Version: Yes

Genome Coverage: 110.0x

Sequencing Technology: Illumina HiSeq

##Genome-Assembly-Data-END##

##Genome-Annotation-Data-START##

Annotation Provider: NCBI

Annotation Date: 03/26/2018 23:45:36

Annotation Pipeline: NCBI Prokaryotic Genome

Annotation Pipeline

Annotation Method: Best-placed reference protein set; Gene MarkS+

Annotation Software revision: 4.4

Features Annotated: Gene; CDS; rRNA; tRNA; ncRNA; repeat_region

Genes (total): 2,937

CDS (total): 2,872

Genes (coding): 2,773

CDS (coding): 2,773

Genes (RNA): 65

rRNAs: 1, 1, 1 (5S, 16S, 23S)

Completer RNAs: 1, 1, 1 (5S, 16S, 23S)

tRNAs: 59

ncRNAs: 3

Pseudo Genes (total): 99

Pseudo Genes (ambiguous residues): 0 of 99

Pseudo Genes (frame shifted): 45 of 99

Pseudo Genes (incomplete): 39 of 99

Pseudo Genes (internal stop): 29 of 99

Pseudo Genes (multiple problems): 13 of 99

CRISPR Arrays: 1

##Genome-Annotation-Data-END##

FEATURES: Location/Qualifiers source-1.38

/organism= "*Lactobacillus rhamnosus*"

/mol_type= "genomic DNA"

/strain= "ARJD"

/isolation_source = "milk"

culture_collection = "MCC: 3594"

/db_xref="taxon: 47715" /country="India: Kolhapur" /collection_date="2016"

/collected_by= "Mr. Abhinandan Patil"

WGS: PYRN01000001-PYRN01000038

Thus, *Lactobacillus rhamnosus* is designated as sample B (Lr) for the further experimental processes.

Acknowledgments

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Reference

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Genomic-Based Restriction Enzyme Selection for Specific Detection of *Lactobacillus rhamnosus and Lactobacillus plantarum* strain by 16S rDNA PCR-RFLP

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Abstract

Probiotics are discovered from the various natural sources from time to time as the functional food. Lactobacillus (LAB) is found because the most preferred genera during this direction with its utility within the dairy and allied sciences. The two isolates of *L. plantarum* and *L. rhamnosus* were isolated from the sheep milk. These strains are identified by Genomic-Based Restriction Enzyme Selection by digestion of *Hind III, EcoR1, BAM H e*nzymes to get a restriction profile using PCR technique. The PCR-RFLP method by using *Hind III, EcoR1, BAM H1* enzymes assist to establish the good correlation between the two Lactobacillus strains.

Keywords: *L. plantarum*, *L. rhamnosus*, PCR technique, Sheep milk.

1. Introduction

The current science of the nutraceutical world deals with the investigation of the new sorts of functional foods as an isolate for the betterment of the mankind [1]. The utilization of the microbes as functional food has explored its use in life science [2,3]. Probiotics are discovered from the various natural sources from time to time because the functional food lactobacillus (LAB) is found as the most preferred genera during this direction with its utility within the dairy and allied sciences [4–7]. The foremost difficult task to use these microbes because the functional food is studying the expansion parameters along side the genomic analysis [8]. The current study deals with the genomic analysis of microbial samples obtained from sheep milk and comparing the results with the existing culture of Lactobacillus.

