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Pathogenic Microorganisms

Activities of Citrullus Lanatus

Highlights

Ovarian Leiomyoma Associated

Multidrug Resistant Pathogens

Discovering Thoughts, Inventing Future

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Preparation of Microcapsules Containing Grape Polyphenols and A-Tocopherol by Spray-Gelling Method

By Shinji Arakawa, Yoshinari Taguchi & Masato Tanaka
Niigata University, Japan

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*Keywords: sodium alginate microcapsule, multiple emulsion, grape polyphenol, spray-gelling method, dual core materials, **α**-tocopherol.*

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Shinji Arakawa [α], Yoshinari Taguchi [σ] & Masato Tanaka [ρ]

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Keywords: sodium alginate microcapsule, multiple emulsion, grape polyphenol, spray-gelling method, dual core materials, α-tocopherol.

I. Introduction

Hitherto, many kinds of microcapsules have been prepared and applied in the various fields such as cosmetics, food, drugs, paintings, adhesives, textile, electric materials and so on [1-3].

The main functions of microcapsules are to protect the core materials from environment for a long time, to optionally release the core materials according to stimuli, to modify the surface of core material [1]. The microcapsules with these functions can be prepared by selecting the core and the shell materials with the desired chemical and physicochemical properties and by developing the microencapsulation procedure. If a few core materials with the different chemical and physicochemical properties could be microencapsulated at the same time, the multiple functions may be given to the microcapsules. For an example, in the case of applying the microcapsules to the food, cosmetics, drugs and textile, the nontoxic materials have to be used as the shell material. Furthermore, if one of the core materials tried to microencapsulate at the same time is hydrophilic and the other hydrophobic, the newly devised microencapsulation procedure has to be developed by using the designated core and shell materials.

It is well known that grape polyphenol is water soluble and has a few physiological effects such as anti-aging effect and anti-oxidation effect, but has a few defects such as a bitter taste and reaction activity. Accordingly, if grape polyphenol could be microencapsulated with some edible shell materials, the application fields of grape polyphenol may be considerably extended [4].

On the other hand, α-tocopherol (vitamin E) has a few physicological effects such as anti-aging effect and prevention effect of lifestyle related disease, but has a few defects such as light and heat destruction and easy oxidization [5-7]. Accordingly, if α-tocopherol oil could be microencapsulated with some nontoxic materials, various application fields for α-tocopherol may be expected. Furthermore, the microcapsules with the multiple functions can be prepared by microencapsulating these core materials at the same time. However, as α-tocopherol is hydrophobic and grape polyphenol is water soluble, in order to prepare the microcapsules containing these core materials as much as possible, it is necessary to develop the preparation method by using the nontoxic shell materials.

The spray methods such as spray drying, spray chilled and spray gelling for preparing the various kinds of microcapsules are well known to be the effective methods, because the selectivity for the core and the shell materials is extremely wide and the continuous production is capable [8,9]. Also, the diameters of microcapsules can be easily controlled by changing the volumetric flow velocity of feed and the spraying pressure [10-12]. Accordingly, the spray method may be suitable to the preparation of microcapsules containing the dual cores with the different chemical and physicochemical properties at the same time.

The purposes of this study are to develop the preparation method of microcapsules containing the hydrophilic and the hydrophobic core materials, to characterize the microcapsules and to discuss the formation mechanism.

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II. Experimental

a) Materials

Materials used to prepare the microcapsules containing the dual core materials were as follows.

Grape Polyphenol (GP) (Sunprite Ind. Co., Ltd, Tokyo, Japan) was used as the first core material and α-tocopherol (α-oil) (Wako Pure Chemical Ind. Co., Ltd, Tokyo, Japan) was used as the second core material. Sodium Alginate (Alg•Na) (Wako Pure Chemical Ind. Co., Ltd, Tokyo, Japan) was used as the shell material. Calcium chloride (Wako Pure Chemical Ind. Co., Ltd, Tokyo, Japan) was used as the gelling agent for sodium alginate. Poem PR-100, DAO-7S and Soy bean Lecithin (SL) (Riken Vitamin Ind. Co., Ltd, Tokyo, Japan) were used as the oil soluble surfactants. ML-750 (SY Glystar : Sakamoto Yakuhin Ind. Co., Ltd, Tokyo, Japan) was the water soluble surfactant.

b) Verification of effect of oil soluble surfactant species on stability of (W/O) emulsion and (W/O)/W emulsion

In order to increase the content of core materials as much as possible, it is necessary to stabilize the (W/O) emulsion and the (W/O)/W emulsion with the help of surfactant species. For this purpose, the effect of oil soluble surfactant species on the stability of (W/O) emulsion and the (W/O)/W emulsion was estimated as follows.

The GP aqueous solution of a given volume was poured into the α-oil dissolving the oil soluble surfactant and homogenized to form the (W/O) emulsion.

Then, the (W/O) emulsion was observed by visual confirmation at the constant time intervals. If the stability of (W/O) emulsion is lower, the (W/O) emulsion may be broken and the phase separation may be observed.

Furthermore, the (W/O) emulsion thus prepared was poured into the Alg•Na aqueous solution and homogenized to form the (W/O)/W emulsion. The stability of (W/O)/W emulsion was observed similarily as stated just above. If the stability of the (W/O)/W emulsion is lower, the (W/O)/W emulsion may be broken and the phase separation may be observed.

c) Preparation of microcapsules

The flow chart and the schematic diagram of experimental apparatus for preparing the microcapsules are shown in Figure 1 and Figure 2, respectively.

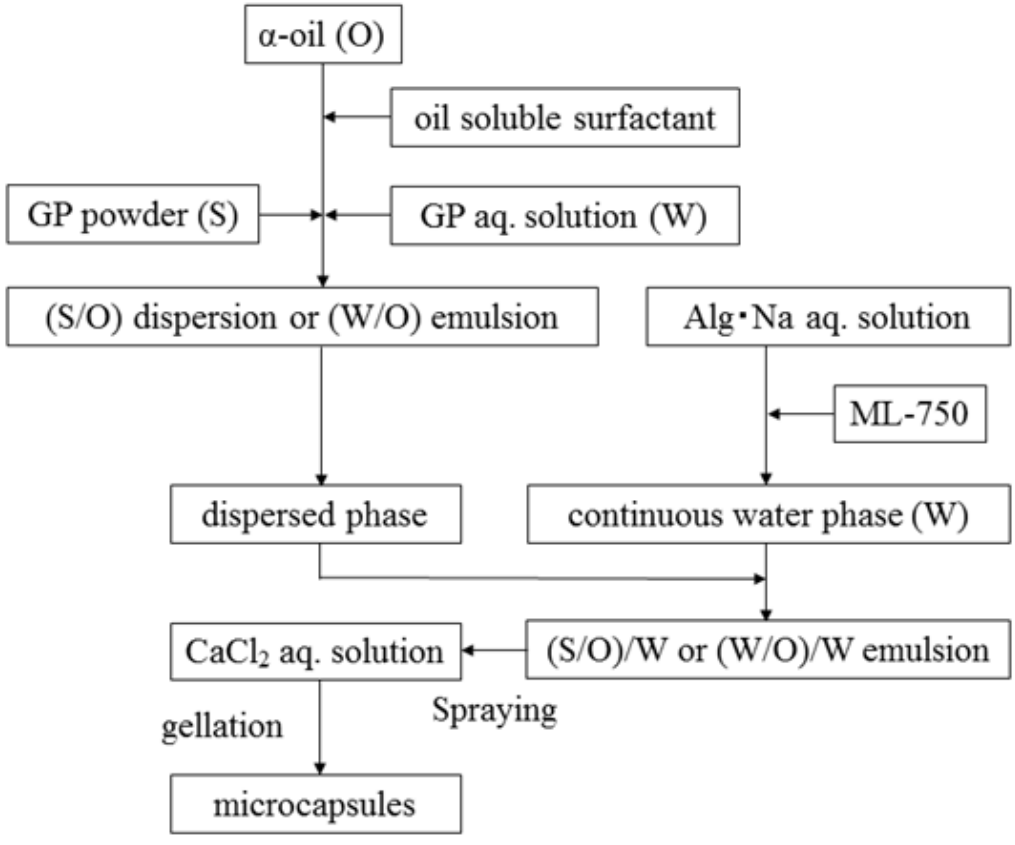

Figure 1 : Flow chart for preparing microcapsules

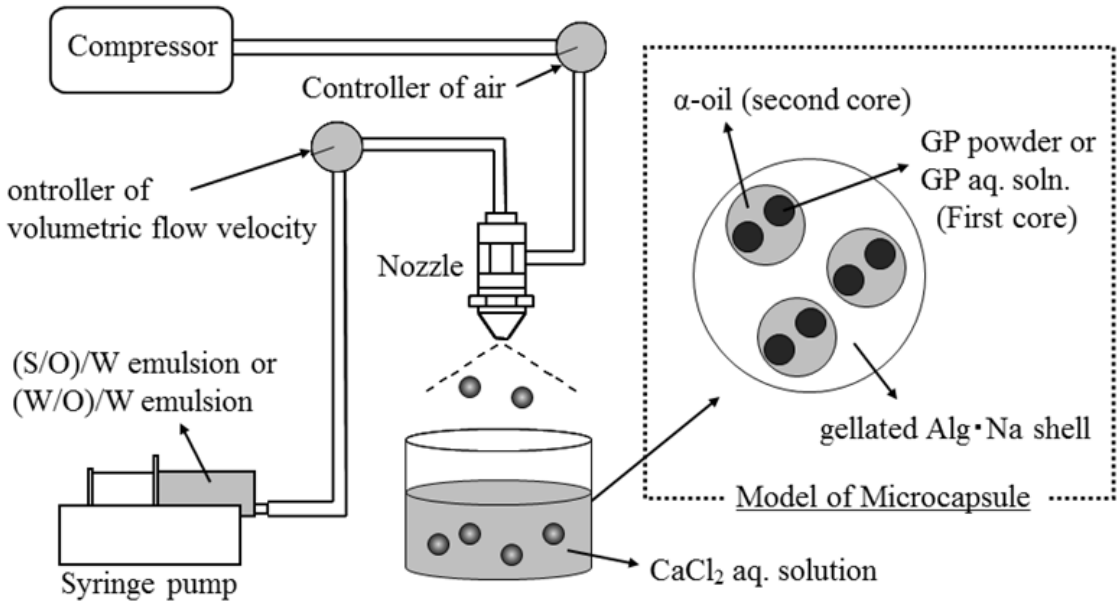

Figure 2 : Schematic diagram of experimental apparatus

GP of a given weight was dissolved in distilled water of 4.0cm3 to prepare the aqueous solution of 20wt%. The GP aqueous solution was poured into the α-oil together with the oil soluble surfactant to form the (W/O) emulsion by homogenizing with the rotor-stator homogenizer (Nihon Seiki Seisakusho Co., Ltd, Tokyo, Japan).

On the other hand, two kinds of the (S/O) dispersions, where GP powder was dispersed in the α-oil, were prepared as follows.

First, the (S/O) dispersion was prepared by directly adding GP powder into the α-oil and stirred. Second, the (S/O) dispersion was prepared by adding the GP aqueous solution into the α-oil, forming the (W/O) emulsion and then, by removing water from the (W/O) emulsion as shown in Figure 3. In this operation, the time for removing water from the (W/O) emulsion was changed from 0 to 12h at 50℃ in order to change the diameter of inner water droplets, namely the diameter of GP powder. Furthermore polyethylene glycol (PEG 600) was dissolved in the inner water phase in order to try to investigate whether the content of GP could be increased or not.

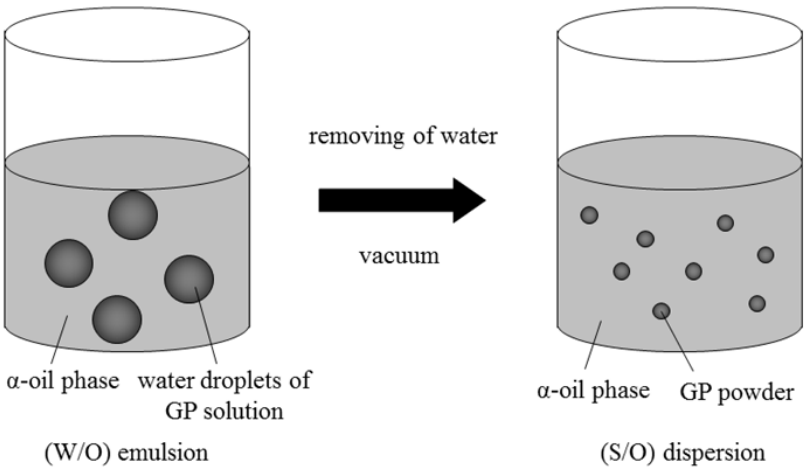

Figure 3 : Scheme for changing (W/O) emulsion to (S/O) dispersion

Then, the (W/O) emulsion or the (S/O) dispersion thus prepared was poured into the Alg·Na aqueous solution dissolving the water soluble surfactant (ML-750) and stirred to form the (W/O)/W emulsion or the (S/O)/W emulsion by homogenizing.

The (W/O)/W emulsion or the (S/O)/W emulsion was sprayed into the calcium chloride aqueous solution through the nozzle with the diameter of 1 mm to prepare the microcapsules containing the (W/O) emulsion or the (S/O) dispersion.

In this fundamental experiment, the spraying conditions such as the volumetric flow velocity of feed and the spraying pressure, the oil soluble surfactant species and the time for removing water from the (W/O) emulsion were changed stepwise.

The experimental conditions are shown in Table 1.

Table 1 : Experimental conditions

Preparation of (W/O)emulsion : 60°C, 12000rpm, 5min		
water phase	20wt% GP aq. soln.	4.0 g
oil phase	α-oil	14.4 g
surfactant	Poem PR-100, SL, DAO-7S	1.60 g
Preparation of (W/O)/W emulsion or (S/O)/W emulsion : 60°C, 8000rpm, 5min		
dispersed phase	(W/O) emulsion, (S/O) emulsion	10.0 g
continuous phase	Alg.Na aq. soln.	89.1 g
surfactant	ML-750	0.9 g
Preparation of (S/O) dispersion		
Time for removing water		0, 2, 4, 6, 8, 10, 12 h (60°C)
Spraying conditions		
gellation solution	2wt% $CaCl_2$ aq. soln.	4500 g
	Spraying pressure	P=0.1~0.5 MPa
	Volumetric flow velocity	V_R=1~5 ml/min

d) Characterization

Mean diameter

The diameters of inner water droplets and GP powder were measured by the instrument for measuring the particle size (Otsuka Denshi Co., Ltd, Tokyo, Japan: ELS-8000).

The diameters of microcapsules were measured by the particle size analyzer (Shimazu Seisakusho Co., Ltd, Kyoto, Japan: SALD-3000)

Here, the mean diameters (dp) were obtained as the mean Sauter diameters.

Microencapsulation efficiency

The microcapsules of a given weight were added into distilled water of 100 cm3, broken by the homogenizer and then, irradiated supersonic for 10 min to perfectly dissolve GP in the water phase.

The amount of GP dissolved in the sampled water was measured by the spectrophotometer.

For this measurement, the calibration curve between absorption degree and the concentration of GP was obtained beforehand.

The microencapsulation efficiency (Fc) was estimated by the following equation.

Observation of emulsion and microcapsules

The (W/O)/W emulsion, the (S/O)/W emulsion and the microcapsules were observed by the optical microscope (OLXMPUS Co., Ltd, Tokyo, Japan: BHT-MV) and their photographs were taken by digital camera. From these photographs, the stability of the multiple emulsions and the formation of microcapsules were observed.

III. Results & Discussion

a) Effect of oil surfactant species on stability of (W/O) emulsion and (W/O)/W emulsion

Figure 4 shows the results investigating the effect of oil soluble surfactant species on the stability of the (W/O) emulsion (Figure 4a) and the (W/O)/W emulsion (Figure 4b). Here, the transient feature of each emulsion was measured to estimate the stability. From Figure 4a, the following valuable results were obtained.

Just after preparing the (W/O) emulsion, in the case of PR-100 and SL, the (W/O) emulsion was brown color, but in the case of DAO-7S, the (W/O) emulsion was dark brown color. Here, the dark brown color means that the (W/O) emulsion is broken and the GP aqueous solution is separated.

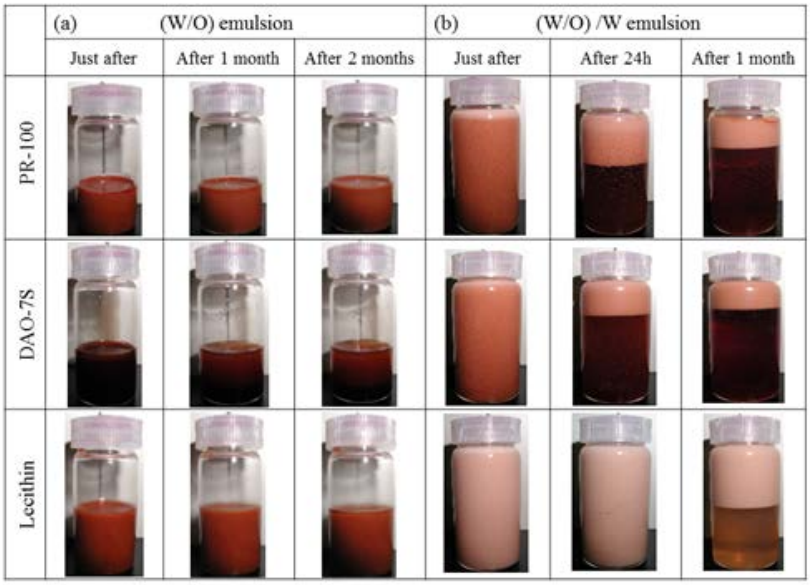

Figure 4 : Effect of oil surfactant species on stability of (W/O) emulsion and (W/O)/W emulsion

After 1 month and 2 months, the (W/O) emulsions prepared with PR-100 and SL were stable and brown color. Similarly, from Figure 4b, the following results were obtained.

Just after preparing the (W/O)/W emulsion, in the case of SL, the (W/O)/W emulsion was stable and brown white color due to keeping the (W/O)/W emulsion, but in the case of PR-100 and DAO-7S, the (W/O)/W emulsions were quasi stable and brown color.

After 24h, the (W/O)/W emulsions prepared with PR-100 and DA O-7S were broken and the GP aqueous solution was separated. However, the (W/O)/W emulsion prepared with SL was stable and brown white color. After 1 month, the (W/O) droplets was kept and floated on the Agl•Na aqueous solution, although a little GP aqueous solution dissolved in the Agl•Na aqueous solution.

From these results, it was found that the (W/O) emulsion and the (W/O)/W emulsion prepared with SL were stable. Hereafter, SL as the oil soluble surfactant was used for preparing the (W/O) emulsion and the (W/O) /W emulsion.

b) Observation of (W/O)/W emulsion and microcapsules

Figure 5 shows the optical microscopic photograph of the (W/O)/W emulsion. The GP aqueous solution droplets are dark color and observed in the α-oil droplets which are dispersing in the Alg•Na aqueous solution. The GP aqueous solution droplets were found to stably disperse in the α-oil droplet.