2. Material and method

Milk of different animals were used to isolate the lactic acid bacteria. Frozen isolates were revived from the glycerol stock, thawed and re-inoculated into freshly prepared sterile MRS lactobacilli broth and incubated at 37 °C for 24 h [9]. After confirmation of purity, 10 µl of active broth culture was reinoculated into 10 ml sterile MRS broth and incubated at 37 °C for 10 h. Two-milliliter aliquots of active log phase cultures from this broth were then used to isolate genomic DNA from the processed sample (MRS lactobacilli broth). The bacteria were collected by centrifugation at 5200 rpm for 5 minutes in a refrigerated centrifuge. The supernatant was decanted from the medium along with washing of pellet using 2 ml of NaCl thrice with EDTA (25 mM NaCl, 4 mM EDTA, at pH 8.0). Later, 100 ul freshly prepared lysozyme solution was mixed (concentration 10 mg/ml in NaCl-EDTA) and incubated at 37 °C for 60 min. 4 ul of RNase-A solution (10 mg/ml, working concentration 100 µg/ml) was used to remove the RNA before incubation. The final volume was made up to 500 microliters by addition of NaCl-EDTA, 12 ul of proteinase solution (17 mg/ml) and 50 ul of a 10% SDS solution. The contents were completely mixed and incubated at 50 °C for 55 min. After incubation, an equal volume of phenol saturated with Tris (pH 8.0) was mixed and centrifuged at 11,000 rpm at 21 °C for 600 s. The upper aqueous phase was carefully removed free from proteins and cellular residues. This phase was repeated once with a new aliquot of the phenol-chloroform mixture (1: 1) with a collection of the supernatant. The DNA in the supernatant was precipitated using 0.7 volumes of isopropanol along with 0.3 M sodium acetate (pH 5.5). The obtained DNA was pelletized by centrifugation at 9,000 rpm at 4 °C for 6 min. The obtained DNA in precipitated form was collected and dried by washing with a 71% ethanol solution. Furthermore, the granules formed were dissolved in 50 ul of Tris-EDTA (10: 1, pH 8) and stored at -18 ° C. In about three isolates were tested by using a commercial kit with the protocol suggested by the manufacturer. The DNA was stored frozen at -20 °C until use [10].

A) Amplification of 16 S rDNA region of LAB

2 µl of genomic DNA was mixed with 48 µl of PCR mixture as per the instruction mentioned in the manufacturer user kit [11]. 50 µl of the final reaction mixture was taken to the PCR steps (Bio-Rad T 100 PCR, USA). The amplification was carried by using the EGE1 forward and EGE2 reverse primer of 16S rDNA region from the isolates. The forward primer is complementary to the 5'end of 16S rDNA and the reverse primer is complementary to the 3' end of the 16S rDNA region.

Forward Primer: EGE1: 5'-AGGAGTTTATCCTGGCTCAG-3'

Reverse Primer: EGE2: 5'CTTACGGCACCTTGTTACGA-3'

The PCR Conditions:

Step 1: 94°C for 360 s

Step 2: 94°C for 60 s (denaturation)

Step 3: 56°C for 60 s (annealing)

Step 4: 72°C for 60 s (elongation)

Step 5: 72°C for 600 s

Table 1. PCR mixture composition

Sr. no	Media ingredient	Formula (µl)
1	Mg-freeTaq DNA polymerase buffer	5
2	MgCl2 (25Mm)	3
3	Sterile deionized water	33
4	Oligo forward 10 picomole/µl	1
5	Oligo reverse 10 picomole/µl	1
6	dNTP (2 mM each)	5
7	DNA	2
Total		50

B) Separation of amplified PCR products

a) Preparation of agarose gel

0.8% prepared agarose gel was dissolved in boiling 100 ml TAE buffer. The gel was cooled at 45 °C with the addition of 15 µl ethidium bromide solution (9.9 µg/ml) was added. The agarose gel was placed into the gel casting stand along with the combs. The combs were removed after getting the rigid gel and used for loading the DNA samples [12].

b) Loading of Agarose Gel

2 µl loading dye was added to 5 µl of PCR products which was introduced into wells. The first well was loaded with the DNA size-marker (1 kb, Fermentas) to observe the amplification range.

c) Electrophoresis of the products

The electrophoreses of the PCR products were carried out at 79 mA for 40 min. The visualization of the PCR products was carried out by a gel documentation system (Vilber-Lormat). The amplication of the DNA fragments observed in the range 1500-2000 bp shows the proper output of the amplification.

d) Purification of PCR products

The restriction enzymes were used for PCR products prior to digestion.
The purification procedure carried out were as follows:

• 50 µl 1XTE buffer was used to PCR product to make up the volume to 100 µl
• Addition and mixing of 200 µl chloroform solution was carried out in duplicate
• The formed solution was centrifuged at 5.000 rpm for 11 min
• The upper aqueous phase was mixed with the 0.l volume of 3 M sodium acetate (pH 5.2) solution
• Addition and mixing of 200 µl 99% ethanol solution was carried out in duplicate along with the centrifugation at 8.000 rpm for 10 min
• The supernatant was discarded and the formed DNA pellets were added with 500 µl of 70% ethanol
• The formed solution was centrifuged at 5.000 rpm for 11 min
• The formed pellet were dried at 37 °C for 10 min
• The formed DNA is dissolved into 50µl 1X TE solution
• DNA was stored as a solution at -20 °C.

e) DNA sequencing

The two isolates were sent out for 16S rRNA gene molecular identification, by illumination Nextseq platform, Netherlands. The results obtained after sequencing were then BLAST using the BLASTn algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and identification of individual isolates were done. To elucidate the phylogenetic relation of our isolated *Lactobacillus*, we retrieved the 16S rRNA sequences of other *Lactobacillus* species from GenBank nucleotide database and the phylogenetic tree was constructed using the Maximum likelihood method.

C) Restriction fragment length polymorphism (RFLP)

11 µl of purified amplification PCR products were used for each of the restriction enzyme digestion. Three different enzymes *Bam HI*, *Hind* III, and *EcoR*I are used for enzyme digestion study. 50 µl final reaction volumes were used for digestion; for *Bam HI* at 65 °C, for *Hind* III and *EcoR*I, it was 37 °C. All of the reactions were performed overnight and additionally *Bam HI* restriction reactions were overlaid with mineral oil to avoid evaporation[13].

Table 2. RFLP reaction mixture composition

Sr. no	Media ingredient	Formula (µl)
1	Restriction enzyme buffer	5
2	Sterile deionized water	34.5
3	Restriction enzyme (5U)	0.5
4	DNA	10
	Total	50

a) Electrophoresis of restriction fragments

1.6% agarose gel was used for separation of restricted fragments.

b) Preparation of agarose gel

2.4 g agarose gel was dissolved in boiling 150 ml TAE buffer. The gel was cooled at 45 °C with the addition of 22 µl ethidium bromide solution (9.9 µg/ml) was added. The agarose gel was placed into the gel casting stand along with the combs. The combs were removed after getting the rigid gel and used for loading the DNA samples

c) Loading of agarose gel

In electrophoresis tank, the solidified agarose gel was placed with the pouring of 1.5 X TAE buffer containing 300 µl of ethidium bromide. 2 µl of gel loading dye was added to 11 µl solution of digestion products. The loading of the samples was carried out from the second well on the gel. 2 µl (500 ng) of DNA was loaded to the first well on the gel with the molecular weight marker from 100 bp to 1kb.

e) Electrophoresis of the products

The electrophoreses of the samples were carried out at 60 mA for 29 min and at 80 mA for 4 h. The visualization of the amplified products was carried out by a gel documentation system (Vilber-Lormat).

f) Interpretation of results

The images obtained by gel documentation were modified in Adobe Photoshop 7.0. using BIO-ID.++software (Vilbeer-L'ourmat). The strains similarities were observed automatically by using the formula of Jaccard. The unweighted_pair_group method was used for strain clustering studies using arithmetic averages and UPGMA, BIO-ID++ techniques. 10% of homology were prepared for the homology coefficient studies.

3. Result and Discussion

Evaluation of amplification and digestion regions of 16S r DNA region of LAB

Genomic DNAs of the isolates are visualized by agarose gel electrophoresis under UV-light (Fig. 1). Then they are taken to the PCR step.