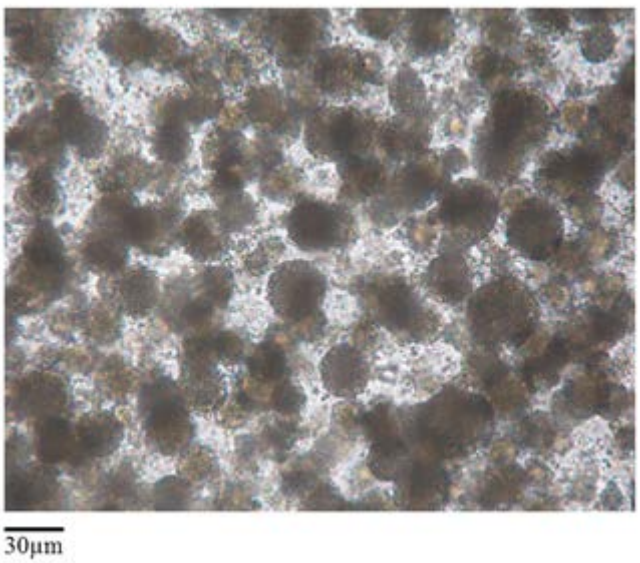

Figure 5 : Optical microscopic photographs of (W/O)/W emulsion

Figure 6 shows the optical microscopic photographs of microcapsules prepared under the same conditions as in Figure 5. From these photographs, it was found that there were spherical and irregular microcapsules and many α-oil droplets were microencapsulated well with the gelled Alg•Na shell.

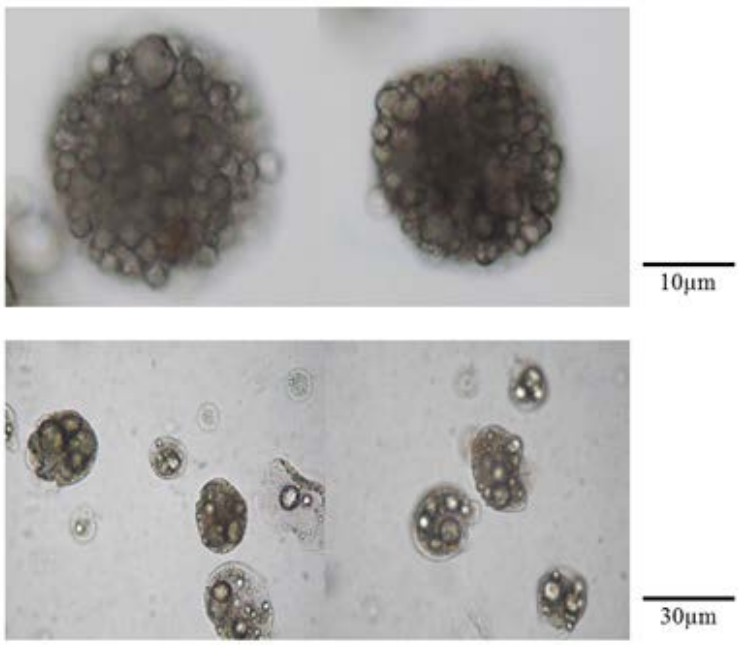

Figure 6 : Optical microscopic photographs of microcapsules

c) Effects of spraying pressure and volumetric flow velocity on mean diameters of microcapsules and microencapsulation efficiency

Figure 7 shows the dependences of the mean diameters (dp) and their dispersion degree (σ/dp) of microcapsules on the spraying pressure (P). Here, σ is the standard deviation of distribution of diameters. The mean diameters and the dispersion degrees decreased from 63 μm to 25μm and from 3.2 to 1.7 with increasing the spraying pressure, respectively and almost become constant at P=0.3[MPa]. From these results, it was found that the smaller and more uniform microcapsules could be prepared by increasing the spraying pressure. Figure 8 shows the dependence of the microencapsulation efficiency (λ) on the spraying pressure. The microencapsulation efficiency was almost kept constant (λ=30%) in spite of increasing the spraying pressure. This lower microencapsulation efficiency should be attributable to the unstable (W/O) emulsion.

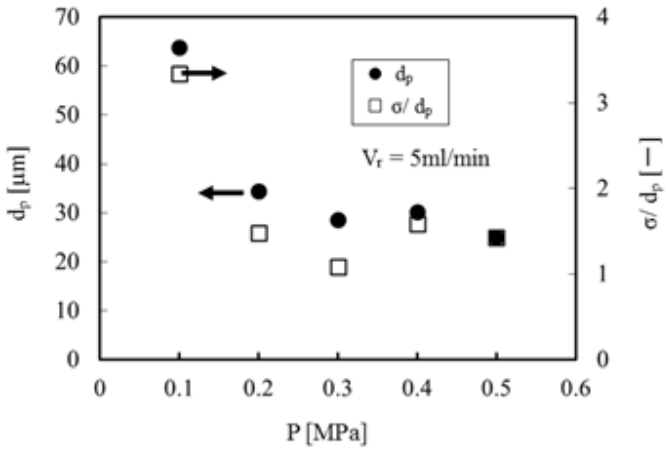

Figure 7 : Depandences of mean diameter and dispersion degree on Spraying pressure

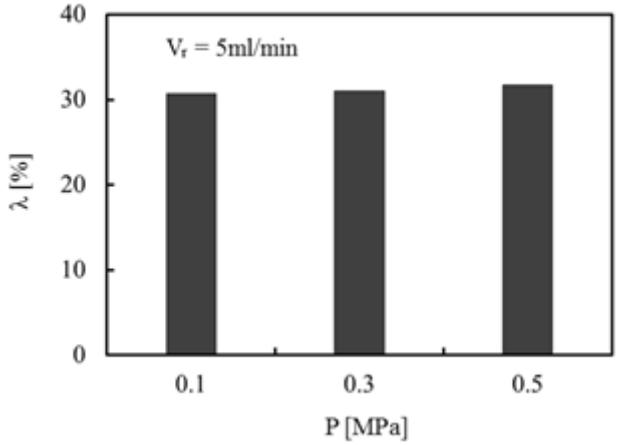

Figure 8 : Depandences of microencapsulation effciency on spraying pressure

Figure 9 shows the dependencies of the mean diameters and their dispersion degrees of microcapsules on the volumetric flow velocity (VR) of the (W/O)/W emulsion. With increasing the volumetric flow velocity of the (W/O)/W, the mean diameter increased from 15μm to 25μm and the dispersion degree decreased from 2.0 to 1.2. From these results, it was found that the larger and more uniform microcapsules could be prepared by increasing the volumetric flow velocity of the (W/O)/W emulsion.

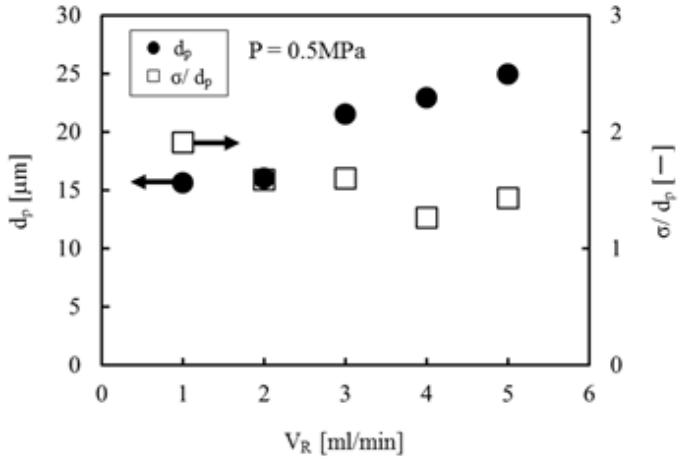

Figure 9 : Depandences of mean diameter and dispersion degree on volumtric flow velocity

Figure 10 shows the dependence of the microencapsulation efficiency on the volumetric flow velocity of the (W/O)/W emulsion. The microencapsulation efficiency slightly increased from 28.0% to 32.0 %. However, the microencapsulation efficiency is very low because of the unstable (W/O) emulsion, too.

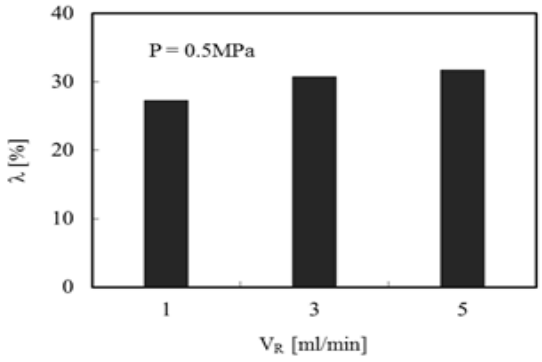

Figure 10 : Depandences of microencapsulation effciency on volumtric flow velocity

d) Effect of changing the (W/O) emulsion to the (S/O) dispersion on microencapsulation efficiency

It was confirmed that the lower microencapsulation efficiency was due to the unstability of the (W/O) emulsion. So, we tried to change the (W/O) emulsion to the (S/O) dispersion by removing the water phase from the inner water droplets as shown in Figure 3. Namely, after preparating the (W/O) emulsion, the water phase in the inner water droplets was removed by heating to form the (S/O) dispersion.

Figure 11 shows the transient water droplet diameters (dpW) with the time for removing the water phase. From this figure, it was found that the mean diameters of inner aqueous droplets were gradually decreasing with the removing time and become constant at the point of elapsing 6h and the (W/O) emulsion was changed to the (S/O) dispersion. Accordingly, the mean diameters become equal to the diameters of GP powder.

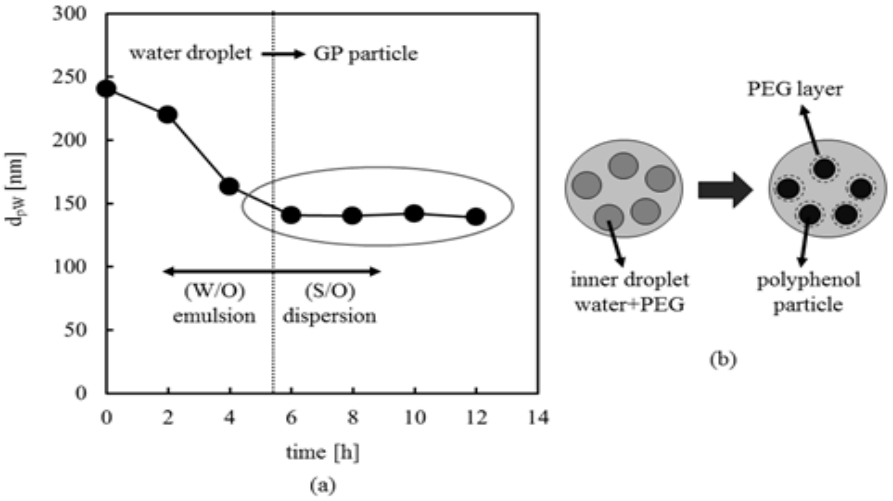

Figure 11 : Transient inner water droplet diameters

Figure 12 shows the dependence of the mean diameter of inner water droplets on the concentration (CL) of SL (Figure 12(a)) and that of the mean diameters (dpS) of GP powder particles on the mean diameter of inner water droplets (Figure 12 (b)). The mean diameters of inner water droplets decreased with the surfactant concentration and the GP powder particle diameters was in proportion to the mean diameters of inner water droplets. It is well known that the content of core materials can be increased by decreasing the diameters of core materials [12-13]. Accordingly, the stability of the (S/O) dispersion could be increased by increasing the surfactant concentration. As a result, the microencapsulation efficiency could be increased.

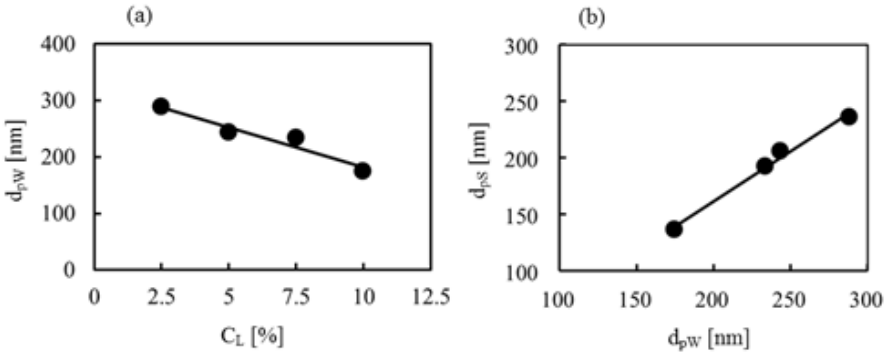

Figure 12 : Dependence of water droplet diameters on surfactant concentration and dependence of GP powder particle diameters on water droplet diameters

Figure 13 shows the dependence of the microenc-apsulation efficiency (λ) on the mean diameters (dpW) of inner water droplets and the GP powder particle diameters (dpS), respectively. From Figure 13, it was found that the microencapsulation efficiency could be increased by decreasing the diameters of inner water droplets and GP powder particles and the microencapsulation efficiencies in the case of the (S/O) dispersion was larger than those in the case of the (W/O) emulsion. Furthermore, the addition of

polyethylene glycol (PEG 600) in the inner water phase could considerably increase the microencapsulation efficiency. The effect of addition of PEG may be attributable to the fact that the adsorption layer of PEG on the surface of GP powder particles was formed as shown in Figure 11 (b) and the stability of GP powder in the α-oil could be increased due to increase in affinity between GP powder and the α-oil. In order to increase the content of core material, the formation of finer water droplets dissolving the hydrophilic core material, the formation of the (S/O) dispersion by removing water from the inner water and the formation of adsorption layer of stabilizer on the surface of powder particles were found to be considerably effective.

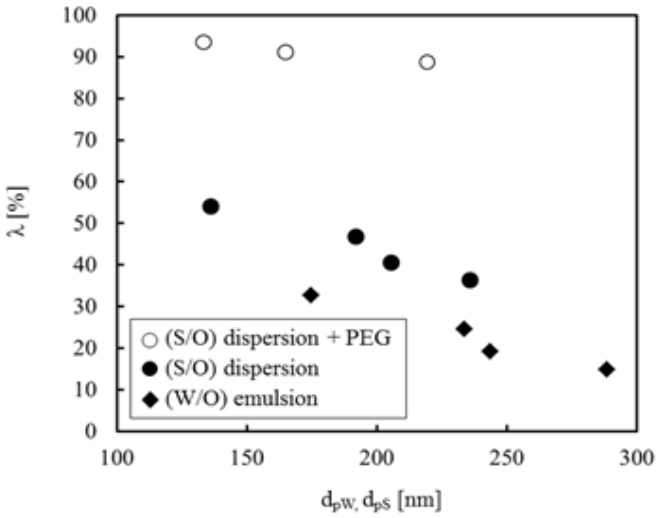

Figure 13 : Dependence of microcapsulation efficiency on inner water droplet diameter and particle diameter

IV. Conclusion

It was tried to prepare the microcapsules containing the dual cores, namely the (W/O) emulsion or the (S/O) dispersion by the spray-gelling method. The following results were obtained.

The microcapsules with the mean diameters from 15μm to 70μm could be prepared.

1. The mean diameters decreased with the spaying pressure and increased with the volumetric flow velocity.
2. The microencapsulation efficiency in the case of the (W/O) emulsion as the core was about 30% at most.
3. The microencapsulation efficiency was considerably improved by changing the (W/O) emulsion to the (S/O) dispersion and by dissolving poly ethylene glycol in the inner water phase.

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Effect of Varied Culture Conditions on Bacteriocin Production of Four Lactobacillus Species Isolated From Locally Fermented Maize (Ogi)

By Onwuakor, C.E, Nwaugo, V.O, Nnadi, C.J & Emetole, J.M.
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Abstract- *Background:* Lactic acid bacteria (LAB) predominates the micro flora of fermented products. They produce metabolites that inhibit the growth of food borne pathogens and spoilage microorganisms.

Materials and methods: Four (4) isolates of bacteriocin producing lactobacillus species (L. lactis, L. fermentum, L. casei and L. plantarum) with antibacterial activity against Salmonella typhimurium (ATCC 14028) and Shigella dysenteriae (ATCC 23351) were subjected to varied growth medium conditions. Bacteriocin production was tested at different physical and cultural conditions such as temperature (25, 30, 35 and 400C), pH (5, 6, 7 and 8), sodium chloride (NaCl) concentration (2, 4, 6 and 8%) and incubation duration (12, 24, 48 and 72 hours).

Aim: To isolate and identify LAB species from fermented maize (Ogi) and to determine the effect of varied culture conditions on bacteriocin production and antibacterial activity against indicator organisms.

Keywords: *bacteriocin, optimum, varied, culture, condition, fermented, maize, ogi.*

GJMR-C Classification : *NLMC Code: QW 50*

Strictly as per the compliance and regulations of:

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Results: The optimum bacteriocin production judged by their different zones of inhibition was recorded at temperature, 30^{0}C and then 35^{0}C. There were significant differences between all the incubation temperatures at $P<0.05$. Duration of incubation showed highest bacteriocin activity after 72 hours. Furthermore, optimal conditions for bacteriocin production were observed to be highest at pH 6.0 followed by 5.0 and then in 2% NaCl concentration. There were significant differences between the zones of inhibition of bacteriocins produced against the indicator organisms at various media pH and salt concentrations at $P<0.05$.

Conclusion: These bacteriocins may have a potential use as food preservative and may help in improving the gastro-intestinal tract by fighting off pathogenic bacteria.

Keywords: bacteriocin, optimum, varied, culture, condition, fermented, maize, ogi.

I. Introduction

Food is any substance or mixture of substances both solid and liquid, which are intended for human consumption or ingestion for their nutritional support for the body or pleasurable benefits. It usually consists of plant or animal origin, which contains essential nutrients such as carbohydrates, fats, proteins, vitamins or minerals and is ingested and assimilated by an organism to produce energy, stimulate growth and maintain life [1] [2].

The lactic acid bacteria (LAB) are a group of Gram positive bacteria, non–respiring, non–spore forming, cocci or rods, which produce lactic acid as the major end product of the fermentation of carbohydrates. They are the most important bacteria in desirable food fermentations, being responsible for the fermentation of sour dough bread, sorghum beer, all fermented milk, cassava (to produce garri and fufu) and most "pickled" (fermented) vegetables [3] [4]. Lactic acid bacteria occur naturally in several raw materials like milk, meat and flour used to produce foods. LAB is used as natural or selected starter cultures in food fermentations in which they perform acidification due to production of lactic acids. Protection of food from spoilage and pathogenic microorganisms by LAB is through producing organic acids [5]. The LAB produces an array of antimicrobial substances (such as organic acids, diacetyl, acetone, hydrogen peroxide, reuterin, anti-fungal peptides and bacteriocins [6] [7] [8]. Bacteriocins are ribosomally synthesized antimicrobial peptides or proteins [9].

Ogi (Akamu) is a product of fermented maize (Zea mays) widely eaten in Africa [10] [11]. Similar maize preparations in Ghana are referred to as "Akana" or "Kenkey". Ogi is often marketed as a wet cake formerly wrapped in leaves but presently in transparent polythene bags. Gelatinized Ogi (a porridge) called "pap" is mainly used as a breakfast meal for adults and weaning food by low income earners who cannot afford the more expensive imported weaning foods [12]. In most parts of Africa especially in Nigeria, children are fed with mashed adult foods. These foods are bulky and this therefore reduces food intake by a child, often resulting in malnutrition. The development of nutritionally balanced calorie less dense, low bulk and easily digestible weaning food becomes necessary. This involves the use of simple but time consuming traditional technology called fermentation [13]. The

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traditional fermentation method employed in Ogi production is a wild process and microorganisms are not controlled [14]. Microbiological analyses have shown the presence of several genera of bacteria, moulds and yeasts in the fermented maize product-Ogi [15] [16].

In the present study, different culture conditions were adjusted for bacteriocin production using *Lactobacillus* isolates from locally fermented maize (Ogi) to determine optimal fermentation conditions for bacteriocin production.

II. Material and Methods

a) Fermented Products

Ten (10) fermented maize (Ogi) samples bought from Oshodi and Odo markets, Lagos State, Nigeria were analyzed.

b) Test Organisms

Pure strains of pathogenic gram negative bacteria responsible for food infections; *Salmonella typhimurium* (ATCC 14028) and *Shigella dysenteriae* (ATCC 23351) were obtained from the Nigerian Institute of Medical Research laboratory (NIMR) Yaba, Lagos, Nigeria and maintained on agar slants at 4^0C in the refrigerator.

c) Isolation of lactic acid bacteria from Ogi

One (1) gram of a 72hrs fermented Ogi was transferred into 5ml peptone water (Merck, Germany) and serially diluted (10 fold dilutions). Then 1ml of each of the dilution was aseptically transferred into sterile Petri dishes (Pyrex and Anumbra) and 15ml of de Man Rogosa Sharpe (MRS) medium (Merck, Germany) was added using pour plate technique then incubated at 370C for 48hrs in an anaerobic flask (Oxoid). After incubation, colonies with different morphologies were randomly selected using a flamed platinum wire loop, streak plated and sub – cultured on MRS agar plates to obtain pure colonies. All isolates were examined for Gram reaction, production of catalase and oxidase activity.

d) Identification of LAB Isolates

Isolates were identified using the following tests: ammonia production from arginine, CO2 production from glucose and growth at different pH values, growth at different NaCl concentrations and carbohydrate fermentation. LAB isolates were tested for charact-eristics of Gram staining, cell morphology, colony morphology, motility, carbon dioxide production from glucose, growth at 100C and 450C, growth at pH of 4.4 and 9.6, growth in 6.5% and 18% NaCl, catalase reaction by 3% hydrogen peroxide and carbohydrate fermentation [17].

e) Detection of inhibitory activity of crude bacteriocin from selected isolates

Selected LAB isolates were grown in MRS broth at 37°C for 24 hrs. Cell free supernatant of each isolate was obtained by centrifugation at 3,000xg at 4°C for 20 min. The supernatant was adjusted to pH 6.5 with 1M NaOH and subsequently filter sterilized through a 0.2μm membrane filter (Whatman, Germany). Inhibitory activity was determined using agar well diffusion assay [18]. Inhibitory effect of the hydrogen peroxide in the supernatant was eliminated by reacting with 5mg/ml catalase added. Suitable agar medium containing 1% agar (45°C) was inoculated with each of the two indicator strains. Agar wells of 5 mm diameter were cut and the filter-sterilized supernatant (20μl) was added into each well. The plates were incubated at 37°C for 24hrs. The inhibition zones around the wells were measured.

f) Optimization of Culture Conditions

The selected lactic acid strains were subjected to different culture conditions to derive optimum conditions for bacteriocin production

g) Effect of varying culture conditions on bacteriocin activity

To study the effect of varying culture conditions, growth and bacteriocin production was estimated at varied temperatures (25, 30, 35, and 400C) pH (5.0, 6.0, 7.0 and 8.0), sodium chloride (NaCl) concentrations (2.0, 4.0, 6.0 and 8.0% w/v) and duration of incubation (12, 24, 48, and 72hrs) in MRS broth. All samples were collected after 48hrs, except for those measuring incubation time effects before inhibitory activity was determined by agar well diffusion assay as described above.

h) Data presentation and statistical analysis

Data were represented as means ± standard error of mean as well as bar charts. Two – way analysis of variance and Bonferroni's multiple comparison tests using GraphPad Prism (version 6.0) software were used to analyze data. Values were considered significant when $P<0.05$.

III. Results

a) Isolation of lactic acid bacteria

In this study, Lactobacillus strains producing antimicrobial compounds were isolated from fermented maize (Ogi). Out of a total of seven (7) isolates that met basic characteristics of Lactobacilli, only four (4) showed antibacterial activity against both indicator organisms. They include *L. lactis, L. fermentum, L. casei and L. plantarum*.

b) Optimization of Culture Conditions

The broth medium containing each isolate was incubated at various temperatures; 25.0, 30.0, 35.0 and

40.0^{0}C and the bacteriocin harvested were tested against *Salmonella typhimurium* and *Shigella dysenteriae* (Figure 1 and 2 respectively).

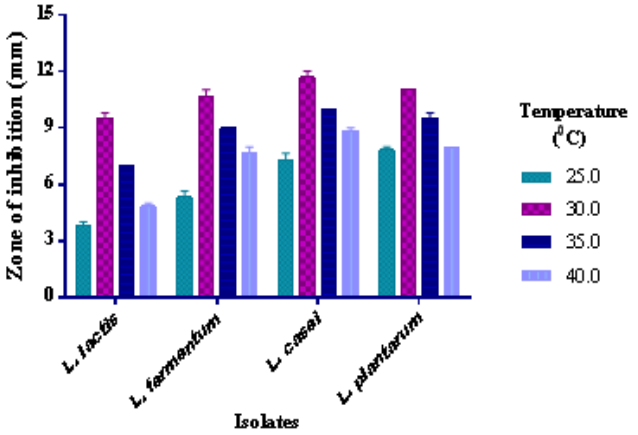

Fig. 1 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied temperatures (^{0}C) against *Salmonella typhimurium*

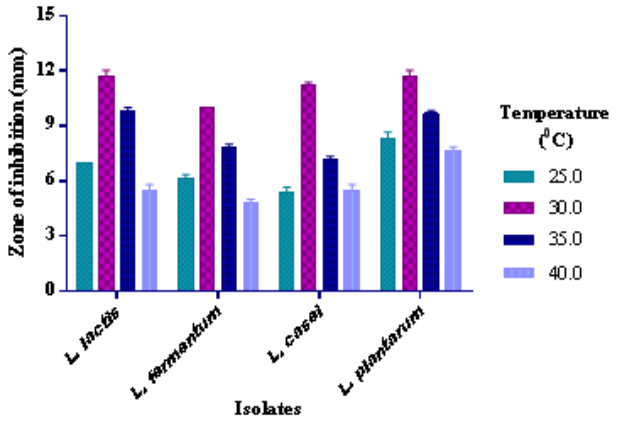

Fig. 2 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied temperatures (^{0}C) against *Shigella dysenteriae*

The effect of varied incubating duration (Hrs) on bacteriocin production was noted as different zones of inhibition were observed among the various isolates against the indicator organisms (Figures 3 and 4).

The effect of varied medium pH and Sodium chloride concentration on bacteriocin production was affected as different zones of inhibition were observed among the various isolates against the indicator organisms (Figures 5 and 6; Figures 7 and 8) respectively

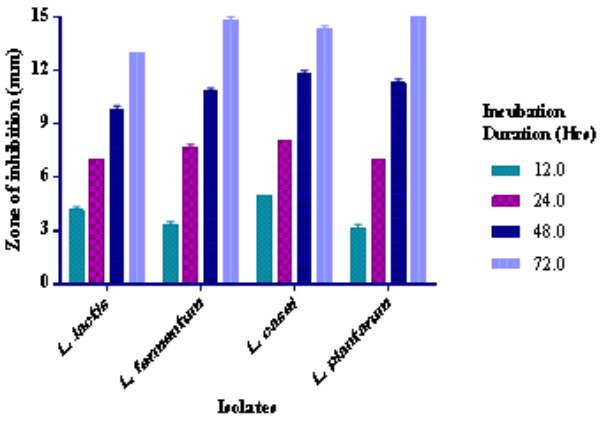

Fig. 3 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied incubation durations (Hrs) against *Salmonella typhimurium*

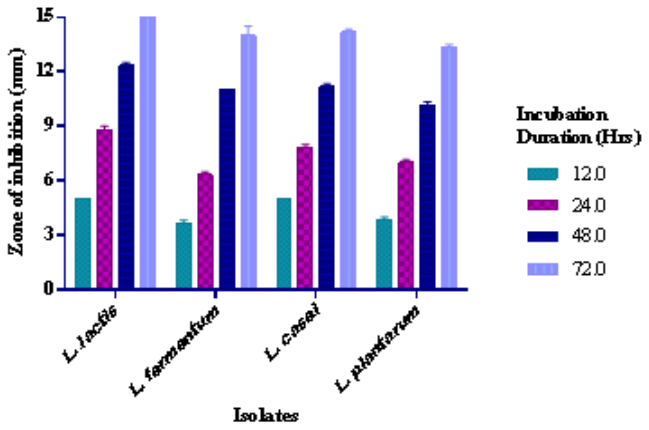

Fig. 4 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied incubation durations (Hrs) against *Shigella dysenteriae*

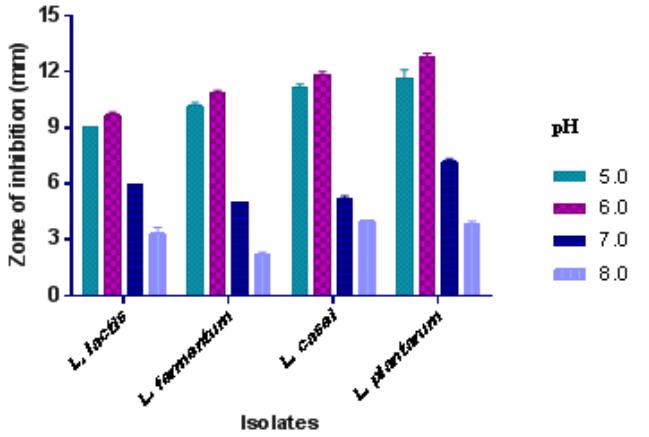

Fig. 5 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied media pH against *Salmonella typhimurium*

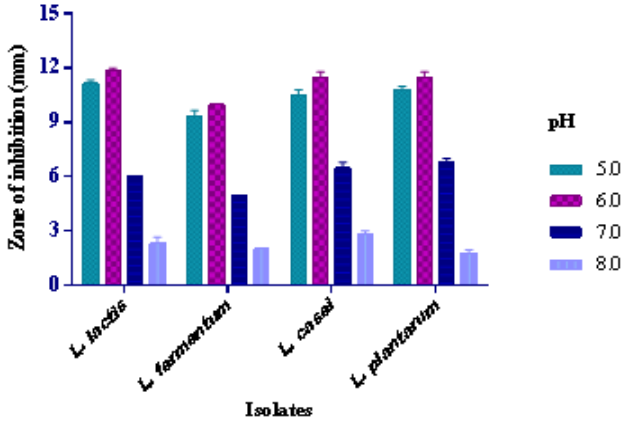

Fig. 6 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied media pH against *Shigella dysenteriae*

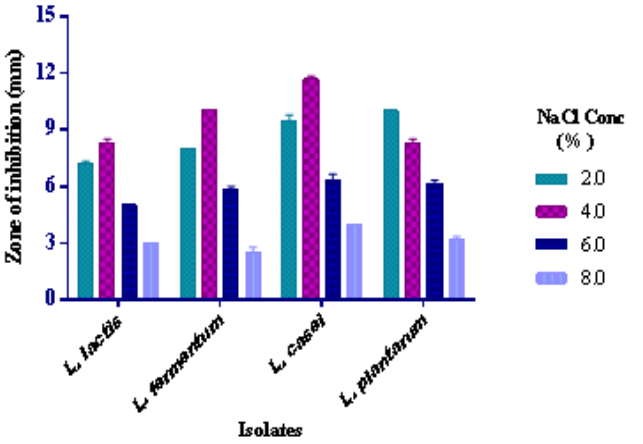

Fig. 7 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied media sodium chloride concentration against *Salmonella typhimurium*

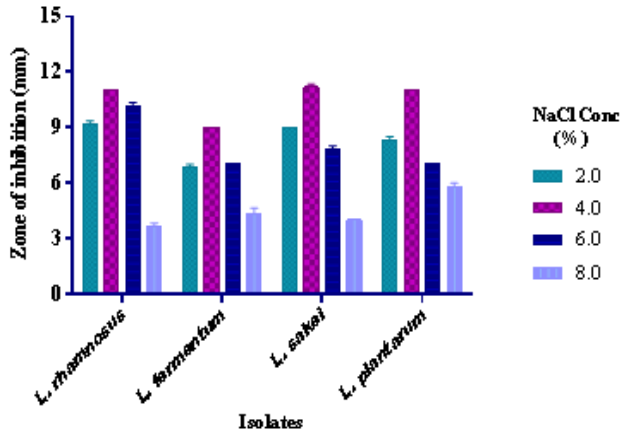

Fig. 8 : Zone of inhibition (mm) of crude bacteriocin from different *Lactobacillus* isolates cultured at varied media sodium chloride concentration against *Shigella dysenteriae*

IV. Discussion

These identified *Lactobacillus* species were in agreement with those earlier identified from similar fermented food products by [19]. The isolates were then tested for antibacterial activity against the indicator organisms (*Salmonella typhimurium* and *Shigella dysenteriae*). The bacteriocin activity of the isolates that showed antibacterial activities were further tested after the culture conditions were varied to determine the effect of different cultural conditions on the antibacterial action of bacteriocins produced. Parameters such as incubation duration and temperature, media pH and salt concentrations were varied.

The effect of varied incubating temperatures on bacteriocin production by the isolates in de Man Rogosa Sharpe broth was determined by its antibacterial activity against the indicator strains. The result showed that antibacterial activity was highest at 30.0^{0}C followed by 35.0^{0}C as seen in figures 1 and 2. There were significant differences between the different zones of inhibition produced for all the incubating temperatures (at $P<0.05$). This was in agreement with the work of [20] and [21], which showed that bacteriocin production was affected by different incubating temperatures. The maximum bacteriocin activity recorded at 30^{0}C suggests that ambient growth temperature is most ideal for bacteriocin production by *Lactobacillus* species.

The effect of varied incubating duration (Hrs) on bacteriocin production was affected as different zones of inhibition were observed among the various isolates against the indicator organisms (Tables 3 and 4). Optimum bacteriocin production was observed after 72 hours judged by the zones of inhibition against the indicators. There were observable reduction bacteriocin activities as incubation time dropped. There were significant differences between the various incubation times (at $P<0.05$). This result was in complete agreement with [22], which showed that incubation time affects bacteriocin production.

The results obtained in this study regarding bacteriocin activity from media incubated at varied pH values (Figures 5 and 6) showed optimum activity at pH 6 followed closely by pH 5 for both indicator organisms. There were clear significant differences between pH 7 and 8 but not in pH 5 and 6 as both showed similar zones of inhibition for all the isolates against indicator organisms. This result was consistent with the reports of [23], which showed influence of pH on growth of vaginal Lactobacilli. This pH tolerance is an extremely important feature since the isolates have the ability to survive, grow and produce bacteriocins under acidic and alkaline conditions.

Every microorganism has a minimal, a maximal and an optimal pH for growth and metabolism. Microbial cells are significantly affected by the pH of their

immediate environment because they apparently have no mechanism for adjusting their internal pH.

Furthermore, the effect of varied medium percentage sodium chloride (NaCl) concentration on bacteriocin production and activity was also evaluated. Highest zones of inhibition and consequent optimum bacteriocin production was observed at NaCl concentration of 4%, but started reducing as salt concentration increased further (Figures 7 and 8). There was significant difference between zones of inhibition obtained at all the salt concentrations. This was in agreement with [22] who studied cultural parameter for bacteriocin production.

Besides the strong acid medium in the stomach, the probiotic microorganisms taken orally have to defend against the bile salt in the gastrointestinal tract [24]. Hence, bile tolerance is considered to be one of the important properties required for high survival and as a consequence of probiotic activity. The decrease in bacteriocin production as salt concentration increased could be attributed to stress on the isolates.

Microbial food safety is an increasing public health concern worldwide [25] [26] and many gram negative bacteria like *Escherichia coli, Salmonella* serovars, *Campylobacter* species, *Shigella* species etc, have been implicated in food borne diseases [19]. Alternate methods for controlling pathogenic bacteria by the production of antimicrobial peptides called bacteriocins are now highly considered. Bacteriocins from lactic acid bacteria have attracted much attention and have been the subject of intensive investigation due to their ability to act as a bio-preservative agent, which led to their incorporation into foods, particularly in the dairy foods and also in human therapeutics [27] [28] [29] [30].

V. Conclusion

This study thus suggests the selection of bacteriocin – producing *Lactobacillus* strains as starter cultures for controlled fermentations at optimum cultural conditions. Bacteriocins from *lactobacillus* species harnessed under different culture conditions have shown different antimicrobial potencies. These findings could be applied in the food and pharmaceutical industries to further enhance maximum bacteriocin production at optimal levels to replace conventional antibiotics in combating pathogens that are vastly acquiring antimicrobial resistance.

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Pap Smear and Histopathological Study of Cervical Lesions

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Abstract- *Background:* Conventional cervical cytology is the most widely used cervical cancer screening test in the world. squamous intraepithelial lesion (SIL) and cervical cancer remain important health problems for women worldwide.

Aim and Objective: To study various types of cervical lesions with relevant factors such as age, parity, to classify cervical lesions into malignant & benign groups and to correlate the cytological with histopathological findings.

Materials and Methods: This study was conducted on 200 cases of Pap smears and cervical biopsies, along with resected specimens. After fixation and staining, smears and cervical biopsies were processed and examined under microscope.

Results: Age wise maximum number of patients were in fourth decade (54.50%), followed by fifth decade. On cytology, 59% were inflammatory smears and frank malignancy was reported in 10% cases. LSIL and HSIL were reported in 9% and 8.50% respectively.