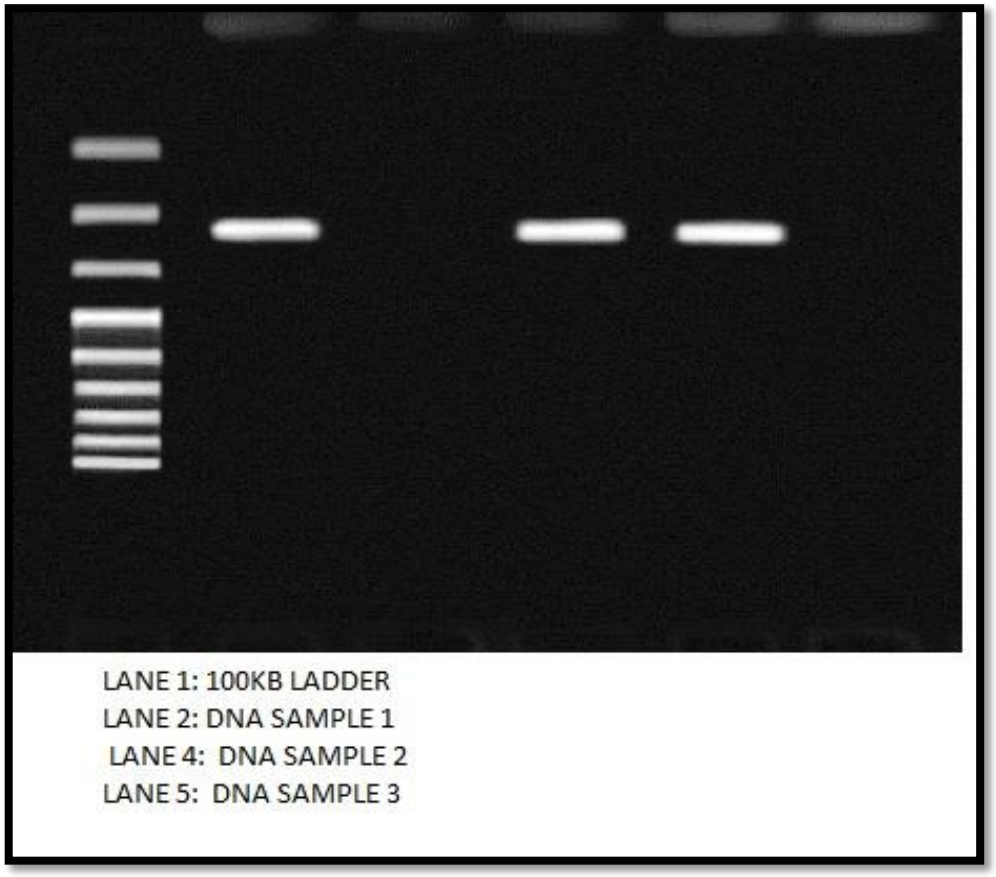

Fig. 1. DNA isolation Lactobacillus with the reference strain

b) Amplification of 16S rDNA region

After DNA isolation the 16S rDNA region is amplified by PCR protocol [14,15]. Then 50 µl of PCR products are visualized by agarose gel electrophoresis under UV-light. The length of the amplification products varied from 1850 to 2000 bp (Fig 2).