Keywords: *malignant, cervical cancer, pap smear, cervical biopsy.*

GJMR-C Classification : *NLMC Code: WP 475*

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Pap Smear and Histopathological Study of Cervical Lesions

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Results: Age wise maximum number of patients were in fourth decade (54.50%), followed by fifth decade. On cytology, 59% were inflammatory smears and frank malignancy was reported in 10% cases. LSIL and HSIL were reported in 9% and 8.50% respectively. Maximum number of cases on biopsy were those of infections (57.50%), 27% cases were those of frank malignancy; most common being invasive squamous cell carcinoma (23%) and adenocarcinoma in 2%. Mean age among cancer cases was high (51.94±12.30 years) compared to those who did not have cervical cancer (39.53±9.66 years). Cervical cancer was seen in 39.65% of patients having ≥3 children. 10% cases diagnosed on cytology turned out to be malignant on biopsy.

Conclusion: Pap smear followed by cervical biopsy is an effective method for detection of pre-cancerous, cancerous and non-cancerous changes in the cervix.

Keywords: malignant, cervical cancer, pap smear, cervical biopsy.

I. Introduction

Papanicolaou (Pap) smear is a simple, safe, non-invasive and effective method for detection of pre-cancerous, cancerous and non-cancerous changes in the cervix.[1] Conventional cervical cytology is the most widely used cervical cancer screening test in the world and cytology screening programmes in several developed countries have been associated with impressive reduction in cervical cancer burden.[2] Squamous intraepithelial lesions are viewed as precancerous lesions exhibiting many of the morphological characteristics of invasive carcinomas. Identification of these entities is the focus of cervical screening programs that aim to discover them and commence their treatment in order to prevent invasive disease.[3] Though data from the 20 populations based cancer registries in India indicate a steady decline in cervical cancer incidence rates over the last two decades, it still occupies second position and the risk of disease is still high.[3] Cervical carcinoma documents the remarkable effects of screening, early diagnosis, and curative therapy on the mortality rate. Death rate has declined for which the credit goes to Pap test and accessibility of cervix to colposcopy and biopsy. Though, the Pap smear is an effective screening test, yet confirmation of the diagnosis of cervical cancer or pre invasive lesions of cancer requires a biopsy of the cervix.

II. Aims and Objectives

The aims of this study were to study the changes in cervical cytology with relation to age, parity and other presenting features, to classify cervical lesions into malignant and benign groups on cytological and histopathological basis and to correlate the changes observed in cervical cytology with cervical biopsy.

III. Materials and Methods

This study was done on 200 cases of Pap smears and cervical biopsies (including hysterectomy specimens). Most of the patients with symptoms suggestive of cervical disease were selected. However, some having gynaecological symptoms other than cervical disease were also included. Few cases reporting for routine screening were also included. A detailed clinical history especially age, duration of symptoms, parity, menstrual pattern and vaginal discharge were noted. The patients in whom both Pap smear and biopsy was available, were included in the study. The fixed cervical smears were subjected to staining according to Papanicolaou's method. The cytological interpretation of the smears was made according to the New 2001 Bethesda system. After grossing and processing, cervical biopsies were subjected to histopathological examination.

IV. Results

Age wise maximum number of patients were in fourth decade (54.50%), followed by fifth decade (Table-1). Duration of symptoms varied from few months to

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many years. Some patients presented within 1 year (79%), but few mainly cases with discharge and history of prolapse presented late (Table-2). In 200 cases, various symptoms were seen, some patients showed multiple symptoms. Majority of patients (58%) presented with vaginal discharge followed by irregular bleeding (47%). Menstrual changes were also seen in large number of patients. There was seen low usage of oral contraceptive pills in our study group (10.50%). Duration of OCP usage varied from few months to years, but long term usage was not seen in any case. On cytology, 59% were inflammatory smears and frank malignancy was reported in 10% cases, LSIL and HSIL was reported in 9% and 8.50% respectively (Table-3). Maximum number of cases on biopsy were those of infections (57.50%), among them majority had non-specific chronic cervicitis. Squamous intraepithelial lesions were seen in 25 patients. Mild dysplasias correspond to low grade squamous intraepithelial lesions, moderate and severe to high grade intraepithelial lesions. 54 cases (27%) were those of frank malignancy on biopsy (Table-4); most common diagnosis being invasive squamous cell carcinoma (23%) and adenocarcinoma in 4 cases (2%). Distribution of age was correlated with cancer cases. Most of the cancer cases were seen in the age group of 31- 45 years. The mean age among cancer cases was high (51.94±12.30 years) and (39.53±9.66 years) in cases who did not have cervical cancer (Table-6). Cervical cancer was seen in 39.65% of patients with ≥3 children. History of oral contraceptive use was present in 21(10.50%) women. Of which 14.29% had cervical cancer and 85.71 % did not have cervical cancer, showing poor correlation between oral contraceptive use and cervical cancer (p= 0.165). 20 cases diagnosed on cytology turned out to be malignant on biopsy showing strong correlation between cytology and histopathology (p<0.001). Some of the cases were obscured by blood and inflammation which were missed on cytology but proved to be malignant on biopsy.

Table 1 : Age Distribution of Cervical Lesions

Age group (Years)	Distribution (n=200)	
	No.	%age
18-30	29	14.50
31-45	109	54.50
46-60	41	20.50
> 60	21	10.50
Total	**200**	**100**

Table 2 : Duration of Symptoms

Duration (Years)	Distribution (n=200)	
	No.	%age
Upto 1	158	79.00
1-3	25	12.50
4-6	11	05.50
>6	06	03.00
Total	**200**	**100**

Table 3 : Cytological Diagnosis

Diagnosis	Distribution (n=200)	
	No.	%age
Unsatisfactory smear	08	4.00
Inflammatory	118	59.00
ASCUS/H	19	9.50
LSIL	18	9.00
HSIL	17	8.50
Frank malignancy	20	10.00
Total	**200**	**100**

Table 4 : Histopathological Diagnosis

Diagnosis	Distribution (n=200)	
	No.	%age
Infections	115	57.50
Carcinoma	54	27.00
Dysplasia	25	12.50
Benign tumors	06	03.00
Total	**200**	**100**

Table 5 : Correlation of Cytological Diagnosis and Histopathological Diagnosis

Histopathological Diagnosis	No.	Cytological Diagnosis					
		Unsatisfactory	Inflammatory	ASCUS/H	LSIL	HSIL	Ca
Infections	115	-	108	07	-	-	-
Carcinoma	54	08	-	-	12	14	20
Dysplasia	25	-	04	12	06	03	-
Benign tumors	06	-	06	-	-	-	-
Total	**200**	**08**	**118**	**19**	**18**	**17**	**20**

Table 6 : Showing Mean Age

Variable	Cervical Ca (n=54)		No Ca (n=146)	
	Mean	SD	Mean	SD
Mean	51.94	12.30	39.53	09.66
T	7.469			
Df	198			
P	< 0.001			
Significance	Highly Significant			

V. Discussion

Cancer cervix is considered to be an ideal gynaecological malignancy for screening as it meets both test and disease criteria for screening. It has a long latent phase during which it can be detected as identifiable and treatable premalignant lesions which precede the invasive disease and the benefit of conducting screening for carcinoma cervix exceeds the cost involved. [4]

Despite the success of cervical cancer screening programs, questions remain about the appropriate time to begin and end screening. This review explores epidemiologic and contextual data on cervical cancer screening to inform decisions about when screening should begin and end. The incidence and mortality rates from, cervical cancer that have had a Pap smear within 3 years have decreased since 2000.

In this study, more than half (54.50%) were aged between 31 to 45 years followed by 20.50% between 46 to 60 years. The mean age of patients with cancer in the present study was 51.94 years. This is close to that found by Biswas et al[5] and Missaoui et al.[6] Although, invasive cancer cervix is reported at all ages; it has two peaks, one at about 35 years and another above 50 years. The highest age of cervical cancer in the present study was 73 years and the lowest was 26 years. The mean age for non-cancer cases was 39.53 years. In this study, the most common symptoms was (58%) followed by irregular bleeding in 47% of the patients. Patients with cancer also presented with post-coital bleeding and in cases of older age group post menopausal bleeding was seen. Symptomatic presentation was similar to some extent as seen by Ikram et al [7].

In this study, 59% patients had the cytological diagnosis of benign/ inflammatory and carcinoma was present in 10% of the cases. This is comparable to Saha and Thapa[8] in which benign cases were 51.16% and carcinoma was diagnosed in 6.97% of the cases. Most common cancer in the present study was squamous cell carcinoma (85.18%). This study showed results similar to those seen by Ikram et al[7] (83.33%).

As regards the various histopathological varieties of SCC, the present study found an incidence of 67.39% for moderately differentiated SCC, 23.91% for well differentiated, 8.70% for poorly differentiated. Thus, the findings of the present study are consistent with that of Missaoui et al [6] in that moderately differentiated large cell non-keratinizing variety is the commonest variety.

VI. Conclusions

It is concluded that most commonly seen problem, infection, can be controlled with good hygiene. Cervical carcinoma is seen in large number of patients. Pap is a relatively less invasive and a simple procedure to diagnose cervical lesions in developing countries. But sometimes, there can be obscuring of the cellular details by blood, especially in malignant cases. In such cases, biopsy is helpful and confirmatory.

VII. Acknowledgments

We thank the patients and technical staff of Pathology Department GMC Patiala for processing the Pap smears and cervical biopsies.

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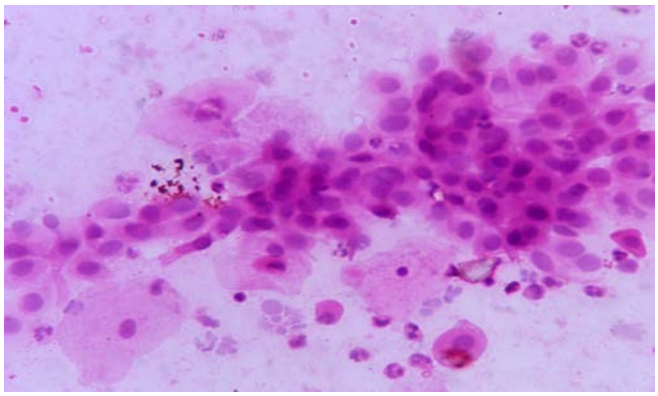

Figure 1 : Photomicrograph of **HSIL** showing group of hyperchromatic parabasal cells exhibiting nucleomegaly and overlapping nuclei (PAP X 400)

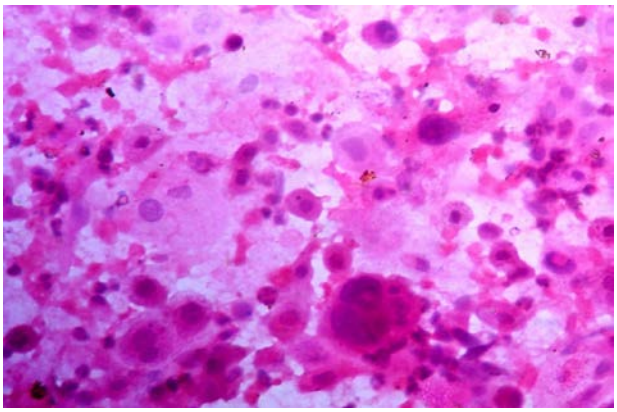

Figure 2 : Photomicrograph of **Squamous cell carcinoma** showing tumour diathesis, malignant cells with nucleomegaly, hyperchromatism and irregular nuclear margins (PAP X 400)

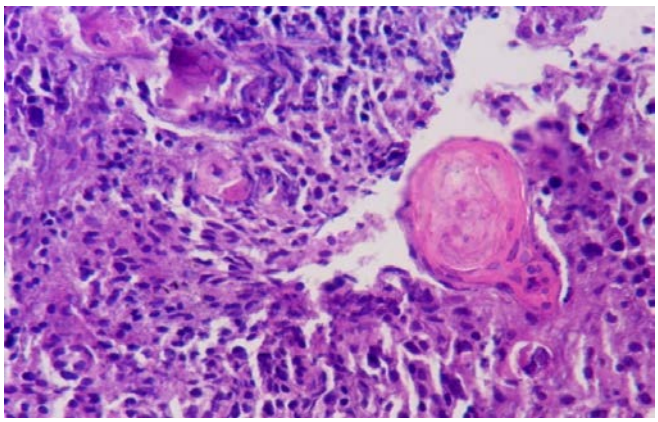

Figure 3 : Photomicrograph of **Well differentiated SCC** (H & E X 400)

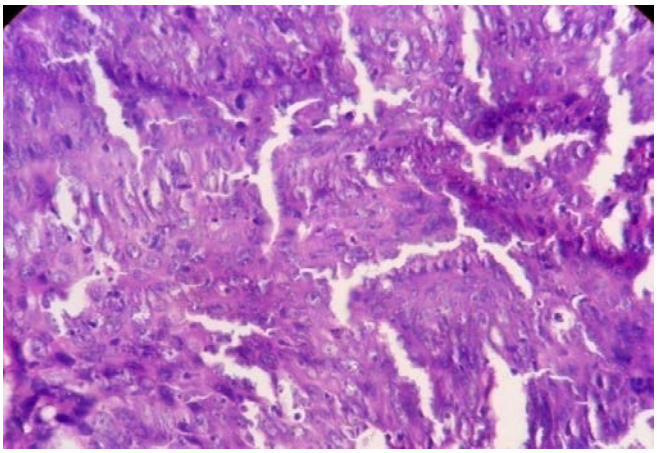

Figure 4 : Photomicrograph of **moderately differentiated SCC** (H & E X 400)

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Phytochemical Analysis and Antibacterial Activities of Citrullus Lanatus Seed against some Pathogenic Micro-organisms

By Nwankwo, I.U, Onwuakor, C.E, & Nwosu V.C
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Abstract- *Aim:* To evaluate the phytochemical components and antibacterial potentials of *Citrullus lanatus.*

Materials and Methods: This was carried out by the crude extraction of the seeds with hot water, ethanol and methanol. The extracts were used to determine the presence of phytochemicals. Stock cultures of test organism such as *Staphylococcus aereus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, Proteus mirabilis* and *Streptococcus pyogenes* were used to test the antibacterial effects of the extracts using the agar well diffusion method.

Results: The extracts showed presence of antibacterial activities which were compared to antibacterial activity of a commercial antibiotic (Ciprofloxacin) against the test organisms.

Keywords: *citrus lanatus seed, phytochemical analysis, antibacterial activity, pathogenic microorganisms.*

GJMR-C Classification : *NLMC Code: QW 190, QZ 65, QV 350*

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Nwankwo, I.U [α], Onwuakor, C.E [σ], & Nwosu V.C [ρ]

Abstract- Aim: To evaluate the phytochemical components and antibacterial potentials of *Citrullus lanatus*.

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Results: The extracts showed presence of antibacterial activities which were compared to antibacterial activity of a commercial antibiotic (Ciprofloxacin) against the test organisms. At 62.5mg, ethanol extract showed a weak inhibitory effect against *Proteus mirabilis* (3mm), *Staphylococcus aureus* (2mm) and *Streptococcus pyogenes* (2mm). Antibacterial activity of the extract was pronounced at higher concentrations (100, 500 and 250mg) for all the extracts. Hot and cold water extracts showed the presence of phenol and methanol extracts exhibited the highest bacterial activity. The phytochemical analysis showed the presence of phenol, saponin, tannin, flavonoid, alkaloid and cyanogenic glycoside.

Conclusion: From this research, watermelon seeds when properly extracted and purified, acts as antibiotics which can be used in treatment of infections caused by pathogenic bacteria.

Keywords: citrus lanatus seed, phytochemical analysis, antibacterial activity, pathogenic microorganisms.

I. Introduction

Citrullus lanatus (water melon) is the fruit of a plant originally from a vine of Southern Africa. It produces about 93% water; hence name "water" melon [1]. C. *lanatus* is a prostrate animal plant with several herbaceous, firm and stout stems. The leaves are herbaceous but rigid, becoming rough on both sides. The leaf stalks are somewhat having and up to 150 mm long. The tendrils are rather robust and usually divided in the upper part. They are monoecious with the flower stalk up to 4mm long and to 20mm in diameter; the fruit still is up to 50mm long [2].

C. *lanatus* seeds are increasingly used for their oil in semi-arid regions and also the use of the oil in the cosmetic and pharmaceutical industry is increasing. There are also prospects for use of the seeds in the improvement of infant nutrition in review of their high protein and fat content [3]. In Chinese traditional medicine, watermelon rind is extensively applied to clear away heat to eliminate toxic substances and its extracts are available in powdered form [4]. In Nigeria, watermelon rind is fermented, blended and consumed as juice. High antioxidant activities have been reported on food products in microbial fermentation [5].

One generous slice of watermelon (about 1/16th of a melon) contains large amounts of vitamin C and Beta-carotene which may help against various forms of cancer due to their antioxidant properties. Watermelon is also high in potassium which helps regulate heart function and normalize blood pressure. It is a good source of fiber also which helps maintain bowed regularity and works to prevent colon and renal cancer [5]. Emulsion obtained from the seed water extract of watermelon is used to cure catarrhal infections, disorders of the bowel, urinary passage and fever [6]. The plant contains large amount of beta-carotene and it is a natural source of lycopene. It is also rich in citrulline, an effective precursor of L-arginine [6]. Phenolic compounds are constituents of both edible and non-edible parts of the plant. The seeds are sources of protein, tannins and minerals [7].

The antimicrobial compounds found in pants are of interest because antibiotic resistance is becoming a worldwide public health concern in terms of food borne illness and nosocomial infections [8]. The plane kingdom has proven to be the most useful in the world's pharmaceuticals [9]. The most important of these bioactive constituents of plants includes phenol, tannin, saponin, alkaloid, flavonoid, steroids, carotenoids, and cyanogenic glycosides [10]. These phytochemicals constitute the antibiotic principals of plants [9]. They are found to be distributed in plants [11]. Leaves, roots, flowers, whole plants, seeds and stems have being examined in many research projects, few reports refers to seeds as sources for pharmaceutical [12]. Chemical

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compounds including alkaloids, lectins and phenolic compounds such as lactones, tannins and flavonoids are present in seeds and seed coat [12], and they probably function in the protection of seeds from microbial degradation until conditions are favorable for germination [13] [10].

Many studies suggest that endogenous antioxidant or exogenous antioxidants supplied by diet can function as free radical scavengers and improve human health [14] [15] [16]. Thus consumption of a variety of plant foods including watermelon seeds may provide additional health benefits [17]. Amongst all the amino acids which the body requires, there are some known as essential amino acids which the body cannot produce *C. lanatus* seeds supply some of these acids including tryptophan and glutamic acids.

Effective health cannot be achieved in Africa, unless orthodox medicine is complemented with traditional medicine. At least, 80% Africans depend on plant medicine for their healthcare [18]. Fruits and vegetables have been recognized as natural sources of various bioactive compounds [19] which could be attributed to their phyto-constituent such as flavonoids, fiber and phenolic compounds.

One of such medicinal plant is *Citrullus lanatus.* Although several of its uses in traditional medicine have been documented, many of these claims are yet to be validated by scientific researchers. Therefore a review of some investigated phytochemical components and therapeutic activities of the plant are highlighted in this present study.

II. Materials and Methods

a) Collection and Preparation of the Seeds of C. lanatus

C. lanatus was bought from Ariaria International Market Aba, Abia State. They were stored in a conductive atmosphere prior to analysis. The seeds were washed and dried in a SMO5E SHEL LAB oven at 300C for 3 days to avoid contamination. The seeds were then grinded with a warring blender and subjected to various extraction techniques.

b) Extraction of C. lanatus seed

The extraction of *C. lanatus* seed were carried out with hot water, cold water, ethanol and methanol leading to the formation of hot water, cold water, ethanol and methanol extracts respectively. About 50g of *C. lanatus* seed were added with 4 conical flasks of 25ml each (with filter paper imbedded) then 60ml of hot water, cold water, ethanol and methanol were added respectively and allowed to settle for some time. The filtrate of the extracts was obtained by separation of the suspension in the filter paper. Ethanolic and methanolic extracts were allowed to evaporate and stored in an airtight conical flask. The hot and cold water extracts were then neatly separated and also stored.

c) Phytochemical Analysis

The phytochemical analysis was performed using universal laboratory techniques for qualitative determination [20] [21]. The phytochemical analyzed includes phenols, saponin, flavonoid, alkaloids, tannin and cyanogenic glycoside.

i. *Phenol Analysis*

2g of the sample was emerged in 20ml of methanol, extracted by filtration through filter paper. 1ml of the filtrate was testes by adding 1ml of Folin-concalteon plus 1ml of 20% NaCO3, the presence of dark blue color shows the presence of phenol.

ii. *Saponin Analysis*

About 20ml of water was added to 10.25g of the specimen in 100ml beaker and boiled gently on a hot water bath for 2 minutes. The mixture was filtered hot and allowed to cool and the filtrate used for frothing test.
Frothing Test

About 5ml of the filtrate was diluted with 20ml of water and shaken vigorously. A stable froth (foam) upon standing indicates the presence of saponins.

iii. *Flavonoid Analysis*

10ml of ethylacetate was added to about 10g of the sample and heated in a water bath for 3 minutes. The mixture was cooled, filtered and the filtrate used for ammonium test.

Ammonium Test

About 5ml of filtrate was shaken with1ml of solute ammonia solution. The layers were allowed to separate and the yellow colour in the ammonical layer indicates the presences of flavonoids.

iv. *Tannin Analysis*

About 5g of the specimen was boiled with 40ml of water, filtered and used for the ferric chloride test.

Ferric Chloride Test: About 3ml of the filtrate was added to few drops of ferric chloride solution. A greenish black precipitate indicates the presence of tannin.

v. *Cyanogenic Glycoside Analysis*

Fehling's Test: About 5ml of mixture of equal parts of Fehling's solution I and II were added to about 3ml of the filtrate and boiled for 5minutes. A more dense brick red precipitate indicates the presence of glycoside.

vi. *Alkaloid Analysis*

Meyer s ' test: Meyer's reagent (mixture of mercuric chloride and potassium iodide dissolved in water) was added to a 5ml of the specimen's filtrate, a greenish white precipitate was formed indicating the presence of alkaloids

d) Test Organisms and their Screening for Viability

Stock cultures of the test organisms were collected from the Microbiology Unit of Abia State University Teaching Hospital, Aba. The test organisms are *Staphylococcus aureus, Klebsiella pneumoniae,*

Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus, Proteus mirabilis and *Streptococcus pyogenes*. The isolates were screened to confirm their identities. They were sub-cultured on nutrient agar and stored on slant before use [22].

e) Sensitivity Test

The antibacterial activity of the four (4) extracts of the *C. lanatus* seeds were tested using the Agar well diffusion techniques standardized inocula culture of the respective test organisms was spread evenly on the surface of nutrient agar plates. Wells of 6mm were aseptically punched on the agar using a sterile cork borer allowing at least 30mm between adjacent wells and the Petri dish. Different concentrations of the 4 different extracts (1000, 500, 125 and 62.5mg) of *C. lanatus* seeds were then introduced into the wells. Each extract was screened separately. The plates were incubated at 37^0C for 24hours [23]. Activity was determined by measuring the diameter of the zone of inhibition produced by the extracts against the test organisms.

The different concentrations were used for determine the minimum inhibitory concentration using Mueller Hinton Agar.

III. Results

Table 1 shows the phytochemical components of watermelon seed extracts. The presence of phenol, saponin, tannin, flavonoid and cyanogenic glycosides were observed. Amongst the observed phyto-components, only cyanogenic glycoside was not present in the ethanol extracts.

Table 1 : Phytochemical analysis of *C. lanatus* seed extracts

Component	Cold Water Extract	Hot Water Extract	Methanol Extract	Ethanol Extract
Phenol	+	+	-	+
Saponin	-	+	+	+
Tannin	-	-	+	+
Flavonoid	+	+	+	+
Alkanoid	+	+	-	+
Cyanogenic glycoside	+	-	+	-

Key: + = present, - = absent

Table 2 shows the zone diameter of growth inhibition of the test organisms by methanolic extracts at different concentrations. There was no inhibitory effect observed against any of the test organisms at 62.5mg/ml concentration. At 125mg/ml, *B. cereus, P. aeruginosa* and *Proteus mirabilis* were not inhibited. There were inhibitory effects against all the test organisms at concentrations of 250-1000mg. The MIC value range from 125-250mg/ml. the zone diameter of growth inhibition of test organism by ethanolic extracts at different concentrations are shown in table 3. Concentrations of 250, 500, and 1000mg/ml inhibited all the organisms. Only *B. cereus* was not inhibited at 125mg/ml concentration while at 62.5mg, only *S. aureus, Proteus mirabilis* and *Streptococcus pyogenes* were inhibited. The MIC value ranged from 62.5-250 mg/ml.

Table 2 : Inhibitory effect of methanol extracts of *C. lanatus* seed against pathogens

	Diameter Zone Inhibition (mm)					MIC (Mg/ml)
	Concentrations (mg/ml)					
Pathogen	1000	500	250	125	62.5	
Staphylococcus aureus	30	17	9	3	0	1.25
Klebsiella pneumoniae	28	18	9	1	0	250
Escherichia coli	31	19	8	3	0	125
Pseudomonas aeruginosa	29	15	6	0	0	250
Bacillus cereus	25	14	8	0	0	250
Proteus mirabilis	20	9	3	0	0	250
Streptococcus pyogenes	24	18	8	4	0	125

Table 3 : Inhibitory effect of ethanol extracts of *C. lanatus* seed against pathogens

	Diameter Zone Inhibition (mm)					MIC (Mg/ml)
	Concentrations (mg/ml)					
Pathogen	1000	500	250	125	62.5	
Staphylococcus aureus	29	19	9	5	2	6.25
Klebsiella pneumonia	29	19	8	2	0	125
Escherichia coli	30	18	8	3	0	125
Pseudomonas aeruginosa	20	16	7	2	0	125
Bacillus cereus	28	15	7	0	0	250
Proteus mirabilis	32	21	7	6	3	62.5
Streptococcus pyogenes	30	22	9	5	2	62.5

Table 4 shows the zone diameters of growth inhibition of pathogens by hot water extracts at different concentration. The MIC ranged from 125-250 mg/ml. At 125mg/ml, *S. aureus* and *B. cereus* were inhibited with diameter of growth inhibition 2mm. at 62.5mg, no test organism showed sign of inhibition.

Table 4 : Inhibitory effect of hot water extracts of *C. lanatus* seed against pathogens

	Diameter Zone Inhibition (mm)					MIC (Mg/ml)
	Concentrations (mg/ml)					
Pathogen	1000	500	250	125	62.5	
Staphylococcus aureus	27	13	7	2	0	125
Klebsiella pneumonia	25	12	6	0	0	250
Escherichia coli	29	14	7	0	0	250
Pseudomonas aeruginosa	25	12	3	0	0	125
Bacillus cereus	24	12	4	2	0	125
Proteus mirabilis	21	10	2	0	0	250
Streptococcus pyogenes	23	9	4	0	0	250

In table 5, the zone diameter of growth inhibition of the test organisms by cold water extracts at different concentrations were shown. At 62.5mg, the test organisms were not inhibited. At 125mg, *S. aureus* and *B. cereus* showed a negligible zone diameter of inhibition (1mm). The MIC value was 250mg/ml.

Table 5 : Inhibitory effect of cold water extract of *C. lanatus* seed against pathogens

	Diameter Zone Inhibition (mm)					MIC (Mg/ml)
	Concentrations (mg/ml)					
Pathogen	1000	500	250	125	62.5	
Staphylococcus aureus	28	15	6	1	0	250
Klebsiella pneumonia	26	13	5	0	0	250
Escherichia coli	27	13	6	0	0	250
Pseudomonas aeruginosa	24	12	15	0	0	250
Bacillus cereus	23	11	5	1	0	250
Proteus mirabilis	20	9	3	0	0	250
Streptococcus pyogenes	20	10	5	0	0	250

Table 6 presents the comparison of the efficacy of different extracts with the standard antibiotic ciprofloxacin. The diameter zones of inhibition produced by the extracts against the test organism were comparable with that of the antibiotic. Some extracts such as methanol produces the same zone diameter (29mm) with the antibiotic against *B. cereus.*

Table 6 : Sensitivity test result of the different extract and the standard antibiotics (mm)

Pathogen	C.W.E 1000mg	H.W.E 1000mg	M.E 1000mg	E.E 1000mg	CIP 1000mg
Staphylococcus aureus	28	27	30	29	34
Klebsiella pneumonia	26	25	28	29	36
Escherichia coli	27	29	31	30	38
Pseudomonas aeruginosa	24	25	29	30	32
Bacillus cereus	22	24	29	28	29
Proteus mirabilis	20	21	25	32	30
Streptococcus pyogenes	20	23	24	30	39

Key:
C.W.E– Cold Water Extract *H.W.E– Hot Water Extract* *E.E– Ethanol Extract*
M.E – Methanol Extract *CIP – Ciprofloxacin*

IV. Discussion

The phytochemical analysis showed the presence of phenol, saponin, flavonoid, alkaloid and cyanogenic glycoside. The presence of these phyto-components has been linked with the antibacterial activity of plants and plants that contain them in higher amount are considered to be superior in their antimicrobial activity [24] [25] [21].

The result of antibacterial activity of the extract against selected human pathogens indicated that the plant sample was active against a wide variety of human pathogenic bacteria. Ethanol extracts exhibited the highest inhibitory effect followed by methanol, hot water and cold water in that trend. This result agrees with the findings made by [26] where ethanol extract proved active in inhibition of the tested organisms than other extraction solvents. The low inhibition effect shown by the aqueous extracts as compared to ethanol and methanol could be due to the fact that these phyto-components are more soluble in ethanol and methanol than in water or that the hot water could have caused the denaturing of the active components.

However, most of the Gram negative organism e.g. *E. coli* showed high susceptibility than most of the Gram positive. The higher susceptibility of the Gram negative bacteria is difficult to explain in the study considering the observation of [27] that the Gram negative bacteria appear to be more resistant to antimicrobial agents than the Gram positive bacteria. This resistance has been observed to reside in the complex cell wall and cell membrane structure.

More so, more antibacterial activities were observed with high concentration of the extracts than at lower concentrations. Activity even at low concentration indicates high potency of the extract against the microorganism.

V. Conclusion

These results gotten from the phytochemical analysis and antibacterial activity of the watermelon seed extracts supports the application of the extracts in ethno-medicine and will serve as a good source in pharmaceutical productions against some pathogenic microorganisms.

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Antibiogram Analysis and Altering Antimicrobial Susceptibility Pattern of Multidrug Resistant Pathogens

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Abstract- *Introduction:* In the current situation of escalating antibiotic resistance it is essential to identify and report sensitivity pattern of these MDR bacteria in order to tailor empirical therapy and hygienic measures. Because there will be hardly any new antibiotics in the near future, a better understanding is needed on the how to optimize the use of existing antibiotics, alone and in combination with other drugs. To achieve this, periodic monitoring and surveillance of hospital antibiogram is mandatory.

Materials & Methods: Antibiogram surveillance was done for a five year period from Jan-2008 to December 2012 .The report generated was as per CLSI guidelines. A longitudinal analysis of prevalent rates of MDR pathogens-ESBL Enterobactericiae, MRSA, Imipenem resistant Gram negative bacilli isolated from all clinical samples and their sensitivity pattern was done.

Results: The most prevalent MDR gram negatives at our centre were ESBL E.coli & ESBL Klebsiella pneumonia (73% & 61% respectively) and MRSA among Gram positives at 24.5%.

Keywords: *antibiogram, surveillance, changing trends, MDR pathogens.*

GJMR-C Classification : *NLMC Code: QW 1*

Strictly as per the compliance and regulations of:

Antibiogram Analysis and Altering Antimicrobial Susceptibility Pattern of Multidrug Resistant Pathogens

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Results: The most prevalent MDR gram negatives at our centre were ESBL *E.coli* & ESBL *Klebsiella pneumonia* (73% & 61% respectively) and MRSA among Gram positives at 24.5%. *Pseudomonas* was the most predominant Imipenem resistant gram negative bacilli. Uropathogenic *E.coli* strains had better sensitivity to Nitrofurantoin at 63%. Imipenem showed 90-100% sensitivity to *E.coli* & *Klebsiella* and 70-80 % to *Pseudomonas*. MRSA was predominantly from soft tissue infection showing 100% sensitivity to Linezolid & 99% to Vancomycin.

Conclusion: During the study period a narrow spectrum of sensitivity was observed for commonly used antibiotics. An empirical antimicrobial Guideline was drafted following the Antibiogram Surveillance. Infection control measures & antimicrobial stewardship had proven to be modestly effective in our study.

Keywords: antibiogram, surveillance, changing trends, MDR pathogens.

I. Introduction

The bacterial disease burden in India is among the highest in the world [1, 2, 3]; consequently, antibiotics are playing a critical role in limiting morbidity and mortality in the country. But unfortunately antibiotic resistance which is a global concern now, has reached a pandemic proportion fuelled by human need, greed and irresponsibility [4]. This is particularly pressing in developing nations, including India, where the burden of infectious disease is high and healthcare spending is low. And the worst consequence is that , the bacterial strains that acquire resistance to one or more first-line antimicrobials pose numerous challenges to healthcare, including: increased patient morbidity and mortality, increased drug costs, prolonged illness duration, and more expensive disease control measures. The overall take-home message from studies of resistant infections is that resistance levels have been worryingly high wherever studies have been conducted [3, 4]. Management of common and lethal bacterial infections has been critically compromised by the appearance and rapid spread of these antibiotic-resistant bacteria. This resistance is affecting patients and therapeutic outcomes, with concomitant economic consequences. Because the anti Microbial Resistance (AMR) genes can be readily transmitted through a bacterial population, surveillance of AMR trends is critical for the rapid detection of new isolates and continuous monitoring of disease prevalence [5]. Surveillance is central to the control of antimicrobial resistance. Data generated by surveillance activities can be used to guide empirical prescribing of antimicrobial agents, to detect newly emerging resistances, to determine priorities for research and to evaluate intervention strategies and potential control measures aimed at reducing the prevalence of resistant pathogens [6-10].

Antibiogram pattern with specific reference to MDR Organisms is increasingly reported in Indian hospitals [11-15] and worldwide [16-21]. Therefore it is crucial to monitor emerging trends in drug resistance at local level to support clinical decision making, infection control intervention and antimicrobial resistance containment strategies.Antibiogram surveillance and changing trends in antimicrobial resistance at our healthcare setting is monitored periodically by annual cumulative antibiogram.The cumulative antibiogram is done as per the consensus guidelines from CLSI[22]. This report provides an overview of surveillance information on multidrug resistant pathogens at our tertiary care centre for a five year period from 2008 to 2012, and also

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presents data on Sensitivity rates of these drug resistant pathogens, highlighting the probable effective pathogen-drug combinations for most common infections.

II. Materials and Methods

Our super speciality hospital is a 300 bedded tertiary care Post graduate teaching centre with CTVS, Cardiology, Urology, Ophthalmology and orthopaedic units. We analysed antibiogram surveillance reported during the five year period from Jan 2008 to December 2012.The following indices were monitored.

1. Prevalence rates: The number of MDR Organisms-MDR O (as a percentage of all specimens received by our Lab) was determined annually and analysed longitudinally for the five year period. Most of the clinical specimens were urine specimens and predominantly from Urology outpatient Unit. And we specifically looked for
   - Prevalence of ESBL *E.coli* & ESBL *Klebsiella pneumoniae*
   - MRSA Prevalence rate
   - Imipenem resistant *Pseudomonas*, ESBL *E.coli* & ESBL *Klebsiella*
2. Antibiogram: The sensitivity pattern as determined by Kirby bauer disc diffusion for all isolates from all clinical samples was used and interpretation of sensitivity was as per updated CLSI Guidelines (years 2007 to 2011).
3. We analysed the changing sensitivity pattern of most prevalent pathogens of Urinary tract infection , soft tissue infection, and Ventilation associated pneumonia (VAP) during the study period as defined by standard surveillance criteria[1,5].
4. We also analyzed the Antibiotic Sensitivity pattern of Imipenem resistant gram negative bacilli strain(*Pseudomonas aeruginosa*, ESBL *E.coli,* ESBL *Klebsiella pneumoniae*)
5. We documented modifications in the hospital infection control measures and Empirical antimicrobial Guideline was drafted following the Antibiogram Surveillance for Infections from specific bodily sites.

III. Our Hospital Antibiogram Software

Our Hospital cumulative Antibiogram is framed periodically using a Software (LIS) from CSC (previous iSOFT). The data entry and analysis is done by a report generator using this isoft software (based on WHONET 5.6). The generated report is based on consensus guidelines given by CLSI [22].

IV. Results

Table: 1a shows the most prevalent MDR-O at our tertiary care centre and which are *ESBL E.coli, ESBL K. pneumoniae* among Gram negative bacilli and *MRSA* among Gram positive cocci.

Table 1a : Prevalence rates of selected Multi Drug Resistant Pathogens

	ESBL *E.coli*	ESBL.*K. pneumoniae*	*MRSA*
2008	67.3%	61.5%	40.9%
2009	73%	55.5%	11.1%
2010	72.3%	63.5%	34.7%
2011	73%	64.5%	25%
2012	74%	61%	19.3%
Overall Prevalance %	**73%**	**61%**	**24.5%**

Table 1b shows the frequency of Imipenem resistance among selected Gram negative bacilli (*Pseudomonas, ESBL E.coli, ESBL Klebsiella pneumoniae*) at our tertiary care centre.