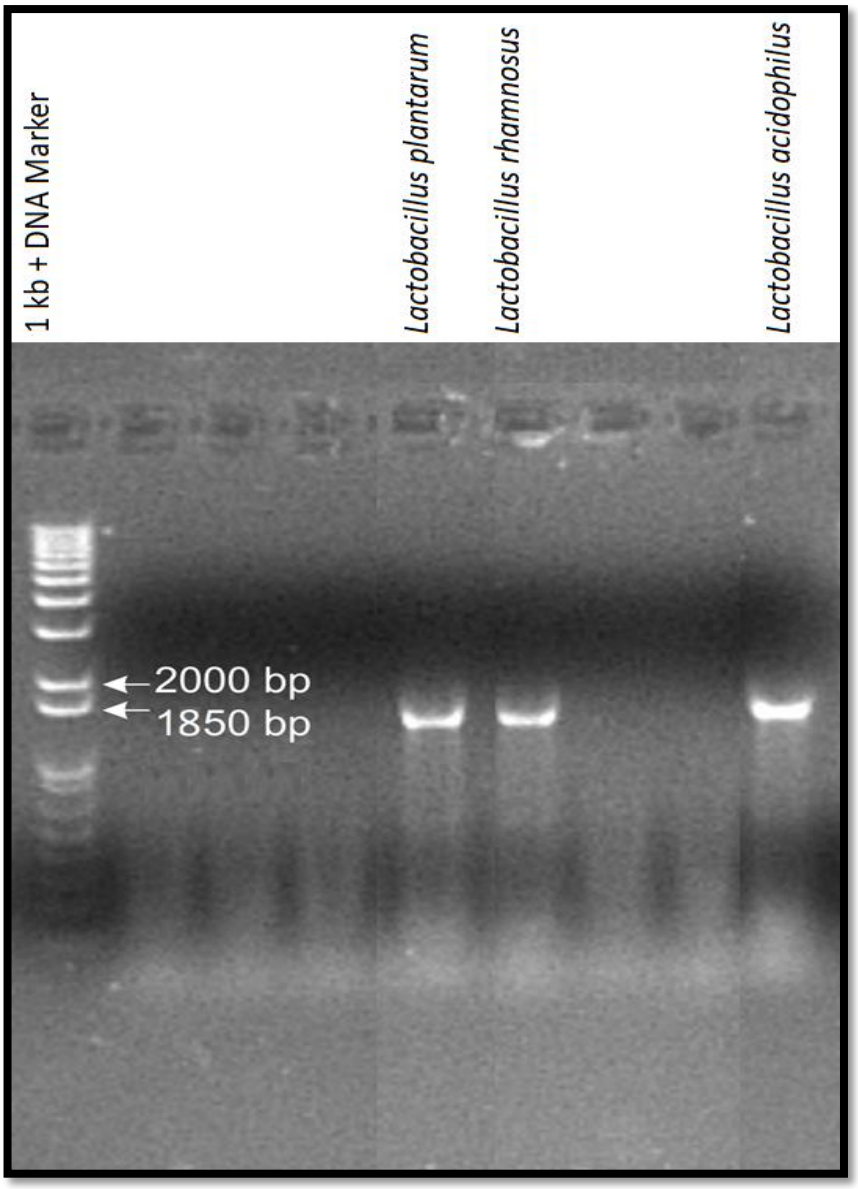

Fig 2. 16S Amplification products of isolates and reference strains

Identification of avian LAB isolates by 16S rRNA sequencing carried out by BLAST (Basic local alignment search tool) shows that sample A with 98% similarity with *Lactobacillus plantarum (Lp)*. Similarly, sample B shows a 96% similarity with *Lactobacillus rhamnosus (Lr)*. The correct identification of LAB with the accurate method and precision; having fast high discriminatory power is achieved by 16S rRNA gene sequencing [14].

Thus *L. plantarum* determined from (lane 5), *L. rhamnosus* (lane 6), and *L. acidophilus* (lane 9) used as the reference standard and are compared with 1kb DNA ladder gene ruler^{TM}.