Table 1b : Percentage if Imipenem Resistance isolates among selected Gram negative bacilli

	ESBL *E.coli*	ESBL *Kleb. pneumoniae*	*Pseudomonas*
2008	1 isolate (1/372) 0.3%	0.00%	37 isolates(37/159) 23.3%
2009	14 isolates(14/527) 2.7%	1 isolate(1/50) 2%	40 isolates(40/166) 24%
2010	6 Isolates(6/440) 1.4%	5 isolates(5/171) 3%	15 isolates (15/128) 11.7%
2011	5 Isolates(5/397) 1.3%	3 Isolates)(3/71) 2.8 %	30 isolates(30/153) 19.6 %
2012	18 Isolates(18/759) 2.4%	8 Isolates (8/116) 6.7%	74 isolates(74/292) 25%
TOTAL	**44 Isolates(44/2496)1.7 %**	**17 isolates(17/359)4.7 %**	**196 isolates(196/899)22 %**

Fig-1: The most frequently isolated pathogen from the urine samples at our centre are ESBL *E.coli*, ESBL *Klebsiella pneumoniae* & *Pseudomonas spp*. The change in antibiotic sensitivity during the five year of all the above mentioned three Uropathogens are analysed in the Fig 1a,1b, and 1c.

Fig-1a shows change in Uropathogenic *E.coli* sensitivity pattern over the time frame for important groups of Antibiotics. Sensitivity to Ciprofloxacin remains constantly low at less than 20% throughout the study period.

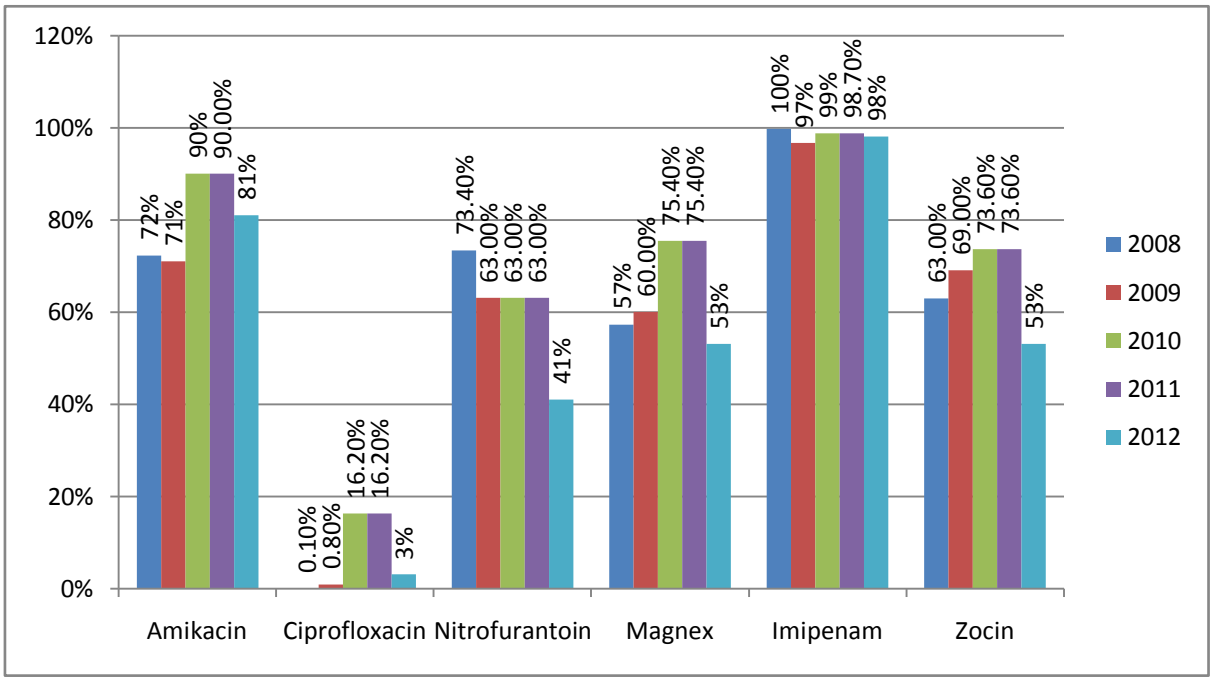


Fig 1 a : Antibiotics percentage sensitivity of ESBL E.coli isolates from urine

Fig-1b shows changes in Uropathogenic Esbl *Klebsiella pneumoniae* sensitivity pattern over time. Sensitivity to Ciprofloxacin and Nitrofurantoin remains constantly low at less than 20% throughout the study period.

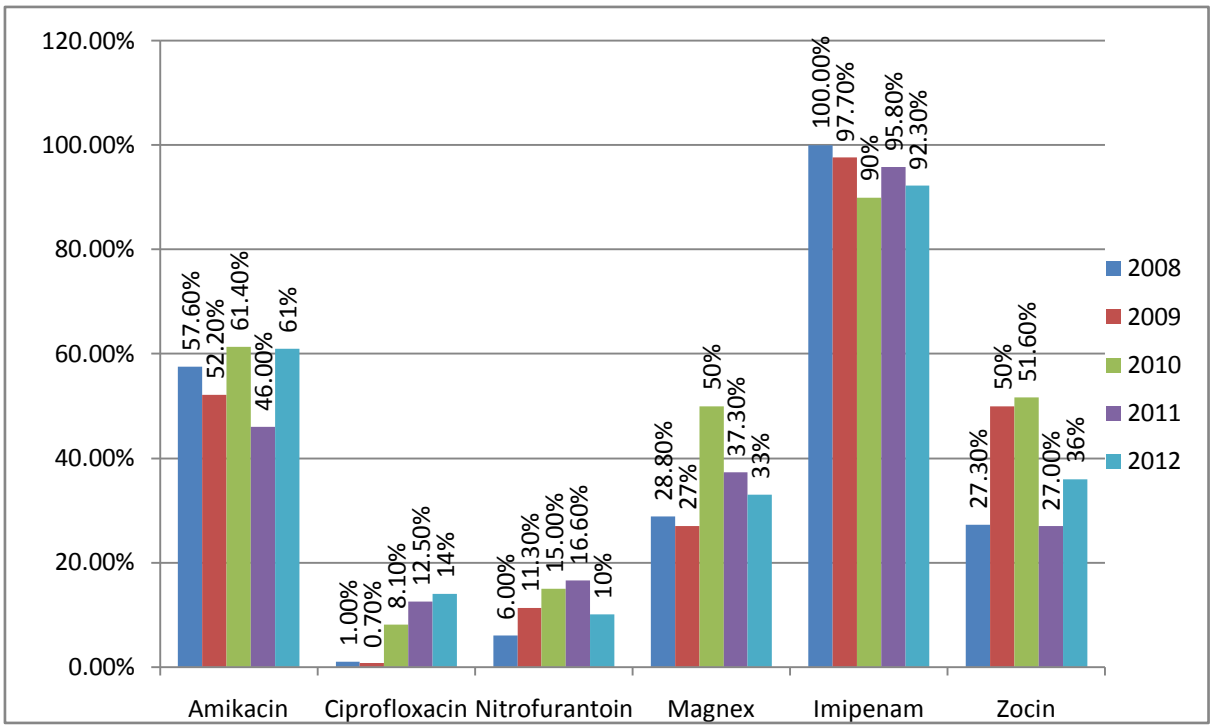


Fig-1c shows Uropathogenic *Pseudomonas spp* sensitivity pattern over time. Sensitivity to ciprofloxacin was at a range between 20-40% and Nitrofurantoin less than 10%

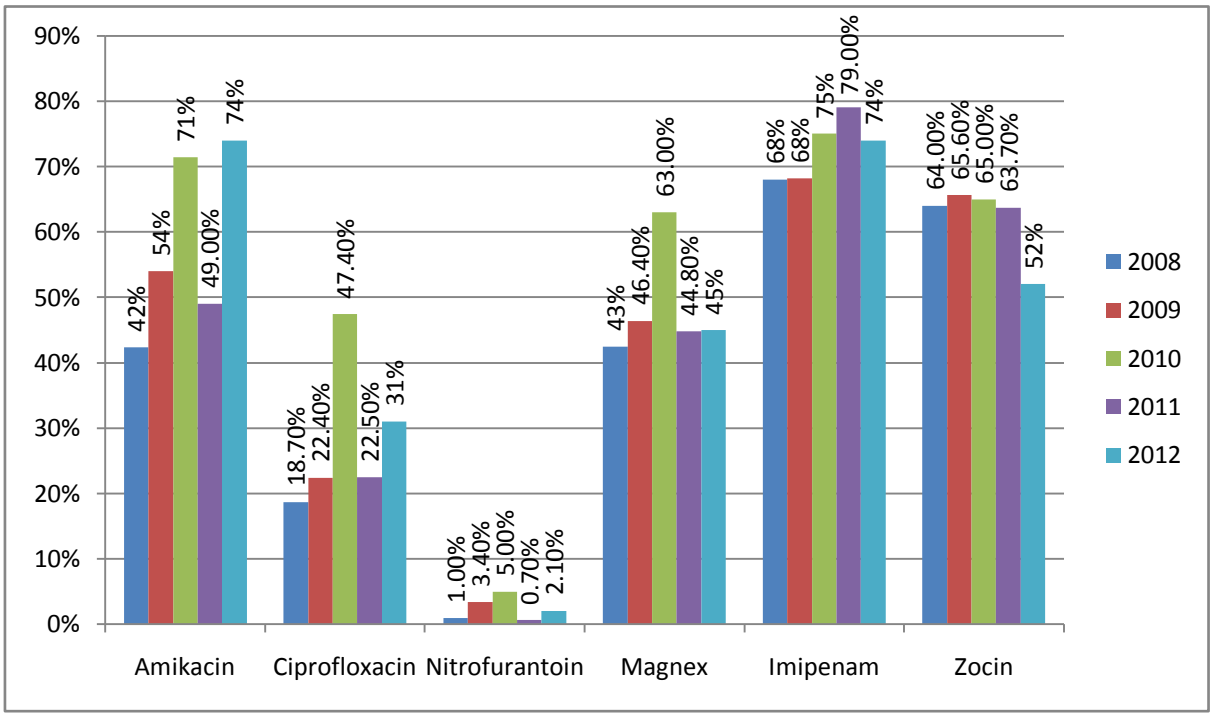


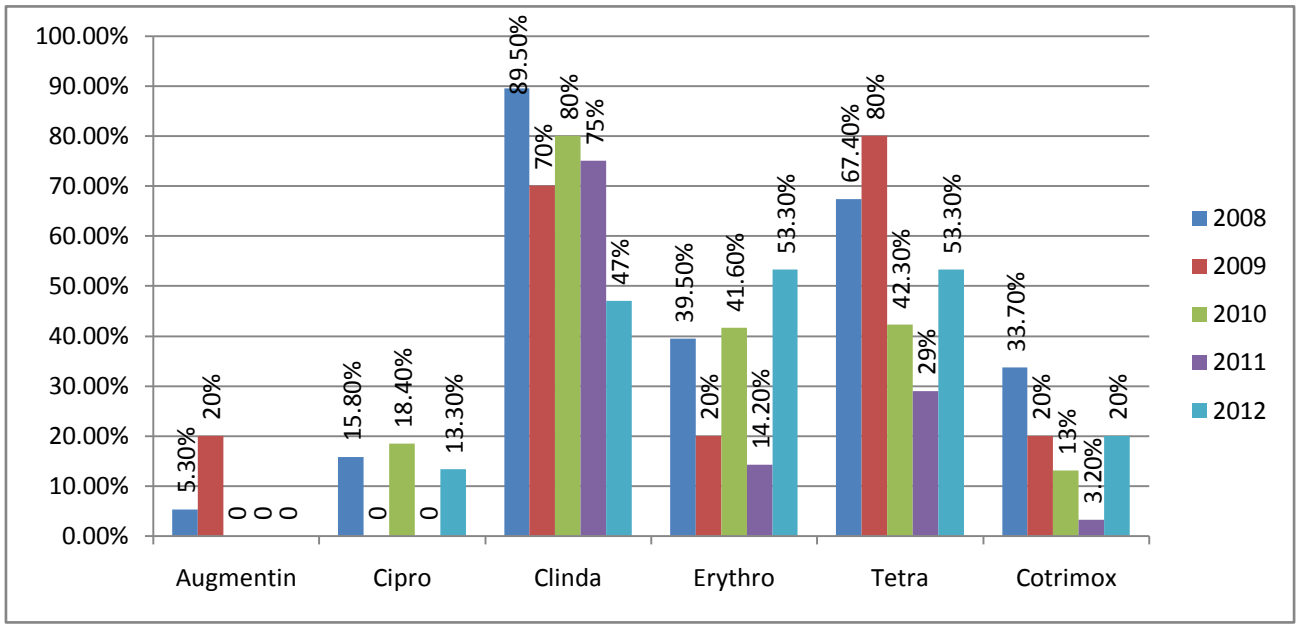
Fig 1c : Antibiotics percentage sensitivity of P.aeruginosa isolated from urine

Table: 2 represent the overall sensitivity rate of *MRSA* isolates from all types of clinical specimens to different class of Antibiotics. Of the Beta lactum groups, Penicillin showed 0% sensitivity throughout the study period.Ampicillin was less than 10% and Augmentin (betalactum+beta lactamase inhibitor) less than 20%

Table 2 : % Sensitivity pattern of MRSA against various antimicrobials

	Sensitivity Percentage				
	2008	2009	2010	2011	2012
Cipro floxacin	11.1	0	13.9	0	8
Ampi cillin	3.7	0	0	9	0
Augmentin	14.8	16.7	0	10	0
Tetra cycline	69.2	76	71.4	68.2	50
Co-Trimxazle	23	16.6	19.4	9	17
Imipenem	88	75	97.2	100	NT
Erythro mycin	42.8	20	48.5	38.1	41
Penicillin	0	0	0	0	0
Vanco mycin	100	100	100	100	96%
Linezolid	100	83.3	100	100	100
Rifampicin	96.3	100	100	100	82%
Clinda mycin	92.5	75	82.3	77.2	50%
Oxacillin	0	0	0	4.5	0
Nitro furantoin	83.3	66.6	NT	14.3	20

Fig-2 shows the changing sensitivity pattern of *MRSA* isolated from wound specimens (MRSA was most frequently isolated from Wound specimens). Augmentin & ciprofloxacin sensitivity percentage consistently declined and came down to 0% during the five year study period. Antibiotics with good sensitivity percentage for Clindamycin, Vancomycin, Linezolid.



**Linezolid 100% throughout the study period & Vancomycin 99.8 %*

Fig 2 : Antibiotic sensitivity % of MRSA from Pus swab/aspirates*

Fig-3: The most frequently isolated pathogen from endotracheal aspirate was *Pseudomonas aeruginosa*. The changing trends in sensitivity pattern of this pathogen in Endotracheal aspitates is shown in Fig-3

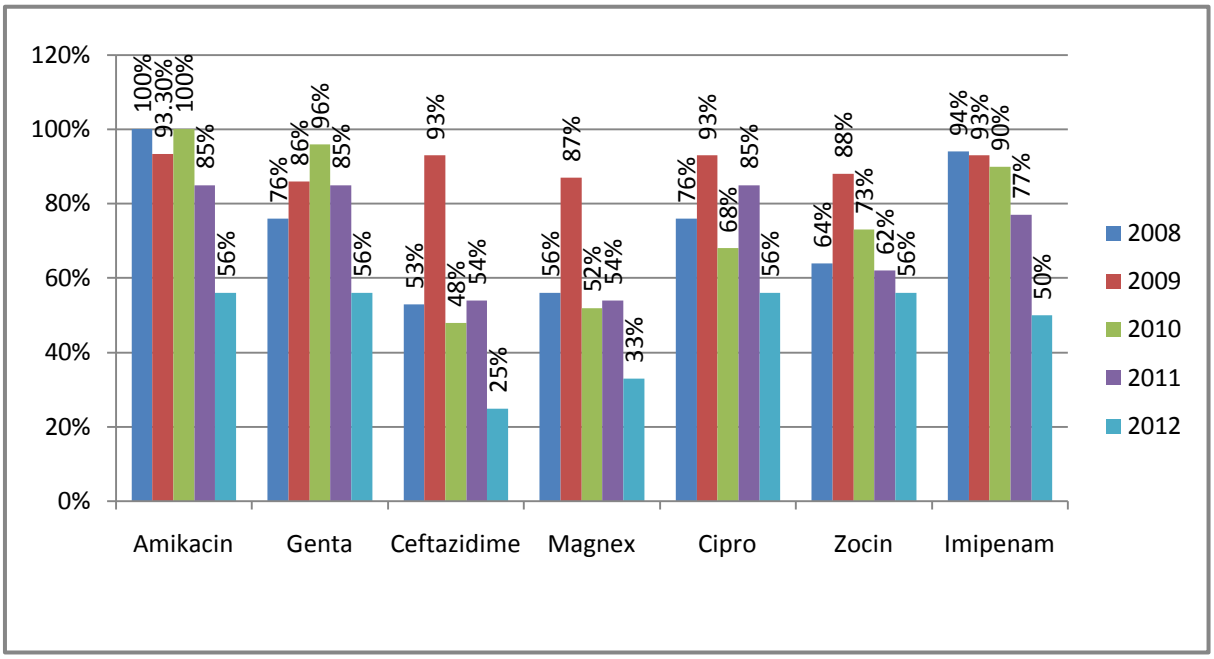


Fig 3 : Antibiotic sensitivity % of Pseudomonas from Endotracheal apirates

Table -3 shows Imipenem sensitivity percentage among selected Gram negative bacilli. Fig-4a & 4b shows Imipenem resistant Gram negative bacilli sensitive to other antibiotics.

Table 3 : Antibiotic Sensitivity pattern of Imipenem resistance strain

	Total no of Imipenem resistance isolates	% of Pan resistant isolates	% of Imipenem resistant isolates showing sensitivity to other antibiotics
ESBL *E.Coli*	44- isolates	47.60%	52.4%-Senitive to other antibiotics* fig -1
ESBL *Kleb. pneumoniae*	17 -isolates	83.30%	16.3%- Sensitive to Amikacin, Nitrofuratoin
Pseudomonas aeruginosa	196-Isolates	68.50%	31.5%- Sensitive to other antibiotics* fig-2

V. Discussion

a) Multi Drug Resistant Pathogens at our tertiary care centre

Our study shows that ESBL producers are the most prevalent Gram negative MDR organism at our tertiary care centre and MRSA is the most prevalent Gram positive pathogen as shown in the Table-1a. Urine samples are the predominantly received clinical sample for culture & sensitivity at our diagnostic microbiology division and the ESBL producers are frequently isolated from all types of Urine specimens submitted at our laboratory. ESBL production among *E.coli* was greater than 70% and *Klebsiella* greater than 60% throughout our study period. This data is consistent with many other centres from India & worldwide [23]. MRSA's are prevalent pathogen from wound specimens. The prevalence percentage of MRSA ranged from 11% - 40% during the study period at our Institute. Literature evidence indicates that the prevalence can range from 3-66% [24, 25] .The prevalence rate started to decline from 2010 in relation to enhanced hospital wide MRSA screening and contact isolation.