c) Digestion of amplified 16S rDNA region by *Hind III, EcoR1, Bam H1*

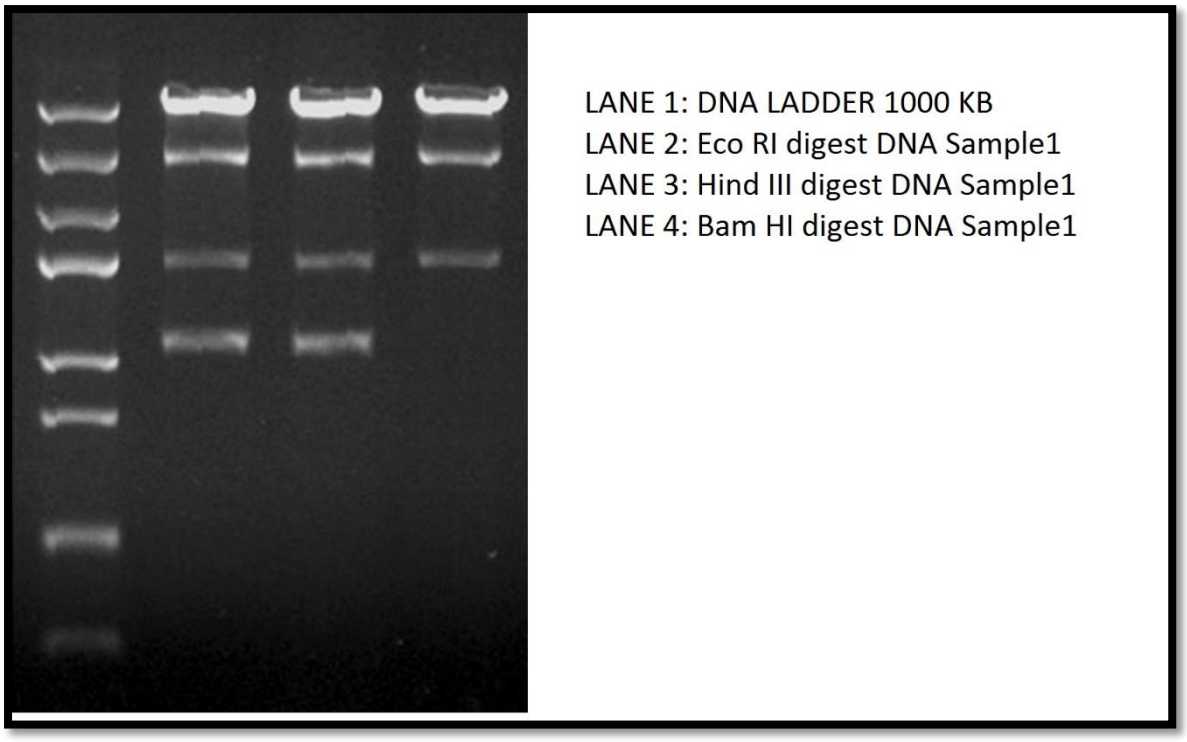

Fig 3. *Hind III, EcoR1, BAM H1* digests of *Lactobacillus plantarum*

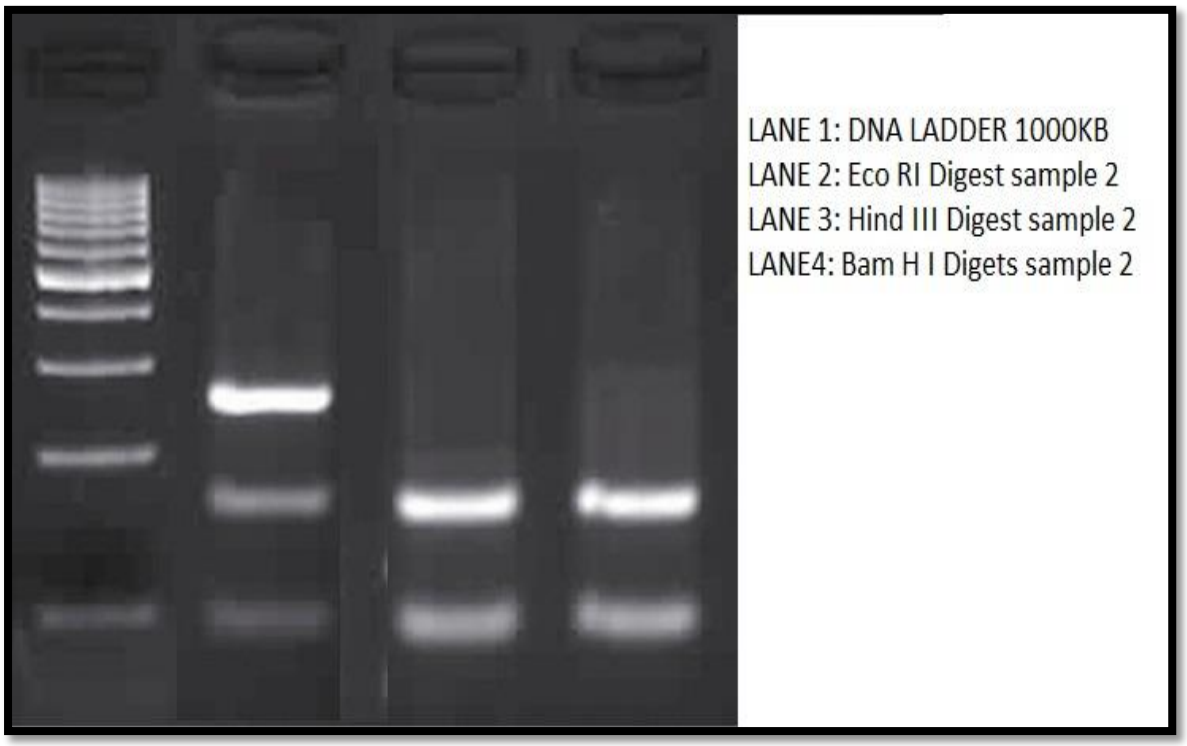

Fig 4. *Hind III, EcoR1, BAM H1*digests of *Lactobacillus rhamnosus*

The two isolates of *L. plantarum* (Fig 3) and *L. rhamnosus* (Fig 4) are digested by *Hind III, EcoR1, BAM H e*nzymes to get a restriction profile. It could be concluded that PCR-RFLP method by using *Hind III, EcoR1, BAM H1* enzymes revealed a good correlation between the two Lactobacillus strains. Because of the absence of some reference strains, isolates were identified by 16SDNA sequencing and BLAST in earlier studies [16].

4. Conclusion

The DNA of the strains were isolated and analysed for the 16 S rDNA investigation by taking standard reference strain of *L. acidophilus* (La). On the basis, amplification and digestion regions of 16S r DNA region of lactic acid bacteria these culture were identified as *L. plantarum* and *L. rhamnosus* and deposited the culture in NCIM, Pune as MCC 3595 and 3594 respectively.

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Characterization and antibiogram of Klebsiella pneumoniae isolated from pus samples in D.Y. Patil Hospital, Kolhapur

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Abstract

Klebsiella pneumoniae opportunistic infection common in hospitalized patient with co-morbid condition; unfortunately, resistant many group of antibiotics is on rise. This study was done to determine the prevalence and antibiotics sensitivity pattern of Klebsiella pneumoniae from pus samples

Material & Method

Total 180 Samples from Various sites were included in this study using standard microbiological techniques Klebsiella pneumoniae were isolated (30%) and identified, antibiotics sensitivity was done by Kirby Bauer's disc diffusion method and interpreted as per CLSI guide lines.

Results

Prevalence of Klebsiella infection more in males and age group affected was above 60 years isolation rate from pus sample was (30%), isolates were sensitive to Levofloxacin (85.29%), Chloramphenicol (73.53%), and Amikacin (52.15%) and highly resistant cefepime (94.11%), Tobramycin (94.11%) and ampicillin sulbactam (94.11%).

Conclusion

This study many be used to select of rational antibiotics therapy to prevent development of multidrug resistance.

Key words

Klebsiella pneumoniae, Pus, Antibiotics resistance Antibiotics susceptibly testing.

Introduction

Klebsiella pneumoniae is plump shaped, capsulated, non- motile, gram -negative organisms belonging to Enterobacteriaceae family. (1) It is the most common causative agent of nosocomial and community acquired infection as it is an aerobic bacterial flora of human gastrointestinal tract and are also found in respiratory tracts of humans and animals. (2)

Klebsiella pneumoniae is a causative agent pneumonia, liver abscess and urinary tract infections in healthy individuals. Recently emergency of multidrug resistant strains of Klebsiella pneumoniae are causing health care associated infections wound, biliary tract, peritoneum and meninges. (3)

Drug resistance of Klebsiella pneumoniae is due to spread of transmissible plasmids and acquiring resistant gene (4) Virulence factors contributing to the pathogenicity of Klebsiella pneumoniae are capsule (K antigen), lipopolysaccharide, fimbriae, (type 1 & 3) and siderophore.

Multidrug resistance of Klebsiella pneumoniae is mainly due to ESBL production encoded by the plasmid. In addition to Beta-lactams it is showing co- resistance to quinolones and aminoglycoside group of antibiotics.

This study was carried out to guide in selection of effective antibiotic for empirical treatment of Klebsiella infections and also to find out prevalence of antibiotics resistance.

Materials & Methods

This retrospective study was conducted in the Department of Microbiology from Jan. 2019 to July 2019. A total of 180 pus samples were collected during this period. Samples were collected with safety precautions and transported to the laboratory without any delay.

The pus samples were either aspirated by disposable syringes or collected onto sterile cotton tipped swabs (5). Samples were obtained from both IPD & OPD of all age groups of both sexes.

Isolation of Bacteria:

Pus samples collected aseptically inoculated on Blood agar and Mac-Conkey agar plates and incubated 18 to 24 hours at 37°c. Klebsiella isolates were identified by their morphology, biochemical reactions. Morphology of Klebsiella identified were mucoid large dome and shaped colonies on Blood Agar and lactose fermenting mucoid colonies on MacConkey agar. Gram negative, short, plump straight rod were seen.