Imipenem resistant *Pseudomonas spp* was the next serious Gram negative MDR pathogen as shown in Table 1b.It shows an overall prevalence rate of 22 % during the five year study period. Even though there was a low prevalence rate of Imipenem resistance seen among ESBL *E.coli & ESBL Klebsiella* (1.7% and 4.7% respectively), it is still a matter of concern. And these three Imipenem resistant pathogens were frequently isolated from urine specimens (41% from mid stream urine, 44 % from catheterised urine). There was gradual increase in the prevalence rate of Imipenem Resistance among *ESBL E.Coli & ESBL Klebsiella* during this five year period from 2008 till 2012(Table-1b). But a gradual decrease in the Prevalence of *Pseudomonas* from 23.3% in 2008 to 19.6 % in 2011 and a sudden increase to 25% in 2012 was documented.

b) Analysis of Sensitivity pattern of ESBL Producers causing Urinary Tract Infection

Ciprofloxacin sensitivity percentage was very low at less than 15% for ESBL producers and at a range of 18-25% for *Pseudomonas spp* among the Urine specimens as shown in Fig 1a, 1b & 1c. This essentially rules out Fluoroquinolones as empiric antimicrobial therapy for Severe & Complicated urinary tract infections at our tertiary care centre.

Nitrofurantoin had a better sensitivity for ESBL *E.coli* at 63%, but a very low percentage for *Klebsiella* & *Pseudomonas* at less than 15% and 10% respectively. Hence a recommendation to use Nitrofurantoin as empiric therapy for Urinary tract infection was kept under reserve.

Carbapenam the drug of choice for ESBL producers showed 95% - 100% sensitivity in *E.coli* & *Klebsiella* UTI and 68-80 % in *Pseudomonas* infections. This emergence of Imipenem resistance among Gram negative UTI is a matter of concern and hence strict antibiotic policy was implemented for the use of Imipenem as empiric therapy for UTI. It was reserved only for clinically severe UTI like Pyelonephritis.

The Beta lactum + Betalactamase inhibitors combination like Magnex(cefaperazone & sulbactum) & Zocin (Pipericillin+ Tazobactum) showed a considerable sensitivity percentage for Esbl *E.coli* at 60-75% AND 73% respectively as shown in Fig-1a. But both the drugs had low percentage sensitivity for ESBL *Klebsiella pneumoniae* at 27%-50% as shown in Figure1b. *Pseudomonas spp* had good sensitivity pattern for Zocin at 65% when compared to Magnex which is around 45% -63%. Hence these two drugs remained useful against *E.coli* UT infection when compared to *Klebsiella & Pseudomonas.*

As the predominant pathogen causing UTIs, empiric treatment strategies generally target *E.coli*. Nitrofuratoin remains effective for *E.coli* isolates with 76% showing susceptibility. This is reassuring as 85% to 90% of all uncomplicated UTI infections are caused by *E. coli*. In brief, the overall better sensitivity pattern for all the three frequent pathogens causing UTI is noted with Amikacin & Imipenem. Amikacin was recommended for patients with good renal parameters. Since emerging resistance was noted with Imipenem, this was reserved for severe upper UTI with compromised renal parameters. Hence the urologists were left with Betalactum and betalactamase inhibitor combination like Magnex & Zocin. These drugs retains value as workhorse dry force , especially for less severely ill UTI and play a valuable role as Carbapenam sparer's in Antimicrobial stewardship programme. The following empiric guidelines were recommended for patients with UTI.

- For Uncomplicated UTI,Ciprofloxacin PLUS Nitrofurantoin

- Complicated UTI (related to instrumentation, ie. catheter, percutaneous nephrostomy (PCN), ureteral stent, and/or recurrent infection with the same organism) amikacin PLUS ciprofloxacin for outpatient OR amikacin q24h if patient can come daily to the hospital for therapy.
- For Pyelonephritis, Amikacin OR Imipenem OR Piperacillin/tazobactam 6'TH hourly. Recommended imaging of upper genitourinary tract with Ultrasound to look for hydronephrosis, obstructing stone, renal or perinephric abscess. To De-escalate to peroral therapy IF possible once fever has resolved.
- Other Infection control measures critical to limiting the spread of ESBL-producing organisms were also addressed (i.e.) Protocols to limit the use of indwelling Foley catheters and protocols for regular catheter changes when they are needed,

c) Analysis on MRSA sensitivity pattern (Most prevalent pathogen causing Soft tissue infection)

As discussed before the most prevalent Gram positive pathogen at our centre was MRSA and the prevalence rate ranged from 11% to 40%. Predominantly 79% of MRSA were from wound swabs, 13% from urine and 9% from Endo tracheal secretions & blood. The overall sensitive pattern of MRSA from all clinical isolate was analysed in TABLE-2. When we look into overall sensitivity pattern both in wards and OPD together, sensitivity to penicillin was Zero percent throughout our study period from 2008 to 2012.This is in accordance with a study by Bandaru etal [26]. Sensitivity to Ampicillin was lowest next to penicillin, followed by Ciprofloxacin, Cotrimoxazole and Erythromycin. Analysis of the changing pattern of Antibiotics for MRSA isolates for the five year period indicated that, the sensitivity percentage for all the above mentioned antibiotics was declining from 2008 to 2012.Ampicillin, Ciprofloxacin & Cotrimoxazole had less than 25 % sensitivity. Erythromycin and Tetracycline percentage was varying during this period. The sensitivity percentage of Clindamycin slowly declined from 92.5 % in 2008 to 50% in 2012 and Rifampicin to 82%.Linezolid had 100 % sensitivity.

In our study 60.5% of MRSA isolates were found to be multidrug resistant, to more than three antimicrobials which are similar to two other studies [25, 27]. Other studies which show less than 50% MDR resistant strains are Majumdar et al (23.2%) [28] And Bandaru et al [26] (32.09%).All the MRSA strains were sensitive to Vancomycin except one in the present study which is in accordance with other studies. [29-31]

Maximum MRSA positive wound specimens were from Ortho department (57%) followed by CTVS (20.4%) and then Plastic surgery (14 %) and Urology (10 %).Wound specimens sent from Orthopedics were predominantly from outpatient clinic. When the sensitivity percentage of MRSA's isolated from pus/ wound aspirates were analysed as shown in Fig-2, a better sensitivity pattern was observed for Erythromicin and ciprofloxacin during the study period. There was a fluctuation in Tetracycline & Cotrimoxazole sensitivity percentage. It consistently decreased to 29% and 3.2% respectively during the year 2011, but an improved sensitivity percentages was observed in 2012. Sensitivity to Clindamycin percentage reduced from 89 %(2008) to 49 % in 2012. Eighty seven percent of non hospitalized MRSA isolates were presumptively identified as CA-MRSA based on Clindamycin susceptibility- a surrogate marker of CA-MRSA. As a result, admission screening for MRSA colonization has been implemented in 2011 in addition to routine infection control measures.

Guidelines & empirical antimicrobial choice for soft tissue/wound infections from different source were recommended based on the above mentioned analysis along with adequate drainage/wound debridement/ cleaning.

- Simple Skin and Soft Tissue Abscess: For Outpatients, Doxycyline OR Clindamycin & For Inpatients, Vancomycin PLUS piperacillin/ tazobactam .
- Cellulitis: Outpatient therapy with clindamycin OR doxycycline
- Traumatic Wounds: Outpatients to start with Doxycycline PLUS Clindamycin PLUS consideration of Amikacin OR Gentamicin. Inpatient with Piperacillin/ tazobactam PLUS Vancomycin OR Ciprofloxacin PLUS Vancomycin .Therapy depends on severity and nature of wound. All wounds should receive adequate cleaning
- Prosthetic Joint Infections: Vancomycin AND Piperacillin/tazobactam OR Vancomycin AND Imipenem.
- PostOperativeSternotomy/SurgicalSiteInfection: VancomycinANDPiperacillin/tazobactam OR Vancomycin AND Imipenem pending cultures.

d) Analysis on sensitivity pattern of Pseudomonas aeruginosa (Most prevalent pathogen causing Ventilation associated Pneumonia)

The most prevalent pathogen from ETA was *Pseudomonas aeruginosa* throughout the study period. Analysis on changing sensitivity pattern of Pseudomonas is as shown in Fig-3. There was a fluctuation in sensitivity percentage of Aminoglycosides, ceftazidime, Beta lactum+ Beta lactum inhibitor combination like Magnex & Zocin till 2010.But the sensitivity percentage started to decline in 2011 and further more in 2012. Imipenem sensitivity alarmingly declined to 50% in 2012.The MDR % ranged more than 60% during this study period , which is low when compared to other studies [32].In a study by Koirala et al [33], *Pseudomonas* had Zero percent sensitivity to Ceftazidime, Amikacin at 22% and Gentamicin at 18%. Sensitivity to ciprofloxacin was at 19% which is very low when compared to our study. Overall ciprofloxacin & Zocin was showing

around 60% sensitivity rate when compared to other antibiotics as shown in FIG: 3.

Pipericillin-tazoactum, Ciprofloxacin, and Imipenem were proposed as empirical antimicrobial choice for patients diagnosed with clinical Ventilation associated pneumonia or Ventilation associated Tracheobronchitis at our centre. These empirical antibiotics were recommended to be given alone or in combination depending on severity of Patient's clinical condition and renal parameters. Also recommendations were made such that antibiotic therapy should be changed and deescalated based on Culture identification report and a specific antibiotic should be chosen based on sensitivity pattern. An Active Surveillance for VAP was also initiated as a measure of Hospital Infection control at our tertiary care centre.

e) Analysis on sensitivity pattern of Imipenem resistant Gram Negative Bacilli

In this study 83.3 % of ESBL *Klebsiella pneumoniae* isolates, 47.60% of ESBL *E.Coli* & 68.50% of *Pseudomonas* isolates were Pan resistant (Table-3). Among the Imipenem resistant *Pseudomonas* strains 21.7 % isolates were sensitive to Amikacin & Zocin (Pipericillin+Tazobactum) as shown in Fig-4a and among Imipenem resistant ESBL *E.coli*strains 33.3 % were sensitive to Nitrofurantoin & Magnex (Cefepe-razone+Sulbactum)as shown in Fig-4 b. This is almost similar to two other studies, Taneja et al[34] and Sasikala et al [35]where in the Imipenem resistant *Pseudomonas* strains had the best in vitro susceptibility to Amikacin and Pipericillin.

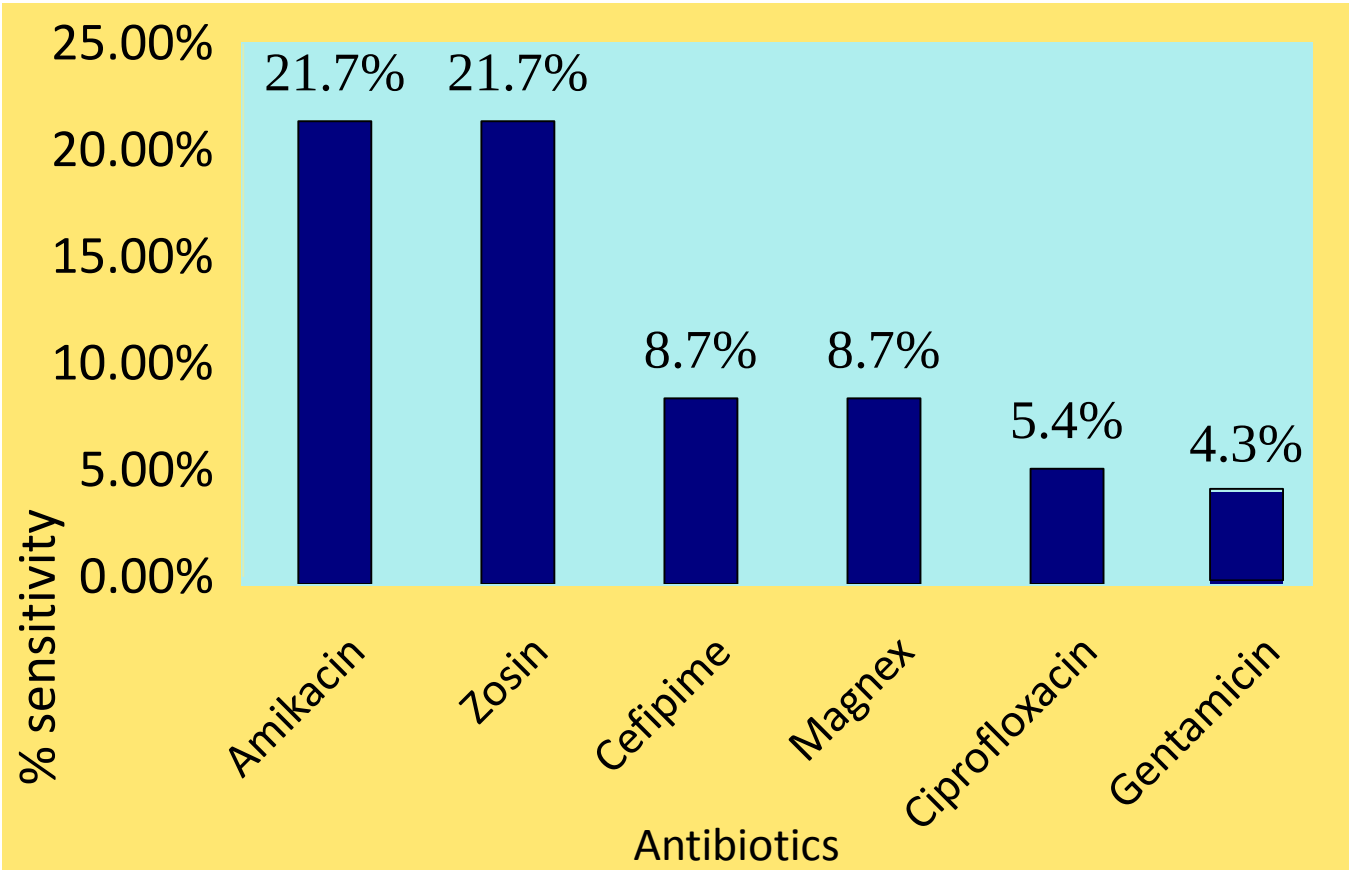


Figure 4 a : Imipenem resistant Gram negative bacilli sensitive to other antibiotics

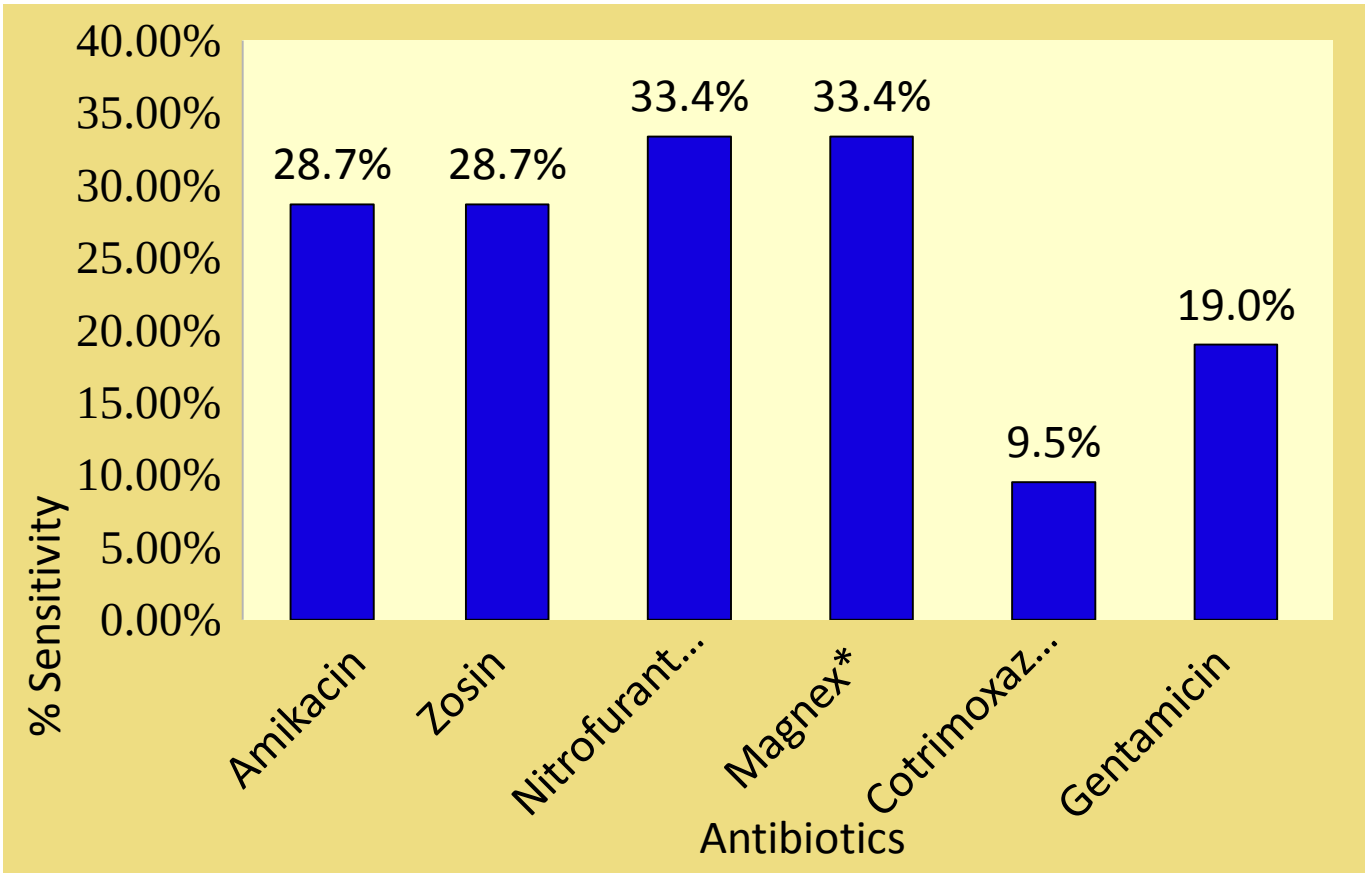


Figure 4 b : Imipenem resistant Gram negative bacilli sensitive to other antibiotics

VI. Conclusions

Our findings suggest that there is a definite increase in the multidrug resistant organisms. This Surveillance study showed that the most prevalent Multidrug resistant Uropathogen at our centre was ESBL producers (E.coli & *Klebsiella pneumoniae*). *MRSA* was the predominant MDRO causing soft tissue infections & *Pseudomonas* prevalent in VAP. We believe that the data analysis on the changing trends in antibiotic resistance from most frequently received clinical samples, is an important pillar in our efforts at improving infection control practices. We proposed a draft Antibiotic guideline in 2012 based on the analysis on the data. The guideline provided recommendations for empiric antimicrobial therapy based on susceptibility pattern and relevant infection control practices for Complicated & Uncomplicated UTI's, for soft tissue infections, VAP's and Blood stream infections. We acknowledge the limitation of disc diffusion antimicrobial susceptibility testing as our tertiary care centre is a charitable institution. Infection control measures including Hand hygiene, antimicrobial stewardship, MRSA screening and restricted use of second line antibiotics had proven to be modestly effective in our study. But still it appears that our MDR Organism antibiograms were largely uninfluenced by infection control measures including institution of Antimicrobial Guidelines in spite of our clinicians adhering to protocols. Probable reasons might be widespread prevalence rates in the community and importation of cases harbouring partially/untreated Multi drug resistant pathogens from other referral hospitals to our tertiary care centre may have negated efforts within our centre.