The Biochemical reactions Voges-Proskauer positive, citrate utilization test, utilization urease test. Acid and gas production from Glucose, lactose, Sucrose, maltose and mannitol sugar fermentation test (1, 6) Sensitivity of Klebsiella pneumoniae was done on Mueller Hinton agar plates by Kirby-Bauer disc diffusion method according to CLSI guidelines 2012 (CLSI 2012). (7)

The antibiotics tested were amikacin (30mcg), Piperacillin/tazobactam (100/10 mcg), ciprofloxacin (5mcg),Cotrimoxazole (30 mcg), Gentamicin (10 mcg), Chloramphenicol (30 mcg), Azithromycin (15 mcg), Polymyxin B (30 mcg), Tobramycin (10 mcg), Cefepime (30 mcg), Ampicillin (10 mcg), Ceftriaxone (30 mcg), Amoxiclav (20/10 mcg), Ampicillin-sulbactam (10/10 mcg), Nitrofurantoin (300 mcg), Levofloxacin (5 mcg).

Result

Present study is includes collection of 180 Pus samples from hospitalized patient which were processed resulting in isolation of Klebsiella pneumoniae 54 (30%) of these isolates 34 (62.97%) were from males and 24 (37.03%) were from female with male to female ratio of 1.7 to 1 isolation of rates was highest in patients age above 6o years (Male 29.41% and Female 35%) followed by age group 45 to 60 (Male 23.52% and Female 30%) isolation rates were lowest in 0-15 age groups (Male 8.82% and Female 5%).

Antimicrobial susceptibility showed isolates were sensitivity to Levofloxacin (82.29%), Chloramphenicol (73.53%), Amikacin (52.95%), Polymyxin B (41.17%) while highly resistant to Tobramycin (94.11%), ampicillin-sulbactam (94.11%), cefepime (91.18%).

Table 1: **Age and Sexwise distribution.**

Age (Years)	Male% N=34	Female% N=20
0-15	3(8.83%)	1(5%)
16-30	4(11.77%)	4(20%)
31-45	9(26.47%)	2(10%)
46-60	8(23.52%)	6(30%)
61 & above	10(29.41%)	7(35%)

Table2: **Antimicrobial susceptibility of Klebsiella pneumoniae**

Antibiotic	Sensitivity (%)	Resistant (%)
Levofloxacin	85.29	14.71
Chloramphenicol	73.53	26.47
Amikacin	52.95	47.05
Polymyxin B	41.17	58.83
Pipercillin-Tazobactum	17.65	82.35
Cotrimoxazole	14.75	85.25
Ciprofloxacin	17.65	82.35
Azithromycin	11.77	88.23

Cefepime	5.89	94.11
Ceftriaxone	8.82	91.18
Tobramycin	5.89	94.11
Ampicillin-sulbactam	5.89	94.11
Amoxiclav	17.65	82.35
Ampicillin	8.82	91.18
Gentamicin	44.11	55.89

Discussion

In the present study we selected pus samples for isolation of Klebsiella pneumoniae because chances of isolation were more in pus sample as compared to urine and sputum samples as shown in many previous studies which is 40% to 50%. (8,9)

Our study showed Predominance of males than females which Co-relates with Namratha et al (9) and Shaha et al. (10) The age group affected Klebsiella pneumoniae above 60 years of age which correlates with previous studies (9) (10). Prevalence of Klebsiella infection in older age group may be due to the fact that Klebsiella species is a opportunistic pathogen and old age is associated with many comorbid conditions like diabetic mellitus, hypertension, postoperative state and catheterizations.

Antibiotics are the backbone of the therapy for the microbial infections unfortunately bacteria are developing resistance to multiple group of antibiotics due to indiscriminate use and inappropriate dose and duration of antibiotics which can be avoided by education of patients and clinicians about appropriate drug dose and duration surveillance of antimicrobial resistance and improved use of immunization.

Multidrug resistance development Klebsiella pneumoniae species may be due to production of extended Beta-lactamases ESBL as well as production of metallo- Beta- lactamases (MBL) which can be chromosomally enclosed or plasmid mediated. (11)

In our isolates highly sensitive to levofloxacin (85.29%) followed by Chloramphenicol (73.53%), amikacin (52.95%) and Gentamicin (44.11%) which correlates previous studies Asatia Rakeshkumar (12) and Bhurle et al (13). Isolates were resistance to Beta- lactams and Cephalosporins.

Conclusion

Antibiotics resistance is becoming threat to public health. Which warrants the proper selection of antibiotics preferably after performing antibiotics susceptibility test is solution for prevention of multidrug resistance and for good clinical outcome.

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Conflict of Interest:

None declared.

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