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Ovarian Leiomyoma Associated with Serous Cystadenoma - A Case Report of an Uncommon Entity

By Dr. Sant Prakash Kataria, Dr. Nitika Chawla, Dr. Gajender Singh, Dr. Sanjay Kumar & Dr. Rajeev Sen

Abstract- *Background:* Primary Leiomyomas of ovary are rare tumors and account for less than 1% of benign ovarian tumors. Only about 60 cases have been reported in literature out of which most presented in child bearing age group.

Case: A 65 year old postmenopausal multipara presented with history of back pain and pain in lower abdomen. Pelvic examination and transvaginal ultrason-ography revealed presence of bilateral ovarian mass. An intra-operative frozen section showed serous cystade-noma in both ovaries with adenofibroma like areas in the left ovary. She underwent Panhysterectomy with Bilateral salpingo-oophorectomy. Histopathologically, a diagnosis of bilateral ovarian serous cyastadenoma with features of ovarian leiomyoma in left ovary was made. Ovarian fibroma-thecoma, cellular fibroma and sclerosing stromal tumour were considered as its differentials and ruled out. The possibility of leiomyos-arcoma was also ruled out.

Conclusions: Although these tumors have a benign course and are very rare, they should be emphasized as a possible differential whenever solid ovarian masses are detected.

Keywords: *ovary, leiomyoma, cystadenoma.*

GJMR-C Classification : *NLMC Code: QW 4*

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Conclusions: Although these tumors have a benign course and are very rare, they should be emphasized as a possible differential whenever solid ovarian masses are detected.

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I. Introduction

Leiomyoma arising primarily in ovary is a rare tumor and less than 60 cases have been reported till date[1]. It accounts for just 0.5 to 1 % of all benign ovarian tumors[2]. The majority of them are small, measure only a few millimeters and most (80%) occur in premenopausal age group[3]. They probably originate from smooth muscle cells in the ovarian hilar blood vessels but there are other possible origins including cells in the ovarian ligament, smooth muscle cells or multipotential cells in the ovarian stroma, undifferentiated germ cells[4] or they may arise from cortical smooth muscle metaplasia, smooth muscle metaplasia of endometriotic stroma, smooth muscle present in mature cystic teratoma and smooth muscle in walls of mucinous cystic tumor as depicted by various cases reported till now[5-7]. We report here a case of relatively large (2.5 x 2.0 cm) ovarian leiomyoma incidentally diagnosed in a 65 year old female with bilateral serous cystadenoma.

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II. Case Report

A 65 year old female presented with backache since 1 month and pain in lower abdomen since 20 days. There were no other complaints. The pelvic examination and transvaginal ultrasonography showed the presence of bilateral ovarian masses. Intraoperative frozen section revealed serous cystadenoma in both ovaries and adenofibroma like areas in left ovary. She underwent Pan hysterectomy with bilateral salpingo-oophorectomy. Grossly, Uterus and cervix measured 5.0 x 3.0 x 2.0 cm. Right fallopian tube measured 5.0 cm in length and lumen was dilated. Right ovary measured 3.0 x 2.0 cm and cut section showed a small cyst measuring 0.4 cm in diameter. A larger cyst measuring 5.0 x 3.5 x 2.0 cm was also found attached to the right ovary. Left fallopian tube measured 1.5 cm in length. Left ovary was replaced by a cystic structure measuring 7.0 x 4.5 x 3.5cm. An attached solid area was also identified measuring 2.5 x 2.0 cm which was encapsulated and grey white. On microscopic examination, endometrium showed changes of cystic atrophy while myometrium was unremarkable. Sections from cystic areas in both ovaries revealed serous cystadenoma . The solid areas in left ovary showed whorling of uniformly spindle shaped smooth muscle cells with eosinophilic cytoplasm and oval bland nuclei. There was negligible pleomorphism, nuclear atypia and only 1-2 mitotic figures per 10 high power fields. A possibility of benign smooth muscle tumor was considered. Special stain like Masson's trichrome showed the presence of smooth muscle. Immunohistochemistry showed positivity for α- smooth muscle actin and desmin confirming the existence of smooth muscle. The final diagnosis of leiomyoma of the ovary with serous cystadenoma was offered.

III. Discussion

Most ovarian leiomyomas are small, measuring only a few millimeters in diameter and are assosciated with ipsilateral or contralateral ovarian lesions[5-7]. But to the best of our knowledge, this is the first case of a primary ovarian leiomyoma assosciated with bilateral serous cystadenoma. Possible origin of this leiomyoma

may be smooth muscle present in wall of serous cystadenoma. Hameed showed that leiomyoma of ovary can arise from smooth muscle of mucinous cystadenoma[10].

Ovarian leiomyomas are asymptomatic and are found incidentally at surgery or at autopsy[2-4]. Some rare cases may be symptomatic and may present with abdominal pain, a palpable mass, hydronephrosis, elevated CA-125, hydrothorax and ascites[8]. In our case, pressure symptoms were due to bilateral serous cystadenoma rather than leiomyoma itself.

Ovarian leiomyoma is associated with its uterine counterpart in 78 % cases[2]. In our case, no uterine leiomyomas were identified even after careful serial sectioning, which makes it a primary tumor of the ovary. Primary ovarian leiomyomas are itself a rare entity and its occurrence in this postmenopausal female makes it more interesting.

Although whorling pattern and shape of smooth muscle cells of ovarian leiomyoma is quite characteristic, but, due its rarity several other tumors should be included in the differential diagnosis. Differential diagnosis of ovarian leiomyoma are fibroma, thecoma, cellular fibroma and sclerosing stromal tumor[9-11]. It can also be confused with tumors arising from broad ligament and extending into the hilum of ovary or wandering leiomyoma. Masson's trichrome stain helps to distinguish smooth muscle from fibrous component in the lesion. Moreover, desmin shows diffuse positivity in leiomyomas whereas fibromatous tumors are negative or only focally positive. But, α-SMA is positive in both leiomyomas and fibromatous tumors and thus can't differentiate between the two[12]. Thecomas do not express α-SMA and are positive for α- inhibin and calretenin. Leiomyosarcoma, although very rare, should also be ruled out using multiple criteria like mitotic count, cytological atypia and tumor necrosis1. Treatment of ovarian leiomyoma is cystectomy or ovariectomy or ovarian wedge resection[10].

IV. Conclusion

To conclude, this case is a primary ovarian leiomyoma considering histopathological and immunehistochemical features. The postmenopausal patient, relatively large size (2.5 cm), absence of uterine counterpart, association with bilateral serous cystadenoma makes this case rarest of its type. Thus, despite its rarity, ovarian leiomyomas should always be considered as a possibility whenever spindle cell lesions of ovary are suspected. Appropriate diagnosis and ruling out a malignant lesion requires extensive tumor sampling and additional immunohistochemical analysis. Overall, since it is a benign tumor, ovary preserving surgery is performed in young females to preserve fertility in these women.

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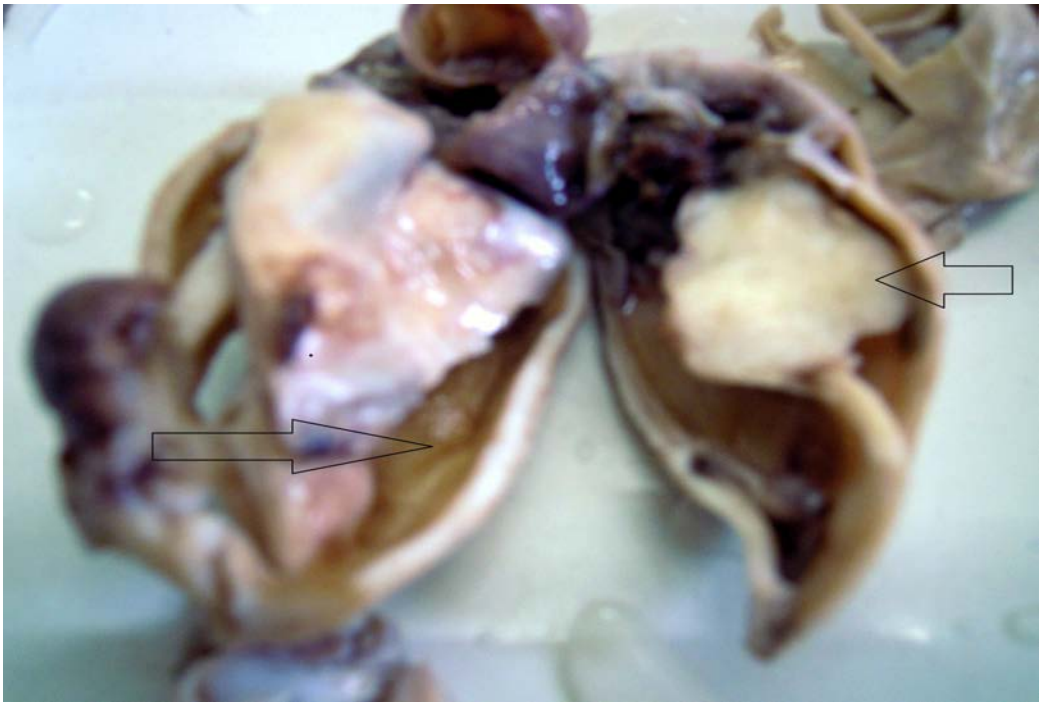

Fig No. 1 : Gross photograph showing cystic (thin arrow) and solid (thick arrow) areas representing cystadeonma and leiomyoma

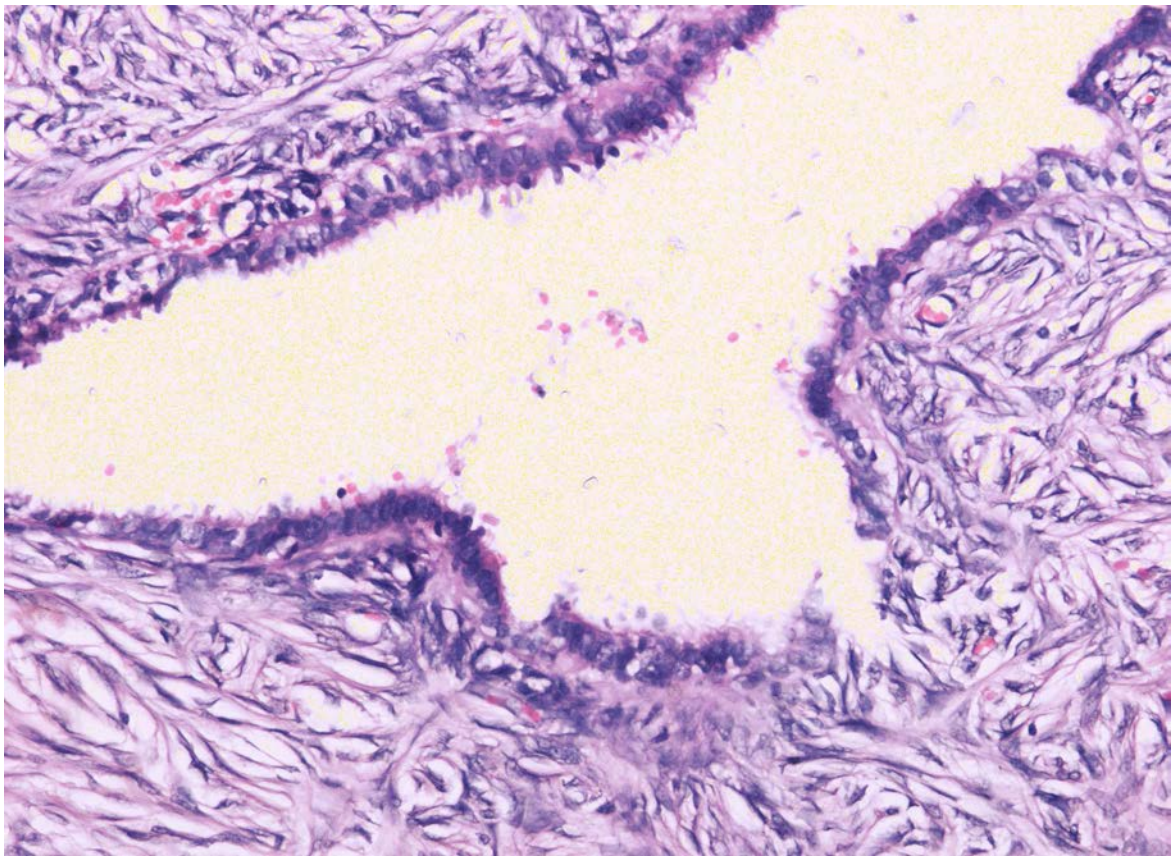

Fig No. 2 : Microphotograph showing a cyst lined by flattened epithelial lining (H& E; 100x)

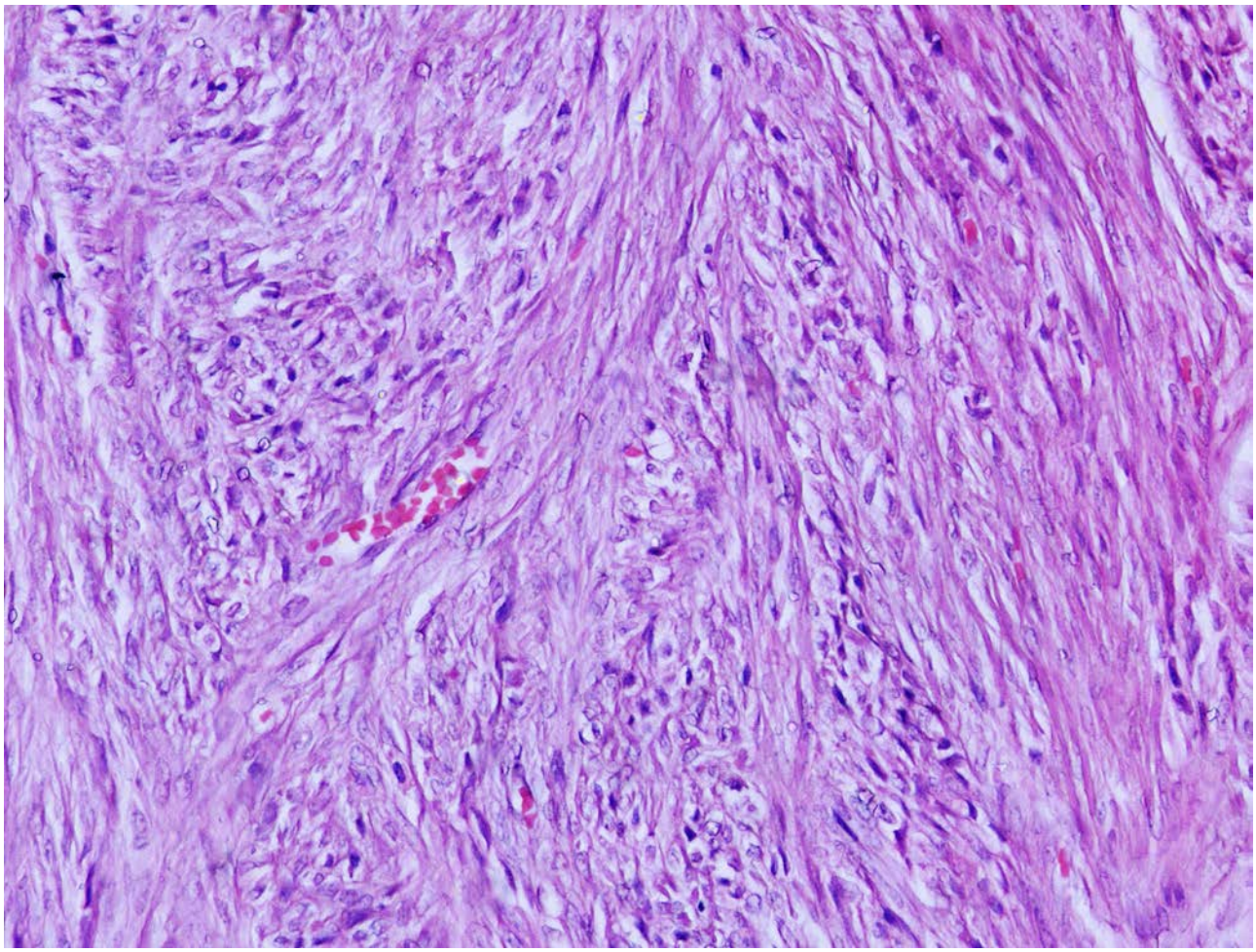

Fig no. 3 : Microphotograph showing whorling appearance of smooth muscle (H& E; 400x)

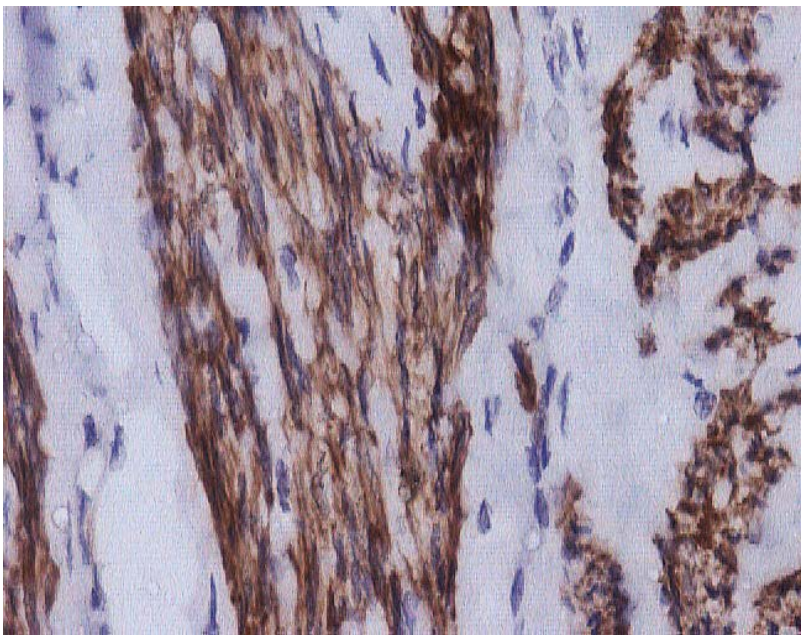

Fig No. 4 : Immunohistochemical Staining Positivity for Smooth Muscle Antigen (SMA; 400x)

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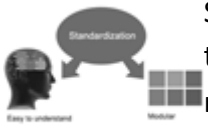

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4. Manuscript's Category,

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12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Administration Rules Listed Before Submitting Your Research Paper to Global Journals Inc. (US)

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

Criterion for Grading a Research Paper (Compilation) by Global Journals Inc. (US)

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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